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Protective Effect of Mesenchymal Stem Cells on Methotrexate-Induced Acute Toxicity on Liver of Adult Albino Rats

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Abstract: Our study aims at studying acute toxic effects of Methotrexate (MTX) on the liver of adult male albino rats and assessing the protective effect of Mesenchymal Stem Cells (MSCs) on the MTX-induced hepatotoxicity. Twenty-four adult male albino rats were divided into four groups, each of six. Rats in group I served as a negative control. Rats in group II received a single intraperitoneal injection of MTX at a dose of 10 mg/kg. Rats in groups III received MSCs therapy at a single intraperitoneal dose of $1x10^6$ cells per rat. Rats in group IV received MSCs therapy at a single intraperitoneal dose of $2x10^6$ cells per rat. Groups III and IV received MTX injection, same dose as group II. All animals were sacrificed on the 10^{th} day. The liver was retained for histopathological and immunohistochemical examination. The sera were used for biochemical analysis. We demonstrated that MTX significantly increased serum concentrations of liver enzymes, reduced the serum antioxidant catalase enzymes levels, significantly increased serum Malondialdehyde (MDA) concentrations. In addition, it increased iNOS expression and caused prominent histological alterations in the liver. On the other hand, treatment MSCs was effective in significantly improving the liver enzymes, ameliorating the elevations of MDA, increasing catalase enzyme levels and decreasing the hepatic nitrosative stress, and ameliorating the pathological hepatic changes induced by MTX. We concluded that MSCs in both doses (one and two million cells/rat) could

ameliorate the hepatotoxicity induced by MTX. Applying such conclusion might have a great impact in future clinical practices.
Keywords: Mesenchymal Stem Cells, Methotrexate, Hepatotoxicity, Oxidative stress.

Introduction

Methotrexate (MTX) is a folic acid antagonist (Türkcü *et al.,* 2015). It has been widely used as an effective chemotherapeutic drug. In addition, it is a well-established treatment for different types of rheumatic diseases, autoimmune and inflammatory diseases (Abdel-Daim *et al.,* 2017; Rjiba-Touati *et al.,* 2017). Acute MTX toxicity is most commonly caused by an accidental overdose of MTX tablets by the patient or physician's prescription error (Madke and Singh, 2015).

Interaction of several factors including the patient's risk factors, type of disease, dosing schedule, and treatment duration, as well as the presence of genetic apoptotic factors can produce Methotrexate-induced cytotoxicity (Abdel-Daim *et al.,* 2017). Unfortunately, different vital organs are adversely affected especially the kidney and liver leading to limitation of the clinical usage of such drug (El-Sheikh *et al.,* 2015).

Several studies have suggested the role of oxidative stress as a mechanism underlying MTX toxicity (Tousson *et al.,* 2014). MTX targets nicotinamide adenine dinucleotide phosphate (NADPH), part of the antioxidant defense system, by reducing its production, in addition, reactive oxygen species (ROS) are increased thus inducing oxidative stress and tissue damage (Yuksel *et al.,* 2017).

Up to 90% of MTX is cleared by the kidneys, and renal impairment; presented as acute kidney injury (AKI), which is attributed to tubular obstruction, due to the precipitation of MTX and its metabolites in the renal tubules (Severin *et al.,* 2016), in addition to the oxidative damage disturbing the balance of oxidant–antioxidant status (Yuksel *et al.,* 2017), can result in impairing its own elimination and increasing its systemic toxicity (Abdel-Daim *et al.,* 2017). The elevated toxic levels of MTX then contribute to multiple vital organ damage, including the liver (El-Sheikh *et al.,* 2015; Severin *et al.,* 2016).

Previous reports have demonstrated the pivotal role of oxidative stress in MTX hepatotoxicity. Excessive generation of reactive oxygen and nitrogen species (ROS/RNS), along with reduced antioxidant defense mechanism promote the development and progression of hepatotoxicity (Abo-Haded *et al.,* 2017). Recent studies have shown the possible role of inducible nitric oxide synthase (iNOS) in mediating MTX-induced hepatotoxicity (Hafez *et al.,* 2015).

Different anti-inflammatory, antioxidant, anti-proliferative and immunomodulatory drugs have been used to decrease the toxic effects of MTX. However, none have provided complete protection against MTX toxicity. Thus, there is a wide field for new agents that can be used to prevent MTX-related damage on normal tissues (Türkcü *et al.*, 2015). There is an urgent need to develop an efficient protective therapy against drug-induced hepatotoxicity.

Therefore, we aimed at studying the acute toxic effects of MTX on the liver of adult male albino rats and assessing the protective effect of Mesenchymal Stem Cells on the MXT-induced hepatotoxicity.

Materials and Methods

This study was conducted in the animal house, Physiology department, Faculty of Medicine, Suez Canal University, Egypt.

Rats

Twenty-four adult male wistar albino rats, weighing 150-250 g were obtained from the Egyptian Organization for Biological Products and Vaccines. All rats were kept for ten days at the experiment site for acclimatization. All rats were kept under optimal environmental conditions (12-hour light-dark cycles, temperature [$20 \pm 2°C$] with moderate humidity [60 ± 5%] and adequate ventilation. Environmental conditions were standardized for all animal groups. They were housed in plastic cages maintained under standard pathogen-free conditions, in a quite non-stressful special room. They were freely fed on a standard pellet chow diet and tap water in clean containers. Necropsy was performed soon after an animal was sacrificed or found dead in a humane manner.

Isolation and characterization of Mesenchymal stem cells (MSCs)

Two Rats were sacrificed by cervical dislocation and the tibia and femur were dissected. Medullary cavities of the bone were flushed with 10 ml of PBS for collection of marrow cells. The cells were centrifuged at 200 g for 10 minutes and the supernatant removed by aspiration. The cells were re-suspended in DMEM supplemented with 15% FBS, 100 U/ml Penicillin/Streptomycin and 50 U/ml Gentamicin and cultured at 37 ^{0}C with 5% CO_2. Adherent cells were harvested when reached 50-60% confluence with 0.25% trypsin.

Experimental Design

The rats were divided into four groups, each of six. Rats in group I served as a negative control, receiving saline only during the entire experimental period. Rats in group II received a single intraperitoneal injection of MTX *(MTX injection (50 mg) vial was purchased as an over-the-counter drug, manufactured by Shanxi*

PUDE Pharmaceutical Company, Ltd, Imported by Techno Pharma.) at a dose of 10 mg/kg bw (mimicking the acute exposure to an acute large dose in humans (Mukherjee *et al.*, 2013). The intraperitoneal LD50 of methotrexate is 6-25 mg/kg for rat (*Datasheet for MTX-New Zealand*).

Rats in groups III received MSCs therapy at a single intraperitoneal dose of $1x10^6$ cells per rat. Rats in group IV received MSCs therapy at a single intraperitoneal dose of $2x10^6$ cells per rat. On the same day of the experiment, rats in groups III and IV received I.P MTX injection, at the same dose used in group II rats. Animals were assigned to the groups in a simple random manner. All animals were sacrificed on the 10^{th} day after blood sample collection via retro orbital plexus of veins.

The liver for all rats were fixed with 10% formalin for histo-pathological and immunohistochemical examination. The obtained blood samples were allowed to clot for 30 minutes then centrifuged at 3000 rpm for 15 minutes in order to obtain clear sera. The sera were then stored at −80°C for biochemical analysis (Liver functions tests and Oxidative stress markers).

Serum Biochemical Analysis

All assays were determined by using UV/VIS Spectrophotometer (Unico S2100 Series, USA) following manufacturer's protocol.

Liver functions tests

The assay kits used to analyze Albumin, Alanine aminotransferease (ALT), Aspartate aminotransferase (AST) were purchased from (Spinreact, Spain). Serum albumin was measured according to method of (Rodkey, 1965). Alanine aminotransferease (ALT), also called serum Glutamate pyruvate transaminase (SGPT), was determined by colorimetric kinetic according to (Murray, 1984a) method. Aspartate aminotransferase (AST), also called serum Glutamate oxalacetate transaminase (SGOT), was determined by colorimetric kinetic according to (Murray, 1984b) method.

Oxidative stress markers

The oxidative stress Kits were purchased from (Biodiagnostics, Cairo, Egypt).

A) Malondialdehyde (MDA) or Lipid peroxide level: Thiobarbituric acid (TBA) reacts with lipid peroxide in acidic PH at temperature of 95°C for 30 min to form thiobarbituric acid reactive product, the absorbance of the resultant pink product can be measured at 534 nm as described by (Yagi, 1982).

B) Catalase: It is an antioxidant enzyme that is present in most aerobic cells containing a cytochrome system. It is considered as one of the body's defense

systems against Hydrogen peroxide (H_2O_2), a strong oxidant that can cause intracellular damage. It was determined as described by (Aebi, 1984).

Histopathological and Immunohistochemical Examination

The specimens from the liver were collected and fixed in 10% formalin solution and embedded in paraffin. Five μm thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H&E) dyes. Stained slides were microscopically analyzed using light microscopy (Olympus CX41). Necroinflammatory score (grade) and fibrosis stage of liver slides evaluated according to ISHAK score (Westin *et al.,* 1999).

For immunohistochemical staining, sections were fixed at 65∘ C for 1 h. Triology pretreatment (deparaffinization, rehydration, and antigen unmasking) was used to enhance standardization of the pretreatment step and produce results that are more consistent. The rabbit polyclonal antibody against inducible nitric oxide synthase (iNOS), was employed as it is (ready to use) according to their manufacturer's specification.

After applying the antibodies, slides were incubated overnight at 4◦C followed by 20 min of Poly HRP enzyme conjugation. Afterwards, DAB chromogen was applied for 2 min and then rinsed, followed by counterstaining with Mayer hematoxylin before examination under the light microscope.

Statistical analysis

Collected data throughout laboratory techniques and outcome measures were coded, entered and analyzed using the based statistics program: Statistical Package for the Social Sciences (SPSS) software version 20.0.

The variables were expressed in the form of means and standard deviation.

ANOVA test was used to determine any significance differences between the mean levels of continuous variables (control and experimental groups). Differences were considered significant at $P< 0.01$.

Compliance with Ethical Standards

Statement on the welfare of animals: All Faculty of Medicine, Suez Canal University, Egypt guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the Research Ethics Committee (REC) at Faculty of Medicine, Suez Canal University, Egypt at which the study was conducted. (Permit number 3681).

Results

Biochemical Findings

Hepatic functional serum marker concentrations

Intraperitoneal injection of MTX significantly increased serum concentrations of ALT, AST enzymes, compared to normal control levels ($p < 0.01$). However there were no observed significant effects on albumin levels. Upon treatment of MTX-injected rats with MSCs (at one million and two million cells/rat) significantly reduced the elevation of ALT levels by 55.4% and 70.3%, AST levels by 30.93% and 33.5%respectively.

Treatment of MTX-injected rat with both doses of MSCs restored serum concentrations of these parameters to normal control levels. However, treatment of MTX-injected rats with two million stem cells/rat was more effective in reducing the elevated serum levels of ALT and AST than treatment with one million stem cells/rat **(Table 1).**

Serum Antioxidant Activity

MTX-injected rats showed a marked reduction in the serum antioxidant catalase enzymes levels, as well as significant increase in serum MDA concentrations ($p < 0.01$). Administration of MSCs (at one million and two million cells/rat) significantly ameliorated the elevations of MDA concentrations in MTX-injected rat ($p < 0.01$). Moreover, this increased activities of CAT enzyme. However, treatment of MTX-injected rats with two million stem cells/rat was more effective than treatment with one million stem cells/rat **(Table 2).**

Histopathological and Immunohistochemical findings

Histopathological examination of Liver

Histopathological examination of liver showed normal structure in all groups (control, MTX, MTX-treated with MSCs) as regards to the lobular architecture, central vein, and radiating hepatic cords **(Figures 1a, 2a, 3a respectively).**

For the MTX group, the liver showed marked dilatation and congestion of portal vein/ sinusoids and central veins**,** portal area showed moderate inflammatory cells infiltrate in some portal areas (point 2), No Periportal or periseptal interface hepatitis (piecemeal necrosis) (point 0), No confluent necrosis (point 0), Five to ten foci per 10 objective of Focal (spotty) lytic necrosis, apoptosis and focal inflammation (point 3) (**Ishak grade "Necroinflammatory score"= 5/8),** bile duct hyperplasia, Fibrous expansion of some portal areas, without short fibrous septa (**Ishak stage 1/6**) **(Figure 2a)**.

On the contrary, with MSCs therapy (two million cells/rat), liver showed mild dilatation and congestion of portal vein**,** kupffer cells hyperplasia, portal area showed mild inflammatory cells infiltrate in some portal areas (point 1), No Periportal or periseptal interface hepatitis (piecemeal necrosis) (point 0), No confluent necrosis (point 0), Two to four foci per 10 objective of focal (spotty) lytic necrosis, apoptosis and focal inflammation (point 2). (**Ishak grade "Necroinflammatory score"= 3/8),** bile duct hyperplasia, no fibrosis (**Ishak stage=0/6**) **(Figure 3a).**

Effects of MTX and MSCs therapy in MTX-injected rats on Hepatic iNOS Expression

Immunohistochemical staining of rat liver sections with iNOS was performed to confirm nitrosative stress. Immunohistochemical staining of control rat liver with iNOS revealed scattered cytoplasmic expression of iNOS in the portal and sinusoidal vascular endothelia, and kupffer cells in between lobular architectures (**Figure 1b**). As regards to iNOs expression in MTX group, there is relative increase in iNOs expression, compared to both MSCs therapy and control groups, in the form of positive cytoplasmic brownish stain for iNOs in kupffer cells scattered in between hepatocyte lobules/ lymphocyte infiltrate in hepatic lobules and portal area **(Figures 1b, 2b and 3b).**

Table 1. Ameliorative effects of mesenchymal stem cells on serum concentrations of renal and hepatic injury markers in adult male wistar albino rats exposed to methotrexate

Group	ALT	AST	Serum Albumin
Control	42.75 ± 1.50	77.25 ± 30.23	3.30 ± 0.35
MTX (10 mg/Kg)	139.80 ± 48.87 $^{\neq}$	137.20 ±13.22$^{\neq}$	3.33 ± 0.40
MTX (10 mg/kg) + one million stem cells	62.40 ± 35.42 *	94.75 ±14.27 *	3.88 ± 0.05
MTX (10 mg/kg) + two millions stem cells	41.50±4.93 *	91.25 ±7.50 *	3.50 ± 0.32

Values are means ± SD.
$^{\neq}$ *Significant change at* p< *0.01 with respect to the negative control group.*
**Significant change at* p< *0.01 with respect to the MTX group as the positive control group.*
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase or transaminase

Table 2. Ameliorative effects of mesenchymal stem cells on serum concentrations of lipid peroxidation and antioxidant markers in adult male wistar albino rats exposed to methotrexate

Group	Malonaldehyde	Catalase
Control	42.57±33.47	948.10±46.66
MTX (10 mg/Kg)	116.61±36.11 $^{\neq}$	557.73±188.50 $^{\neq}$
MTX (10 mg/kg) + one million stem cells	69.74±27.08 *	773.62±45.03 *
MTX (10 mg/kg) + two millions stem cells	46.21±35.2 *	820.40±171.04 *

Values are means ± SD.
$^{\neq}$ *Significant change at* $p < 0.01$ *with respect to the negative control group.*
**Significant change at* $p < 0.01$ *with respect to the MTX group as the positive control group.*

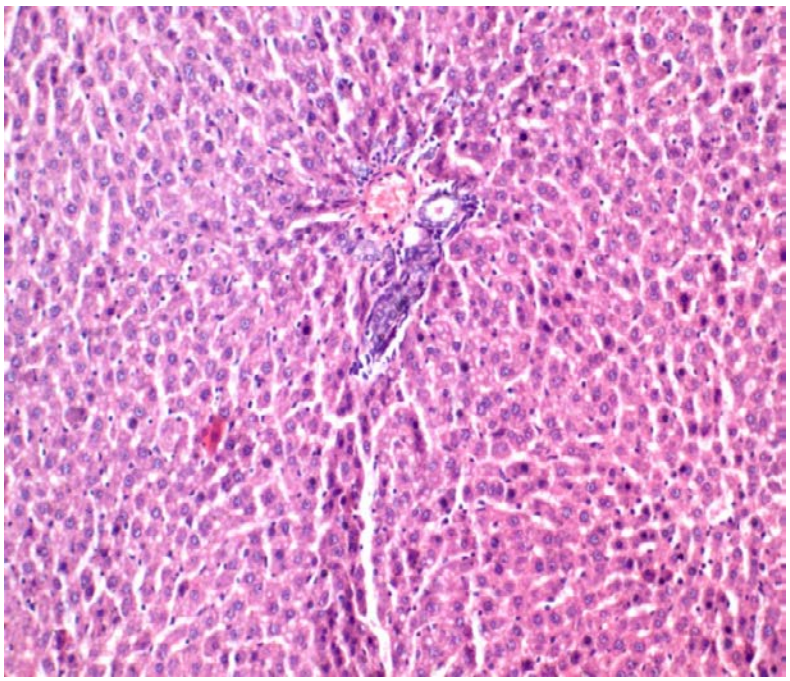

Figure 1a. Normal liver tissue in control group showed preserved lobular arrangement of hepatocytes, intervening sinusoids, Kupffer cells in between, portal area with portal vein, normal hepatic artery and bile duct in portal area. No inflammation or degenerative/reactive changes (H&E X200).

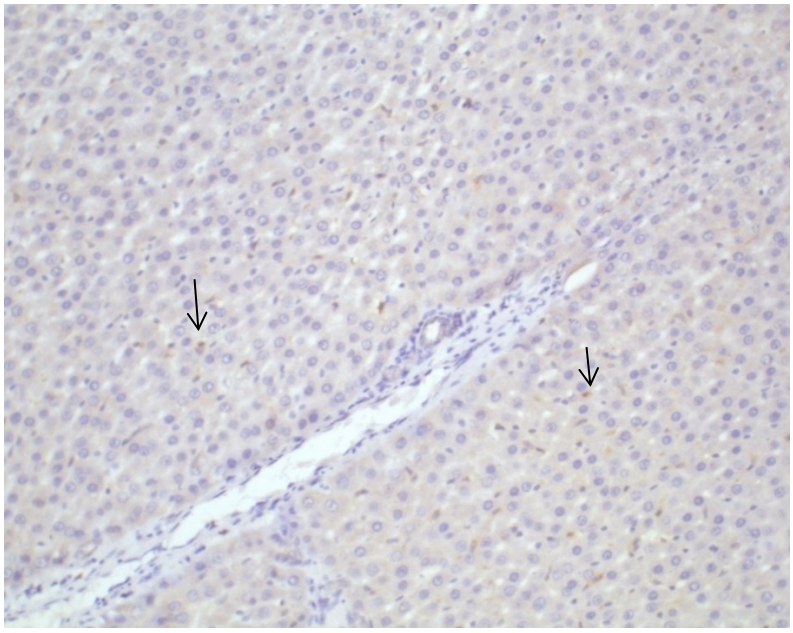

Figure 1b. In control group, scattered faint positive cytoplasmic brownish stain for iNOs in kupffer cells scattered in between hepatocyte lobules and portal and sinusoidal vascular endothelia (arrows) (IHC x 200).

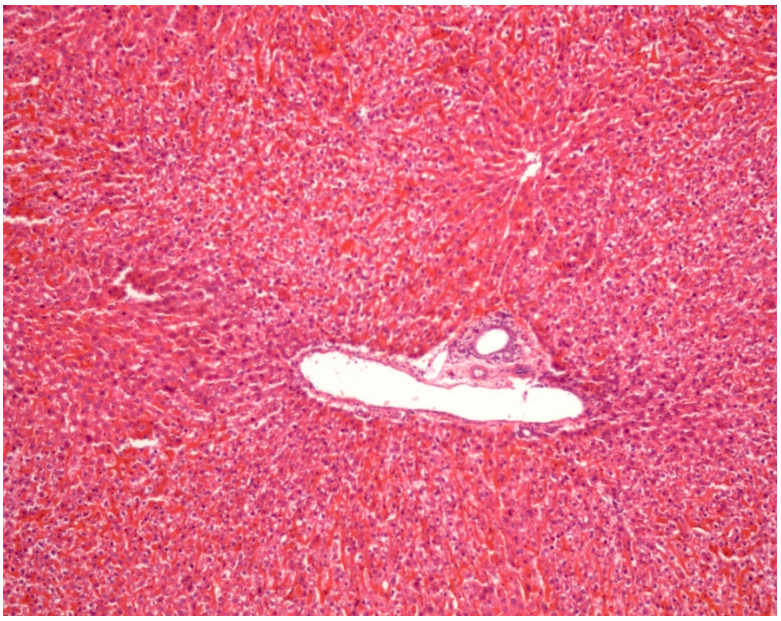

Figure 2a. Liver tissue for MTX group showed preserved lobules of hepatocytes, severe congestion of sinusoids, pronounced lymphocytic infiltrate of hepatocytes lobules and kupffer cells hyperplasia, No Fibrosis (H&E x 200)

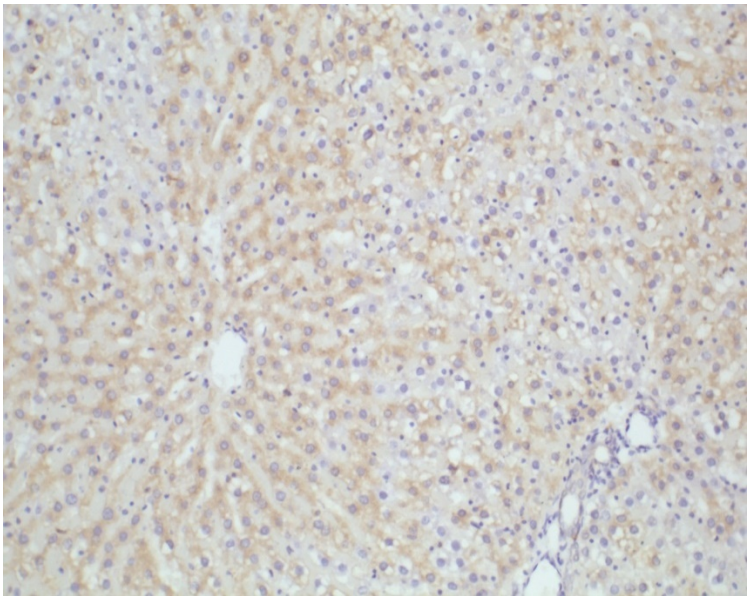

Figure 2b. For MTX group, strong positive cytoplasmic brownish stain for iNOs in kupffer cells in between hepatocyte lobules/ lymphocyte infiltrate in hepatic lobules and portal area (IHC x 200)

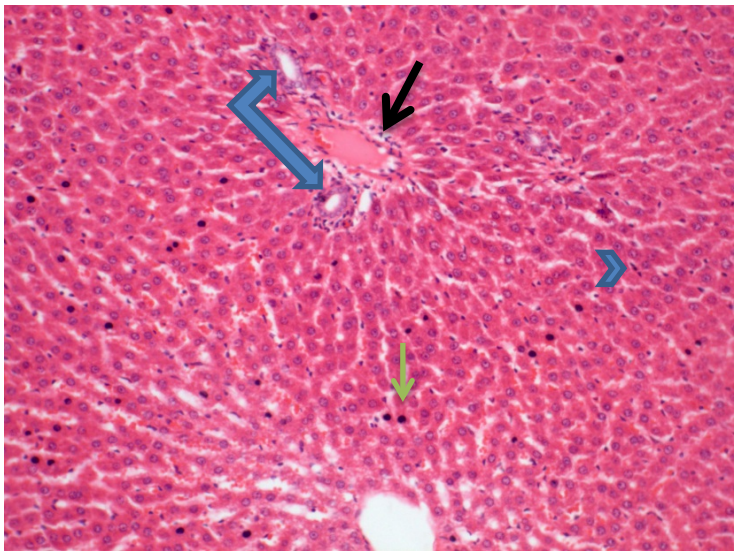

Figure 3a. Liver tissue in MTX group treated with Mesenchymal stem cells (two million cells/rat) showed preserved lobules of hepatocytes, mild congestion of portal vein, sinusoides (black arrow). Focal lytic necrosis of hepatocytes (green arrow) and kupffer cells hyperplasia (arrow head), portal area showed mild inflammatory cells infiltrate (green arrow), bile duct hyperplasia (double headed arrow), no fibrosis (H&E x 200).

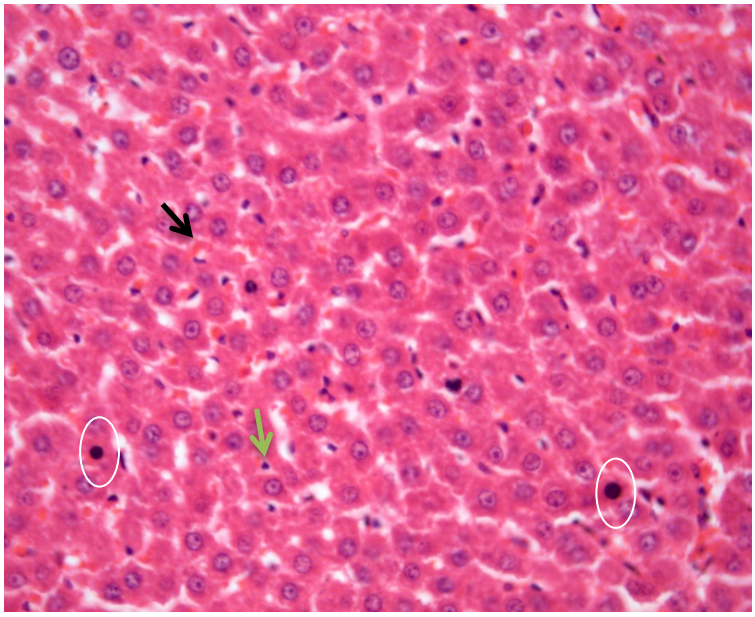

Figure 3a.1. Liver tissue in MTX group treated with Mesenchymal stem cells (two million cells/rat) showed preserved lobules of hepatocytes, mild congestion of sinusoids (black arrow), kupffer cells hyperplasia (green arrow), focal pyknotic nuclei (lytic necrosis) of hepatocytes (white circle) (H&E x 400).

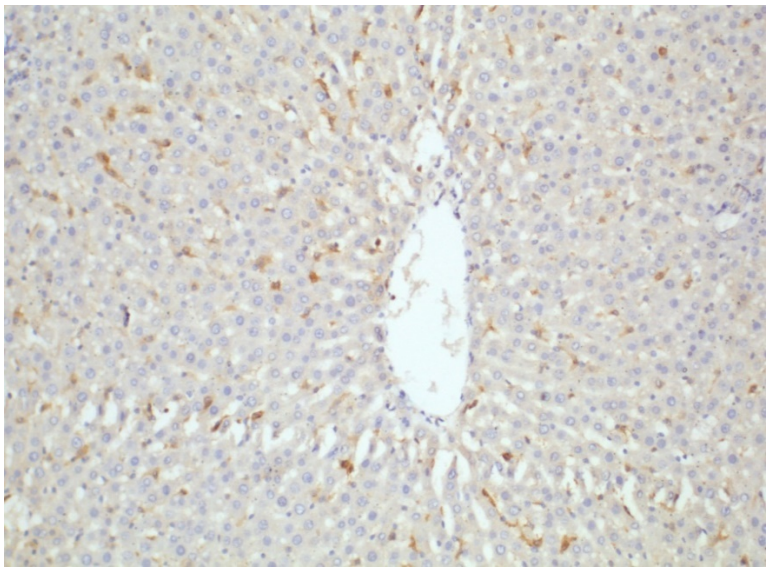

Figure 3b. In MTX group treated with Mesenchymal stem cells (two million cells/rat), positive cytoplasmic brownish stain for iNOs in kupffer cells scattered in between hepatocyte lobules/ lymphocyte infiltrate in hepatic lobules and portal area (IHC x 200).

Discussion

Methotrexate (MTX), despite being a widely used chemotherapeutic and immunosuppressive agent, it has many side effects especially being hepatotoxic. This study addressed the potential protective effect of bone marrow derived MSCs on MTX liver acute toxicity. Role of MSCs in treatment of MTX-induced toxicity is not clear enough to be used in clinical trials. Accordingly, our experimental study may have a great potential to denote that the MSCs could be able to significantly reduce the biochemical and histological alterations, induced by MTX.

As regards to the MTX-induced hepatotoxicity, the mechanisms are still unclear. However, this could be attributed to its cellular pathway. Methotrexate indirectly affects MTHFR (methylene-tetrahydro folate reductase) and hence the generation of methionine from homocysteine (Pandit *et al.,* 2012). Kumari (2016) stated that the increased homocysteine levels increase production of reactive oxygen species (ROS) such as MDA. Altered balance between ROS production and antioxidant defenses leads to a state of "oxidative stress", which leads to various pathological conditions in different organs all over the body (Kumari, 2016). In addition, increased ROS can lead to several structural and functional abnormalities due to the damage they cause to lipids, proteins, and nucleic acids of all cells of the body (Sönmez *et al.,* 2012).

The state of methotrexate induced oxidative stress was evidenced in our study by the significant increase in serum MDA level; an oxidant marker and indicator of tissue lipid peroxidation, caused by ROS, suggesting that lipid peroxidation is an important contributing factor to the MTX-induced tissue damage (Şener *et al.,* 2006), associated with significant decrease of serum catalase; an antioxidant enzyme that converts the potentially harmful hydrogen peroxide into oxygen and water (Weydert and Cullen, 2010), in MTX-induced toxicity rats, when compared with control rats.

The reduction in the level of antioxidant defense system may be attributed to a significant reduction in Glutathione (GSH) level, an important antioxidant protecting against ROS, induced by MTX. This in turn may be explained by the reduction of cellular NADPH pool; important for maintenance of the reduced form of cellular GSH by action of glutathione reductase.

This reduction of the NADPH may be related to the inhibition of the cytosolic nicotinamide adenosine diphosphate (NADP)-dependent dehydrogenases and NADP malic enzymes (Caetano *et al.,* 1997; Babiak *et al.,* 1998; Jahovic *et al.,* 2003; Çakır *et al.,* 2011; Abdel-Raheem and Khedr, 2014; Pınar *et al.,* 2018).

In addition, MTX induces the release of pro-inflammatory cytokines as COX-2, TNF-α, iNOS, MMP 9, interleukin IL-1β and IL-6 as a result of up-regulation of the expression of nuclear factor-kappaB (NF-κB). This leads to tissue damage, apoptosis and endothelial dysfunction (Yamamoto and Gaynor, 2004; El-Gowilly *et al.,* 2015; Natarajan *et al.,* 2018). Methotrexate induced oxidative stress creates a nitrosative inflammatory state in hepatic tissue. El-Sheikh *et al.,* (2015) stated that MTX causes increased hepatic nitric oxide, with upregulation of inducible nitric oxide synthase (El-Sheikh *et al,* 2015), which is proved by the increased cytoplasmic expression of iNOS in hepatic tissue (>50%) in our MTX-induced toxicity rats compared to scattered cytoplasmic iNOS expression (<10%) in normal rats. This result suggested that the peroxidation reaction was higher in MTX group than control group.

Moreover, the MTX induced nitrosative stress leads to marked inflammation in liver tissue. In agreement with El-Sheikh *et al.,* (2015), MTX toxicity induced marked inflammation of the hepatic tissue, associated with significant increase of serum transaminases (ALT & AST) compared with control group. There were prominent pathological changes in the liver tissue of MTX group including congestion of portal vein/ sinusoids and central veins**,** inflammatory cells infiltrate in some portal areas, Focal (spotty) lytic necrosis, apoptosis and focal inflammation , bile duct hyperplasia, Fibrous expansion of some portal areas, without short fibrous septa observed compared to control group. The Ishak score of liver tissue revealed inflammatory and fibrosis score of (5/8, 1/6 respectively) in MTX group. We observed that the histopathological changes of the liver tissues were concomitant with the disturbance in the balance of oxidant–antioxidant status and the increased hepatic iNOS expression.

Ayatollahi *et al.,* (2014) demonstrated that MSCs transplantation has an anti-oxidant protective effect against oxidative stress induced liver injury. That is why, in current study, rats were treated with MSCs; used in two different doses (one and two millions MSCs), aiming to alleviate the methotrexate toxicity effects (Ayatollahi *et al.,* 2014). Our present study showed that transplantation of MSCs improved the imbalance of oxidant/antioxidant capacity, a significant reduction in serum MDA level as well as a significant elevation of serum catalase level in group III and IV (MTX-induced toxicity group treated with MSCs) as compared to group II (MTX-induced toxicity with no treatment). As a sequalae, the restoration of oxidant/antioxidant balance, resulted in decreasing the hepatic nitrosative stress, which was evidenced by marked decrease in iNOS expression in liver tissue (10- <50%) compared to the increased cytoplasmic expression of iNOS in liver tissue (>50%) in MTX-induced toxicity group with no MSCs transplantation.

Moreover, interestingly, MSCs ameliorated the histological alteration in hepatocytes induced by MTX. Supporting to our result, Gazdic *et al.,* (2018), found that MSCs protect from acute liver injury. Moreover, In agreement with Ayatollahi *et al.,* (2014) histopathological assessment of hepatic tissue showed significant improvement of Ishak inflammatory and fibrosis score from (5/8, 1/6 respectively) in MTX-induced toxicity group, to (3/8, 0/6 respectively) in MSCs treated group (in either dose).

The MSCs induced improvement in MTX toxicity was not only structural, but it was also functional. MSCs transplantation (in either dose) caused significant improvement of serum transaminases (ALT & AST) level compared to the levels for MTX-induced toxicity group.

Conclusion

We concluded that MSCs in both doses (one and two million cells/rat) could ameliorate the hepatotoxicity induced by MTX. Applying such conclusion might have a great impact in preventing the MTX-induced hepatotoxicity during MTX therapy strategies.

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Extradition and Human Rights in Somalia

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Abstract
Extradition is the only legal way for a country to surrender any wanted person to another country for the latter to put them on trial or execute punishment imposed on him/her. Avoiding the application of extradition procedures is contrary to human rights standards as it deprives the person of his/her rights under extradition law. The wanted person's rights in extradition proceedings are, in any case, more efficient and useful than the rights in any deportation or expulsion procedure. The compliance with the rights of persons wanted for extradition necessitates priority of extradition procedures over other procedures. These other procedures shall give way to extradition.
Law should clearly distinguish between and regulate provisional extradition arrest, prior to the arrival of the formal request for extradition, and full extradition arrest, after the arrival of the request. The lack of clear rules endangers the right to freedom of wanted persons. It must be borne in might that generally the provisional extradition arrest is a technological necessity. This is why the efforts to bring back fugitive offenders should start with the circulation worldwide the petition for this arrest.
The relative immunity of extraditees must be honoured and guaranteed by the domestic law of the requesting country. They shall not be prosecuted, tried, punished or/and detained for a crime, different from the one for which their extradition was granted and carried out.
There shall be no extradition to a country, which does not exclude any death penalty for the crime, in respect of which the extraditee was surrendered. It is always safer when the exclusion of the death penalty is achievable through the implementation of an explicit legal provision. The exclusion is less reliable when it is a result of some diplomatic assurance, given by the requesting country to the requested one.
Keywords: human rights, extradition, disguised, arrest, death penalty.

Introduction

Historically, extradition is the oldest form of international collaboration between countries, involving cooperation in the matters pertaining to crime control by exchanging fugitive offenders. It is a complex legal-political means allowing a country to surrender a person found within its territory to another in order to be prosecuted or sentenced there for one or more crimes punishable with imprisonment. Each country needs to ensure that criminals cannot evade justice simply by crossing borders. This requires an effective, responsive extradition system that effectively combats domestic and transnational crime, including terrorism, organized crime and corruption, with appropriate human rights safeguards.

Extradition always involves a request by the competent authorities of one interested country to respective authorities of another for the surrender of a fugitive, found in the territory of their country. S/he is either a prosecuted person (accused) or one who has been convicted (sentenced) of a criminal offence in the requesting country.

The need to resort to extradition as a specific means of collaboration is determined by the impossibility of a country interested in having the wanted person physically available to exercise any coercive power on the territory of the foreign country where the person is located, without infringing its sovereignty. The obtaining of the surrender of such person from a foreign country without any impairment of its sovereignty is achievable only if that country freely decides that it is appropriate to grant the request of the requesting country and, therefore, to surrender the person. In so doing, the requested country balances the primary needs of international collaboration in the repression of crime with the protection of the fundamental rights of freedom of wanted persons (Bassiouni, M.C., and Wise, E. M., 1995; Blakesley, Ch. L, 1981; Edmonds-Poli, E and Shirk, D., 2018; Shearer, I. A., 1970; Бойцов, А. И., 2004).

Nowadays, the need of having elaborate legal procedures for extradition is mainly caused by the expansion of organized crime and terrorism phenomena and the increasing possibility of movement and transfer abroad, offered by the continuous development of means of transport and resulting in the ease of movement between countries. The international mobility of offenders, in particular, and the use of advanced technology, along with other factors, make it more necessary than ever to ensure the collaboration between the law enforcement and judicial authorities and provide assistance to the requesting country that has assumed jurisdiction over the criminal case, to assure the fugitive's attendance at the criminal and/or execution proceedings against him/her in the country concerned.

In order to achieve this goal, countries have enacted laws and signed international agreements mandating their authorities to mutually provide such international cooperation.

Results

Specific recomentations are given to improve the Somali national legal framework for extradition and the Criminal Procedure Code of Somalia, in general. The recommendations derive from the legislative experience of foreign countries, mainly from the civil law (Latin) family.

I. Export/ Passive Extradition and Human Rights Violations

1.1 The Disguised Extradition

By granting and executing the extradition request, the requested country surrenders the wanted foreign person who is, usually, not welcome there. In this regard, extradition resembles the domestic (unilateral) procedure of *expulsion* of some foreigner. However, in contrast to expulsion, extradition benefits a specific foreign country. This is the requesting country, which wants the surrender of the fugitive. Therefore, the extradition procedure is designed to ensure the carriage of efficient justice in the requesting country. If the extradition concludes successfully, the person is brought from to the judicial actors who shall prosecute and try him/her, and/or to the prison where s/he shall serve this punishment.

The requested country also benefits from the extradition if it concludes successfully. This country relieves itself of someone who could be a potential source of future problems in its territory. Often, though, the requested country has nothing specific against the wanted person; this made him stay for a longer or a shorter period of time, in its territory. Technically, that country has no legal ground to deport or expel the person and therefore, has no need to get rid of him/her on the basis of the information obtained from the extradition request. However, even if the requested country has some grounds to deport or expel the person from its territory, including grounds from the information in the extradition request, this shall never result in his/her surrender to the requesting country. This is why the requesting country shall not, in turn, expect to directly obtain the surrender of the person through deportation or expulsion. Such a result may be achieved only if the other country carries out the so-called disguised or fraud extradition. This sort of "extradition" is generally not encouraged and should not be expected.

Recently, on 27 May 2019, the African News Agency informed that the "*Authorities in Somalia had arrested and deported a former commander of Ethiopia's notorious Jail Ogaden, located in the eastern Somali regional state.*

Reports said Hassan Ismail Ibrahim known by the alias "Hassan Dhere" was captured in the central Somalia town of Goldogob and was handed to Ethiopian authorities in Jigjiga. Hassan had been on the run since August 2018 when federal forces were deployed by Prime Minister Abiy Ahmed to depose long-serving Somali regional state president Abdi Illey.

*Jail Ogaden is a facility where thousands of prisoners were tortured and abused according to Human Rights Watch investigations through rape, sleep deprivation, and physical assault. Ethiopia closed the facility last year with the promise to transform it into a mus*eum"(African News Agency, 2019).

Obviously, no formal extradition procedures were applied in the surrender of the alleged offender by Somalia to Ethiopia. The Somali authorities disregarded these procedures. What happened is a case of disguised (contrived or fraud) extradition. It is, probably, the most typical violation of human rights in international judicial cooperation.

According to the common understanding, the disguised/ fraud extradition always constitutes a gross violation of human rights standards. It eludes extradition rules and eventually deprives wanted persons of regular extradition proceedings and their possibility to exercise their specific procedural rights of defence, which may result in a decision for refusal of extradition. Most often, wanted persons are interested in taking part in extradition proceedings to state and prove, where applicable, the existence of one or more impediments to their extradition to the requesting country. Such impediments might be:

- ✓ The lack of dual criminality of the offence in respect of which extradition is sought; under Article 11.2 of the Somali Penal Code [further: PC] "*Extradition shall not be granted unless the act which gives rise to the demand for extradition is an offence [15 P.C.] under Somali law and the foreign law*".
- ✓ Although dual criminality exists, the crime is political; under Article 11.3 of the Somali PC „*No person may be subjected to extradition for political offences*"1.
- ✓ If a danger exists that if extradited the wanted person may be tortured in the requesting country; Article 3 (1), Item 3 of the UN Convention against Torture and Other Cruel, Inhuman or Degrading Treatment [Somalia is also a Party as it accessed the Convention on 24 Jan 1990] expressly

1 According to Article 8.3 of the same Code, "*For the purposes of penal law, any crime actuated, in whole or in part, by political motives shall be considered a «political crime»*".

forbids authorities of all requested Parties from "*extraditing a person to another state where there are substantial grounds for believing he would be in danger of being subjected to torture*"2.

The wanted person's rights in extradition proceedings are, in any case, more efficient and useful than the rights in any deportation or expulsion procedure. The compliance with the rights of persons wanted for extradition necessitates priority of extradition procedures over other procedures. These other procedures shall give way to extradition. This is why deportation or expulsion is strongly discouraged in case of existing extradition proceedings, e.g. Article 33 of the Bulgarian Law on Extradition and European Arrest Warrant and Article 77 of the Romanian Law on International Judicial Cooperation in Criminal matters expressly prohibit these administrative actions in substitution of extradition proceedings. To prevent any such abuses from occurring Article 697 (1) of the Italian Criminal Procedure Code [Further CPC] prescribes that the surrender of any person to another country for limiting his/her personal liberty (on the grounds of a detention on remand ordered in the other country or imposed imprisonment sentence there) "*shall only take place by means of extradition*".

The problem with Somalia is that this practice of unlawful deportation, replacing extradition proceedings, is likely to persist as the 'feasible' way out of the situation (as a strange 'act of necessity' in fighting crime, after all). The Somali authorities are 'forced' to such deportations in violation of human rights standards because they cannot carry out normal extradition to Ethiopia and to many other countries as well. In general, normal extradition to them is not possible as there are no legal grounds for it. Somalia has no extradition agreements with most of the foreign countries (except for the Parties to the 1983 Arab Riyadh Agreement for Judicial Cooperation, the Riyadh Convention) and its law does not allow any non-treaty-based extradition. Article 36 of the Somali provisional Constitution [Extradition of the Accused and Criminals] and Article 11 [Extradition] of the Somali PC, though applicable to passive (export) extradition proceedings initiated on foreign requests, have some indirect impact on active (import) extradition proceedings as well. Both Article 36 (1) of the provisional Constitution and

2 According to Article 1.1 of this Convention, "*For the purposes of this Convention, the term "torture" means any act by which severe pain or suffering, whether physical or mental, is intentionally inflicted on a person for such purposes as obtaining from him or a third person information or a confession, punishing him for an act he or a third person has committed or is suspected of having committed, or intimidating or coercing him or a third person, or for any reason based on discrimination of any kind, when such pain or suffering is inflicted by or at the instigation of or with the consent or acquiescence of a public official or other person acting in an official capacity. It does not include pain or suffering arising only from, inherent in or incidental to lawful sanctions*".

Article 11.1 (b) of the PC stipulate that a fugitive "*may be extradited … on the basis of an international treaty or convention which the Federal Republic of Somalia is a party to*".

It is noteworthy that, apart from the direct human rights concerns, the Somali state authorities are not interested in having only this treaty-based extradition either. Because Somali law allows treaty-based extradition only, it is not possible to extradite a fugitive from Somalia under any other (extra-treaty) condition, including reciprocity. This, in turn, considerably narrows the possibilities of obtaining extradition from another country.

Somalia does not contemplate reciprocity relations although it belongs to the Civil Law (Latin) legal family. At the same time, even the Muslim countries from this family extradite under reciprocity – Article 1 of the Iranian Law on Extradition3, Article 52 of the Iraqi CPC, Article 365 (1) (3) (ii) of the CPC of Kazakhstan, Article 2 of the UAE Law on International Judicial Co-operation in Criminal Matters, etc.

It is to be clarified that reciprocity relations are the typical extra-treaty condition for rendering international judicial cooperation. Such relations are invoked if the interested country has already considered (not necessarily granted) an extradition request from the other country; the interested country has just to mention this in the request to the other country. This is how *reciprocity by action* is being invoked. Subsidiarily, if the interested country has not considered in the past any extradition request from the country which it approaches now, this interested country should promise/declare to it its readiness to consider, in turn, its future requests. This is the way to invoke *reciprocity by words*.

The previous consideration by the requesting country of an extradition request from the country, which is being approached now, or the promised future consideration by the requesting country of extradition requests from the country which is being approached now, is sufficient to establish reciprocity relations. The requesting country shall not necessarily have executed the request, or respectively, shall not necessarily promise to execute all future requests from the country, which it approaches. Even if an agreement exists, the requested Party would not be obliged to execute all requests coming from the other Party or other

3 This Article reads: "*If there is an extradition treaty concluded between Iran and foreign states, extradition should be performed according to the provisions of that treaty, and if there is no extradition treaty or an extradition treaty is concluded but it does not include all required points, extradition should be performed according to the conditions of this law subject to reciprocal treatment*."

Parties. It would be obliged to execute only those incoming requests, which meet the applicable legal requirements. The actual obligation of the other country under any possible agreement is to read the request rather than ignore it stating that they do not have any legal obligations to the requesting county in the field of extradition.

When it comes to Somalia, in particular, this country cannot rely on any reciprocity with countries that it has no agreement with. As Somalia does not consider their extradition requests, it cannot expect, in turn, any cooperation from them either. Even if Somalia promises to the country, which it approaches to consider its future extradition requests, no relation of reciprocity with that foreign country would be invoked. The problem is that the Somali promise would not be acceptable as its own laws prevent it from being kept. Therefore, it would be an invalid promise producing no legal consequences.

In theory, Somalia may expect non-treaty based extradition from Common Law (Anglo-Saxon) countries. Usually, they do not work with reciprocity. Their extra-treaty condition derives from the so-called "designated countries list". Such lists are produced unilaterally by the central state authorities of those countries. If the requesting country, including Somalia, is on this list, the judicial authorities there would consider its request. Otherwise, if it is not, then most probably the requested country's judicial authorities will not consider the request at all.

Actually, Somalia may expect effective non-treaty based cooperation from the few countries, which do not restrict themselves to the abovementioned extra-treaty conditions for judicial cooperation. These countries are more flexible and render cooperation also under other extra-treaty conditions. Such, for example, are the following Civil Law countries: Hungary (Section 6, are para. 2 of the Hungarian Law on International Legal Assistance in Criminal Matters), Portugal (Article 6.1, "f" of the Portuguese Law on International Judicial Cooperation in Criminal Matters) and Romania (Article 5, para. 3 of the Romanian Law on International Legal Assistance in Criminal Matters).

The absence of reciprocity is not an impediment to the judicial authorities of these countries if the cooperation: (a) is seen to be advisable in view of the nature of the facts, or in view of the need to combat certain serious forms of criminality;(b) may benefit the person concerned; or/and (c) may serve to shed light on facts related to own nationals. Finally, Article 2 (2) of the Indonesian Law No. 1/1979 on Extradition is in the same sense. It reads: "*In the event that no treaty as mentioned in para (1) above has been drawn, extradition may be initiated based on good relations and if the interests of the Rep. of Indonesia requires it*".

In view of the findings so far, it is recommendable that the Somali legislation sould accept reciprocity as the typical extra-treaty condition for extradition. Probably, the existing restriction to treaty-based extradition only comes from the reception of Article 26 (1) of the Italian Constitution.

The provision reads: "*Extradition of a citizen may be granted only if it is expressly envisaged by international conventions*". However, Italy is not an appropriate example for Somalia in this regard. This European country has a lot of extradition agreements with other countries and the position and capacity to negotiate, sign and ratify many more. Compared to Italy, Somalia has much fewer extradition agreements with other countries (actually, such an agreement is only the 1983 Riyadh Convention4) and is not likely to have many more soon. As a result, Somalia needs to rely on non-treaty based extradition for a long period of time.

Moreover, rather than restricting the Somali authorities to treaty-based extradition the Constitutional provision of Article 36 may be amended to become closer to Civil Law principles. In particular, this Article of the Constitution may follow the example of Article 5.2 of the Somaliland Constitution which reads: "*The extradition of a Somaliland citizen to another country is prohibited*" (also Article 358.4 of the Iraqi CPC, Article 493bis (A), letter "d" of the Libyan CPC, Article 18.1 of the Turkish PC postulating that only foreigners are extraditable).

Usually, this is the issue regulated by Constitutional texts rather than the aforementioned conditions for reciprocity. If the national criminal law is applicable extraterritorially to crimes of own nationals as it the case with Civil Law countries, the extradition of these nationals is not that necessary. Either way, such countries will never extradite any of its nationals to Somalia, even if the Somali authorities extradite theirs.

4 This basic legal instrument of the Arab world was ratified by the Democratic Republic of Somalia on the 21st of October 1985. The other Parties to the Riyadh Convention are as follows: The State of Palestine (ratif. on the 28th of November 1983), The Republic of Iraq (on the 16th of March 1984),The Republic of Yemen [The People's Democratic Republic of Yemen (on the 13th of April 1984) and The Yemen Arab Republic (on the 11th of June 1984)], The Republic of Sudan (on the 26th of November 1984), The Mauritanian Islamic Arab Republic (on the 17th of June 1985), The Syrian Arab Republic (on the 30th of September 1985), The Republic of Tunisia (on the 29th of October 1985), Hashemite Kingdom of Jordan on the 17th of January 1986, The Kingdom of Morocco (on the 30th of March 1987), The Great Socialist People's Libyan Arab Jamahiriya (the 6th of Jan 1988), The United Arab Emirates (the 11th of May 1999), The Sultanate of Oman (the 28th of July 1999), Bahrain (the 23rd of July 2000), The Kingdom of Saudi Arabia (the 11th of May 2000), The Algerian People's Democratic Republic (the 20th of May 2001).

1.2 The Extradition Arrests

Along with the arrest for national criminal proceedings, the Somali CPC contemplates another arrest, which is also for criminal justice purposes. This is the arrest under Article 279 (2, 3) of the CPC:

"*In the cases where the person to be extradited has to be arrested, the President of the Court of Appeal shall issue a warrant of arrest in accordance with normal procedure.*

Such warrant of arrest shall be revoked automatically and the arrested person released if:

a) *within 60 days from the date of the arrest, where the request for extradition was made by an African State; or*
b) *within 90 days from the date of the arrest, where the request for extradition was made by a State outside Africa the Minister of Grace and Justice has not received the documentation in support of the request for extradition.*

Such time-limit may be extended, at the request of the State which asks for the extradition, only once and for a period not exceeding one month. Such extension may be granted by the Supreme Court, upon request by the Minister of Grace and Justice".

Obviously, the quoted Article envisages the provisional extradition arrest of the wanted person pending the official/formal request for his/her extradition. This is the arrest with such strict deadlines. Thus, the incoming requests under letters "a" and "b" are actually for the provisional arrest (detention) of the wanted person – see also Articles 43 and 44 of the 1983 Riyadh Convention, ratified by Somalia on the 21st of October 1985. The respective correction in the text of the quoted Article 279 CPC should be made. It is to be identified there that "the person may be arrested, prior to the arrival of the extradition request, on the basis of separate petition of the interested State describing the crime, including time and place of commission, and mentioning the existence of a detention order for the wanted person"5. Otherwise, a lot of mistakes are likely to be made.

If the official/formal request for the extradition of the detainee arrives within the deadline, s/he shall *per argumentum a contrario* stay in custody. This is the way to secure his/her appearance in court for the extradition proceedings against

5 This is required, for example, by Article 16 of the European Convention on Extradition and by Article 10 of the the Intergovernmental Authority on Development [EGAG] Convention on Extradition.

him/her. His/her new custody is called full extradition arrest (detention). Usually, it lasts until the end of the court proceedings. However, this full extradition arrest should be explicitly regulated rather than come as a conclusion from the provisions on the provisional arrest of the wanted person. Examples of explicit rules of the full extradition arrest are: Article 37 of the Bosnian Law on Mutual Legal Assistance in Criminal Matters, Article 16 of the Turkish International Judicial Cooperation in Criminal Matters and Article 16 (1) of the UAE Federal Law on International Judicial Cooperation in Criminal Matters.

The detention of the wanted person must always be justified. If the extradition is denied, s/he shall be released. However, if the request for his/her extradition is granted, this person shall *per argumentum a fortiori* stay in custody as s/he cannot rely on anything to prevent his/her surrender from taking place and his/her basis interest is to escape. Also, there must be a provision stipulating the release of the person if the requesting country does not take him/her over on. The rule may be different. It may read in the sense that if no representative of the requesting country comes on the day agreed on, the person is released immediately (e.g. Article 502.3 of the Belorussian CPC) or in 15 days extensible up to 30 days (e.g. Article 499.3 of the Albanian PC and Article 708.5 of the Italian CPC), or in 20 days extensible up to another period of 20 days (e.g. Article 61. 2,3 of the Portuguese Law on International Judicial Co-operation in Criminal Matters), or in 30 days (e.g. Article 26, para. 4 of the Bulgarian Law on Extradition and Article 48.3 of the 1983 Riyadh Convention) and never surrendered in relation to the same decision for his/her extradition. Certainly, in cases of *'force majeure'* that prevents the surrender or taking-over of the extraditee, the competent authorities of the two countries shall agree upon a new date of surrender, e.g. Article 57 (6) of the Romanian Law of International Judicial Cooperation in Criminal Matters.

It is noteworthy that Somalia cannot always rely on international agreements for extradition as in the case with the 1983 Riyadh Convention. Some of them refer to the requested country's law on the detention issue and many other issues as well. There are multilateral Conventions, which solely declare themselves a legal basis for extradition. Thus, pursuant to Article 16.4 of the UN Convention against Transnational Organized Crime, "*If a State Party that makes extradition conditional on the existence of a treaty receives a request for extradition from another State Party with which it has no extradition treaty, it may consider this Convention the legal basis for extradition in respect of any offence to which this article applies*." Therefore, even if Somalia becomes a Party to such a Convention, this country would need domestic rules on the full extradition detention. The rules would be used if the issue is not regulated by a respective extradition agreement with the requesting country.

1.3 The Rule of Speciality in Defence of the Extraditee

The aforementioned considerations apply to the Speciality Rule, which is also missing in the domestic extradition law of Somalia. According to Article 278 (2) of the Somali CPC, export (passive) "*extradition shall always be made subject to the condition that the person to be extradited shall not be tried for a different offence, nor be subject to different punishment, other than those for which extradition was offered or granted*". This is the undisputable 'speciality principle' of extradition law (Bernacchi, M. B., 1992; Hedges, R. J., 2014).

The problem is that the only reliable and acceptable in practice guarantee that this principle will be complied with by the requesting country is its law. The law of the requesting country must postulate the immunity of extraditee from prosecution, restriction of his/her liberty, trial or/and punishment for a crime different from the one(s) in respect of which s/he was surrendered. This applies to Somalia as well. Whenever it is a requesting country, the foreign country, it has turned to, would look for applicable rules materializing the speciality principle. If such rules are missing in the international agreement (bilateral treaty or multilateral convention) between Somalia and the requested foreign country, the competent judicial authorities of that country will look for them in the domestic extradition law of Somalia: Articles 278-281 of the CPC and 11 of the PC. Because the competent judicial authorities of the requested country cannot find any such rules, they will most likely reject the Somali extradition request.

Such important agreements with provisions on extradition which do not contain any Speciality Rule are the UN Convention against Transnational Organized Crime (see Article 16.5) and the UN Convention against Corruption (see Article 44.6). This rule must be in the law of the requesting country. Sooner or later, Somalia will become a Party to them but will not be able to make use of them for obtaining extradition of fugitives from other Parties until it inserts the Speciality Rule in its CPC.

The Speciality Rule text might be borrowed from Article 17 of the Angolan Law on International Judicial Cooperation in Penal Matters or Article 721 of the Italian CPC, or Article 39 of the Kosovar Law on International Judicial Cooperation in Criminal Matters, or Article 16 of the Portuguese Law on International Judicial Cooperation in Criminal Matters, or Section 496 of the Slovak CPC.

II. Import/ Active Extradition and Human Rights

2.1 Active Extradition and the Death Penalty Issue

Somalia has not abolished the death penalty. This punishment exists in the Somali penal system by virtue of Articles 90.1 (a) and 94 of the PC.

Apart from all human rights concerns, the existence of this punishment might be a serious impediment to extradition requested by Somalia. The problem would occur when Somalia requests extradition in respect of a crime which carries the death penalty only under Somali law. The requested country's law may not provide for the death penalty either because it has been abolished there, in total, or because its law prescribes it only for the commission of other crimes. As the crime does not carry the same punishment as in Somalia the requested country is expected to require assurances that the death penalty shall not be imposed or, if already imposed (and the extradition is for its execution), that this punishment shall not be executed.

Besides, the requested foreign country would be specifically obliged to look for such assurances if it is a Party to the UN Convention against Torture and Other Cruel, Inhuman or Degrading Treatment [Somalia is also such a Party as it accessed the Convention on 24 Jan 1990]. Article 3 (1), Item 3 of this Convention expressly forbids authorities of requested Parties from "*extraditing a person to another state where there are substantial grounds for believing he would be in danger of being subjected to torture*". As the death penalty is the most serious type of torture, extradition would be refused unless the foreign country gives sufficient assurance that this punishment is ruled out.

The assurances are individual (diplomatic) and normative. The individual assurance is given on an *ad hoc* basis by an authorized body/official of the requesting country. Such assurance is provided for in Article 37 of the 2011 Legal Assistance Agreement on Civil and Criminal Matters between Bosnia and Herzegovina and Iran. This Article stipulates that if the legislation of the requesting Party prescribes death penalty for the offence for which the extradition is requested, whereas the legislation of the requested Party does not prescribe such a penalty or in that Party the death penalty is not executable, then the extradition shall be permitted provided solely that the requesting Party provides the assurances that the death penalty shall not be executed.

The normative assurance seems, in any case, more reliable. For example, there may be a provision in the law of the requesting country that capital punishment shall not be imposed, and if already imposed shall not be put into effect with regard to a person extradited by a foreign country under such condition. In such a case, the death penalty stipulated in the law or imposed shall be replaced by 30 years imprisonment. Before the abolition of the death penalty, Bulgaria had such a provision–Article 38 (3) of the Bulgarian CC [repealed]. The 30-years imprisonment is preferable to the life imprisonment under Article 95 of the PC as some countries deny extradition even in cases where the crime, for which the

extradition is sought from them, carries life imprisonment, e.g. Article 16 (2) of the Kosovar Law on International Judicial Cooperation in Criminal Matters and Article 6 (1) (f) of the Portuguese Law on International Judicial Cooperation in Criminal Matters.

Such a mechanism of eliminating the death penalty in active extradition cases is recommendable to Somalia as well. Its elimination is the lesser evil compared to letting the person go free abroad and eventually, work against the Somali authorities.

2.2 The Fair Trial Issue

If the extradition request of Somalia is for trial the Somali authorities should be aware that most countries in the world are Parties to the UN International Covenant on Civil and Political Rights, 23 March 1976. They consider themselves obliged by Article 14 of the Covenant to reject any extradition request (even in respect of an ordinary criminal offence) whenever their competent authorities find any substantial grounds for believing that the request has been made for the purpose of prosecuting or punishing a person on account of his race, religion, nationality or political opinion, or that person's position may be prejudiced for any of these reasons. The obligation is applicable not only to relations with other Parties of the Covenant but also to third countries as well, incl. Somalia which has not yet ratified it.

The UN Human Rights Committee also noted that 'if a state party extradites a person ..., and if, as a result, there is a real risk that his or her rights under the Covenant will be violated in another jurisdiction, the State party may be in violation of the Covenant' [Communication No. 469/1991, UN Doc: CCPR/C/49/D/469/1991, paragraph 14.2].

A good example of the implementation of this policy is Article 6.1, letter "e" (ii) of the IGAD Extradition Convention. According to this provision, the extradition request shall be turned down if the wanted person "*has not received or would not receive the minimum guarantees in criminal proceedings, as contained in Article 7 of the African Charter on Human and Peoples Rights and Article 14 of the International Covenant on Civil and Political Rights*".

It follows that requested countries will study carefully the Somali criminal justice system and if they find it incompatible with the right to defence standards their decision would be negative to Somalia. These expectations should stimulate the interest of Somali authorities in improving the CPC, accordingly, and in ensuring the factual protection of the right to a fair trial in the country (LAW, 2018).

2.3 The Somali Trials *in Absentia*

If the extradition request of Somalia is for the execution of a punishment imposed in the absence of the wanted person the Somali authorities should be aware that even foreign countries, which also conduct such trials, are very careful and punctual in such cases.

If the Somalia judgment has been rendered *in absentia* (Articles 128D - 128H of the Somali CPC) the requested country is likely to inquire as to whether the wanted person had been notified of the criminal proceedings against him/her and had also the opportunity to defend him/herself. If this is not the case, the other country would require from Somalia as the requesting country assurances, sufficient to guarantee to the person the right to a re-trial, which safeguards the minimum rights of defence and, in particular, the right to be trialled in his/her presence (Article 14, letter "d" of the UN International Covenant on Civil and Political Rights)6. For this purpose, the other country would seek a provision in the CPC of Somalia which is similar, for example, to Article 422 (1). 6 of the Bulgarian CPC: "*A criminal case shall be re-opened where: ... Extradition has been allowed in the case of sentencing in absentia where a guarantee has been provided by the Bulgarian State Authorities for reopening the criminal proceedings over the offence for which extradition has been allowed*". Thus, criminal proceedings are to be opened in accordance with the law of the requesting country: either at a demand of the requested country or on the request of the extraditee, at least. If such a provision is inserted in the Somali CPC, it would make much more realistic the extradition requests of Somalia is for the execution of a punishment imposed in the absence of the wanted persons under Articles 128D - 128H of the Somali CPC.

Judgments *in absentia* are a practical problem if already rendered and also until and to the extent they will possible given Article 35.8 of the Provisional Constitution of Somalia which prohibits them in favour of the accused. This provision reads: "*The accused persons have the right to be present at their trial*."

6 It is also good to know that, according to Section 7 (2) of *the Model Law on Extradition* (UNODC, 2004), "extradition requested for the imposition or enforcement of a sentence may be refused, if the judgment has been rendered in absentia in the requesting State, the convicted person has not had sufficient notice of the trial or the opportunity to arrange for his defence and he has not had or will not have the opportunity to have the case re-trialled in his presence, unless the competent authorities of the requesting State give assurances considered to be sufficient to guarantee to that person the right to a re-trial which safeguards his rights of defence, or unless the person has been duly notified and has had the opportunity to appear and arrange for his defence and has elected not to do so". Hence, if a requested country has followed the quoted model text and its law is applicable, for one reason or another, it is probable that the competent authorities of that country honour the extradition request and surrender the wanted person.

Concluding Remarks

The challenges to human rights are growing steadily, especially in the area of international judicial cooperation in criminal matters as the complexity of this cooperation increases irreversibly. On the one hand, any international such cooperation must become more and more efficient today. Extradition, in particular, is a principal tool to bring criminals to justice; its high efficiency is essential in the global fight against crime, both international and domestic. On the other hand, however, this efficiency must not be achieved at the expense of human rights. Their protection is one of the greatest tasks of our civilization. Breaches of human rights laws are not justifiable and shall be avoided. A successful mechanism in balancing the two interests should be established for the purposes of avoiding unlawful arrests and surrenders of wanted persons, and protecting them against torture, prosecution and punishment for crimes, in respect of which they have not been extradited. Also, this mechanism shall prevent other forms of inhuman or degrading treatment or discrimination on the basis of race, religion or nationality; the risk of double jeopardy. In addition, if the extradition is for trial the right to a fair trial in the requesting country shall be guaranteed, and the risk of a disproportionate sentence be significantly reduced.

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Therapeutic Trials on Bovine Dermatophilosis Involving Topical Application of Mahogany Seed Oil Extract

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Abstract
Therapeutic trials involving topical treatment of bovine dermatophilosis using natural Mahogany seed oil on twelve naturally infected zebu cattle of various ages and sexes. The oil was delivered with a paint-brush, which was vigorously rubbed on the affected surface areas. The treatment was repeated every two days for four weeks. Two of the cattle served as negative control, while treatment was applied on the remaining ten. After four weeks of treatment the animals were observed for the disappearance of lesions. The result showed that seven of the cattle recovered completely and the characteristic lesions disappeared. One of the cattle was partially healed while two were not cured, the reason being attributed to the severity of the infection. Mahogany seed oil was found to be effective and no adverse effect was observed on the animals. The oil also has an insect repelling characteristic. Mahogany seed oil as a topical treatment for dermatophilosis is recommended to the livestock farmers.
Keywords: Dermatophilosis, Mahogany seed, Zebu cattle.

Introduction

Dermatophilosis is a disease that affects mainly cattle, sheep, camels, horse and goats. Etiology is *Dermatophilus congolensis* and occurs as an acute or chronic exudative skin disease. It has been reported worldwide especially in Africa, Australia, United States of America and the United Kingdom (Radostits, 2000). In tropical and sub-tropical areas, the disease can be epizootic and can result in considerable economic losses as a result of lost production, premature culling, treatment costs and down grading of the hides and skins (Lloyd and Sellers, 1976). Many methods that include either of both topical and parenteral medications has been used in the treatment of dermatophilosis with variable results (Oduye, 1975; Park, 1976; Heath, 1989).

Mahogany tree is widespread in dried and wet tropical forest zones. The oil can be extracted from the seed and leaves, which contains bitter alkaloid magazine effective against sore and ulcer (Dutter, 1979). The practical analysis of the oil shows the main fatty acid present as Palmitic, Oleic, Linoleic, Linolenic and Stearic acids.

The oil formulation has antibiotic properties while the characteristic "ferrous" odor and the bitter taste may be due to the presence of the terpenes and flavonoids, which have insect repellant action, corroborating an earlier observation by Geoffrey (1983). Mahogany oil is a remarkable topical healing agent with skin healing, anti-neuralgic, anti-inflammatory, antimicrobial, and antioxidant properties (Shittu and Bualla, 1988). It is against this background that this study was designed with the objective of determining the therapeutic effect of mahogany seed oil extract in the treatment of bovine dermtophilosis as an alternative to orthodox medicine.

Research Methods

Mahogany seed collection and oil extraction

Fresh Mahogany seeds were obtained from a commissioned herbal practitioner in Lafia Nasarawa State, North - Central Nigeria .The seeds were allowed to dry and then subjected to grinding machine to form seed into paste form. The paste was subjected to extractor machine and the oil collected through channel outlet. The oil was allowed to cool before preservation in a stopper bottle.

Study design

Twelve zebu cattle of various ages and sexes naturally infected and clinically diagnosed of bovine drematophilosis were randomly selected from a herd of semi -intensively reared cattle as experimental animals. The clinical diagnosis involved making smears of exudates from typical lesions of the skin on glass slide. The smears were stained with Giemsa stain and examined for the presence of the mycelia or coccoid form of the organisms which stained positive with Gram's stain.

Method of Treatment

Two cattle served as negative control while treatments were applied to ten. Each of the infected cattle was treated by topical application of the Mahogany seed oil delivered with a paint brush on the affected area of the animal body at a dose of 10 ml / 50cm^2 surface areas. The treatment was repeated every two days for four weeks. No other form of treatment was applied before and after the mahogany seed oil therapy. No treatment was given to the two infected cattle used as negative control for this experiment.

Result and Discussion

Mahogany seed oil is acidic (Table 1) and contains Oleic, Palmitic, Stearic, Linoleic and Linolenic acids in varying proportions (Table 2).

Table 1. Specification and analysis of the mahogany seed oil

Colour	**Light peach with slight yellow**
Odor	Heavy, fatty and ferrous odor
Free fatty acid	3.0%
Peroxide value	4.5
Non-saponificable	0.6
Saponification value	180 -200
Iodine value	100 - 112
Specific gravity	0.91
Viscosity	5.8
pH Value	4.1

Table 2. Fatty acid contents of the mahogany seed oil

Fatty acid	**Percentage**
Oleic	41.3%
Palmitic	13.9%
Linoleic	29.73%
Linolenic	0.19
Stearic	13.29%

The effect of sex and age on response to treatment was not determined. Out of the twelve Zebu cattle used for the experiment, treatment was administered to ten, out of which seven recovered completely and were cured of lesions of the disease.

Complete healing was observed in each animal by complete removal of skin scabs and replacement with growth of new hairs on the infected areas. Smears taken from the recovered cattle were again stained with Giemsa stain and examined for the presence of the mycelia or coccoid which tested negative with Gram's stain.

One was partially cured while two in chronic condition were not cured as well as the two that served as the control (Table 3).

No adverse effect was observed on the animal treated with the mahogany seed oil extract.

Table 3. Lesion distribution on the animal bodies, Sex and Age of the animals and Effect of the treatments

S/No	Affected Base	Sex	Age (years)	Response
1	Neck and dew lap	Female	3.5	Cured
2	Base of the tail	Male	3.5	Cured
3	Udder	Female	4.0	Cure
4	Udder	Female	3.8	Cured
5	Dorsal aspect of abdomen	Male	1,6	Not cured
6	Back, head and two hind limbs.	Male	3.8	Partially cured
7	Testis and Neck	Male	0.9	Cured
8	All the neck and right side of the lap	Female	1.2	Cured
9	Udder and neck	Female	1.5	Cured
10	Lateral aspect of the abdomen and neck	Female	4.0	Not cured
11	Neck and back region	Female	3.8	Control (Not cured)
12	Hind limbs and neck	Male	1.5	Control (Not cured)

Treatment with mahogany seed oil extract by topical application on the effected area of the animal body showed 65.2% effectiveness. This percentage represents the seven and half animals' totally cured relative to the experimental unit of ten animals treated in this study.

The recovery rate as obtained in this study may have been influenced by various factors such as severity and location of infection and method used for the treatment. The two cattle that were not cured were in the chronic stage of the infection. This result of the therapeutic effect of the mahogany seed oil extract in treating dermatophilosis further collaborate the findings of Dutter (1979), that the oil can be extracted from the seed and leaves and it is effective against sores and ulcer. This is also in agreement with the findings of Shittu and Bualla (1988), that Mahogany oil is a remarkable topical healing agent with skin healing, anti-inflammatory, anti-microbial and antioxidant properties.

The fast response observed in the calves and the udder of the female cattle may be due to the fact that the lesions on the calves were still fresh, and the skin scab formed at the udder were not thick and allowed for effective treatment. This is in

line with earlier observation of Coleman (1967), that the skin scabs formed in dermatophilosis may be too thick for effective treatment. The implication of this is that the treatment will be more effective with early diagnosis and oil application.

Conclusion

In early diagnosis, topical application of Mahogany seed oil extract is effective, economical and without adverse effect in treating bovine dermatophilosis.

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Marketing Agricultural Produce in Nigeria: Public Sector Marketing Boards or Public Private Partnerships?

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Abstract
Recently, there have been clamour for the re-introduction of agricultural marketing boards in Nigeria. This nostalgic desire for the re-establishment of marketing boards is largely premised on the notion of the "good old days" when marketing boards supposedly catalysed the growth of the agriculture sector in Nigeria. They were reputed to have provided farmers with relevant information, resources, capacity building and quality control that allowed the farmers thrive and the agriculture sector grow. However, these Marketing Boards were scrapped in 1986 after over forty years of operation, due largely to inefficiencies and corruption emanating from its status as a monopoly. The Minister of Agriculture, had requested for a debate on the efficacy of government reintroducing the marketing boards. This paper joins this debate, suggesting that marketing boards are outdated and at odds with the broader government economic policy of market liberalization and deregulation. In the place of marketing boards, this paper suggests a Public Private Partnership structure that would ensure the growth and sustainability of agricultural produce marketing in Nigeria.

Introduction

In recent times, there has been increased clamour for the re-introduction of agriculture marketing boards for the marketing of agricultural produce in Nigeria. First, it was the former Minister of Agriculture and Rural Development, Dr. Akinwunmi Adesina, who announced in 2015 that the government intended to re-introduce the commodities marketing boards to ensure that farmers received adequate prices for their products. According to the Minister, the whole objective was to remove middle men from the sale of produce and give farmers a better deal (The Nation Newspaper, 2018). Subsequently, the desire of government to reintroduce the marketing boards was also reiterated by Chief Audu Ogbeh, the current Minister of Agriculture and Rural Development.

The Minister was of the opinion that the reintroduction of marketing boards would aid the standardization of produce and therefore boost their export (The Vanguard Newspaper, 2018). The term agricultural marketing boards as used in this paper refer to produce buying and selling entities set up by government with broad responsibilities and powers of compulsion over producers and handlers of defined commodities (Abbott, 1967). The agricultural marketing boards in Nigeria were monopolies established by government, primarily for the export of agricultural produce from the country. In carrying out this function, the boards also had the ancillary responsibilities of price stabilization and product standardization ensuring that the produce met the requirements of the export destination countries. It appears that it is the lacunae in the performance of these primary and ancillary functions in the present "free trade" arrangement that currently exists in Nigeria, that is driving the clamour of the re-establishment of the marketing boards (The Vanguard Newspaper, 2018).

It is a fact that the nation's agricultural sector enjoyed significant economic boom during the early era of marketing boards. Indeed, because the marketing boards were used to structure the sale of agricultural produce at the time when agriculture was the main stay of the nation's economy, the success of the sector is usually attributed to the marketing boards. it is however also a fact that prior to the scrapping of the boards in 1986, the country's fortune in the sector had dwindled massively and that the marketing board system had ceased to function at all. This paper explores first whether there is a correlation between the success of agriculture and the existence of the marketing boards. The answer to this question then help determine whether to reintroduce the marketing boards or in the alternative to introduce public-private partnerships to help achieve some of the policy objectives that is currently driving the clamour for the re-introduction of the marketing boards.

History of Marketing Boards

The origin of the marketing boards in Nigeria can be traced to the West African Produce Control Board, established in 1942. This Board was later succeeded by four commodity marketing boards in Nigeria, two in Ghana and one in each of Sierra Leone and Gambia (Kolawole, 1974). In Nigeria, the Cocoa Marketing Board was set up first in 1947, while the Groundnut, Cotton and Palm Produce Marketing Boards were all established in 1949 (Iweze, 2014). In 1954, these marketing boards were reconstituted as marketing boards for the regions, each vested with statutory authority to purchase all the major export crops grown within their regions of influence (Kolawole, 1974). After the creation of the various states within the federation, the marketing boards were once more rearranged along state lines. It is worth mentioning that the Nigerian Marketing

Company was also incorporated in England in 1947 and this was also subsequently owned by the different state marketing boards (Kolawole, 1974). The Nigerian Marketing company served as the sole selling agent for all the produce aggregated by the different marketing boards (Kolawole, 1974).

To say that the marketing boards at their peak dominated the economy of the West African countries, where they operated, is an understatement. They were effectively the very lifeblood of the economies of these countries. This is captured quintessentially by M.D Williams:

"*About the value of the Boards, there is some dispute; about their importance, there is no disagreement. They market the greater part of West Africa's exports. In Gambia, they market groundnuts, almost the only exports; In Sierra Leone, they market palm produce, some half of the exports in 1951… In the Gold Coast, the Cocoa Board is responsible for marketing up to £60m worth of cocoa a year, two thirds of the value of the country's exports; while the newer Agricultural Produce Marketing Board exports coffee and oil seeds. In Nigeria marketing boards are established for cocoa, palm produce, ground nuts and cotton, representing in 1951 some two-thirds of the country's export"* (Williams, 1953).

There were a number of justifications by the British colonial government for the introduction of the marketing boards. The first was that farmers would benefit from a regulated system of marketing in which government fixed crop prices and licensed a few buyers to protect farmers from abuses. Secondly, that the boards would use any excess funds generated to cushion against short and intermediate price fluctuations in world market prices. It is also claimed that some of the funds would be used for other purposes of general benefit to farmers and industries such as research, disease control and eradication and rehabilitation of deceased cocoa trees, the amelioration of indebtedness, the encouragement of cooperation and the provision of other amenities and facilities to producers (Ajayi *et al.,* 2017).

However, there is also the contrary view that the origin of the marketing boards in Nigeria and indeed West Africa can be traced from the attempts by the British Merchant firms like the United Africa Company (UAC) to organise monopolistic cartels to purchase produce to foster the colonial economic policy where Nigeria and the other West African countries served as a ready source of cheap raw materials to feed the growing industries of Britain. Indeed, it is believed that this system of exploitation continued after independence, in that case from the Nigerian led governments against the farmers. It also continued even after the price fixing functions of the regional Boards were taken over by the Federal Government in 1974 and after the state marketing Boards were finally dissolved

in 1977 in favour of a centralized Federal Commodity Marketing Board (Ajayi *et al.,* 2017).

It was these seeming injustice to farmers, coupled with the dwindling fortunes of the agriculture sector that led to the liberalisation of the sector in 1986 with the dissolution of the marketing boards and the introduction of a free market pricing regime for agricultural produce (Omorogiuwa *et al.,* 2014). The free market pricing regime led to the deregulation of produce marketing with a concomitant increase in prices. This meant in general that farmers received more for their produce as prices that were formerly set at very low thresholds by the technical departments of the marketing boards, were now purely market driven (Williams, 1953). However, the present system has also thrown up a number of its own challenges, chief of which is the lack of any entity to superintend the quality control and standardization of produce, leading to the drop in exports (The Sun Newspaper, 2018).

An Assessment of Marketing Boards

As stated above, there have been several conflicting reasons, depending on which side one is listening to, for the establishment of the marketing boards. Officially, the original objective of the West Africa Export Commodity Marketing Boards and its subsequent offshoots was to solve the problem of price instability in the trading of agricultural produce. The idea was to bridge the gap between the prices received by producers and the day to day fluctuations in world prices (The Sun Newspaper, 2018). A corollary to this was also the elimination of seasonal price fluctuations in produce prices (Iweze, 2014). The strategy was to ensure that the surpluses accumulated in years of high world prices are used to maintain the stable prices paid to the producers. (Kolawole, 1974) The way this worked was that the marketing boards fixed prices of the main export crops, then licenced merchants to pay these prices to farmers and then compulsorily purchased the whole of the crops from the licensed merchants. They then sold the export crops, in the case cocoa on the market and in the case of other crops by long-term contracts (Williams, 1953). According to Iweze, while the marketing boards succeeded in some extent in stabilizing seasonal producer's prices, they failed in stabilizing the income of farmers (Iweze, 2014).

Over time it appeared that this price stabilization function became subsidiary to other objective of generating funds to execute government economic development programmes (Kolawole, 1974). The pursuit of this latter objective, led to a disconnect between producer prices and world prices thereby defeating the initial price stabilization objective. As pointed out by Lewis, "clearly the governments have had their hands on the throat of the goose which is laying the golden eggs"

(Lewis, 1967). Right from the colonial period, the marketing boards had a deliberate policy to depress prices paid to farmers. They established stabilization funds ostensibly to protect farmers but it was actually used to accumulate huge reserves for government (Ajayi *et al.,* 2017). These were forced savings that ended up hurting the farmers (Ajayi *et al.,* 2017). Over time this created a disincentive for farmers to continue to increase production (Kolawole, 1974).

The other widely acclaimed function of the marketing boards is the improvement in the quality of crops through the grading and standardization system deployed by the various boards. This is one of the functions which policy makers in Nigeria feel is missing in the present marketing arrangement for agricultural produce in the country and which they would like the marketing boards to come in and perform. However, the success of the marketing boards in performing this function needs to be put into proper context as it must have been much easier to comply with international standards in the days the old marketing boards operated. The standards required for export commodities have certainly gone up in Europe over the years and it would have been equally difficult for a government run statutory body like the marketing boards to maintain present day standards.

Also the differential pricing amongst different boards for different produces within the west African region, encouraged smuggling as traders saw opportunity for arbitrage. Another complaint was the propensity of state governments to raise so much tax from produce tax that they ignored other sources of revenue to the detriment of the state's overall economy.

From the analysis above, the overwhelming view appears to be that the marketing boards never delivered its mandates successfully. This was the case even during the post-independence period. As Iweze argues, the British colonial government only employed the marketing boards to subtly protect her imperial interests at the expense of the Nigerian peasants (Iweze, 2014). And as reflected in the preceding paragraphs above, this exploitative practice never ceased even after independence as Nigerian politicians found them a ready-made instrument for taxing farmers, enriching themselves and financing their political activities. (Williams, 1985) For instance the Western Region Marketing Board later became insolvent due to the excesses of the political leadership. Most of the funds were diverted to finance the regional party, the Action Group and for personal use. For instance, the Coker Commission of Inquiry found Chief Awolowo, the regional and party leader culpable for running down the Western Region Marketing Board due to his failure to adhere to standards of conduct which were required by persons holding public office (Ogbidi, 2012).

The exploitative nature of the Marketing Boards was aptly covered by Gavin Williams:

"*Since their inception, Nigerian Marketing boards have been used to serve various interests and purposes, hardly any of which have benefited the producers. They originated in the second world war and were perpetuated after the war by a Labour government so that they might play their part in meeting British needs…Nigerian politicians found them a ready-made instrument for taxing farmers, enriching themselves and financing their political activities. Their pricing policies from producing export crops rendering the boards redundant."* (Williams, 1985)

In summary, the marketing board system was generally characterized by corruption, bureaucratic red tape, smuggling and discriminatory tax regimes by state governments (Williams, 1985). Indeed the decline in agriculture as a major export in Nigeria was not only as a result of the increase in the price of oil but could also be traced to the marketing boards which denied farmers the incentive to continue to produce export commodities (Williams, 1985). The marketing boards were originally presented as a humanitarian scheme to save farmers from financial strangulation, but ultimately became the avenue for the government to exploit the poor (Ajayi *et al.*, 2017). The Boards effectively put in the hands of the government the sole power to market crops produced by often poor powerless farmers, a power that the government shared with a select number of privileged people and firms (Ajayi *et al.*, 2017).

The Present Situation: Marketing Without the Marketing Boards

The development of markets for agriculture produce is essential to the growth of the agricultural sector. It provides incentives for private sector investors to devote their resources to agriculture projects with the knowledge that there exists a clear path to returns for such investments. Another role that markets play in a sector with seasonal production like agriculture, is to help manage risks associated with demand and supply shocks by helping adjust supply when needed thereby reducing the price variability faced by consumers and producers (Barrett & Mutambatsere, 2005). The liberalization of the sector which occurred in 1986 after the dissolution of the marketing boards, has not resolved all the issues afflicting the market for agricultural produce in Nigeria. The market for agricultural produce has not started operating efficiently to optimally perform some of the functions which the market ought to perform. The sector is still constrained by issues like poor quality control and grading of produce which adversely affects export trade, and the absence of a common method of determining prices. This is why the current Minister of Agriculture, Mr. Audu

Ogbeh has proposed the reintroduction of marketing boards to tackle these issues (The Vanguard Newspaper, 2017).

However, the reintroduction of the marketing boards is not likely to solve the present issues. The reintroduction of these boards is not only outdated but also out of step with the country's present liberalised economic outlook. Therefore, it appears that whilst the country is liberalising and encouraging private sector investment and operation of most of its economic sectors, the government is looking at nationalizing the marketing of agricultural produce. The clamour for the re-introduction of marketing boards is not new and has always been the preference of bureaucrats. Speaking against the clamour for the reintroduction of marketing boards, Gavin had this to say as far back as 1985:

'*Against all the evidence, it (marketing boards) maintains a strong appeal for bureaucrats, technocrats and regrettably many socialists. Socialists have no business defending or reforming such exploitative institutions. De jure state monopolies on the marketing of crops impose high costs on producers, on government budgets and on consumers. They create de facto monopolies for favoured and protected traders and the opportunities for profitable collusion between businessmen and officials, civil police and military. Against such institutions and monopolistic arrangements, socialists should support free trade'* (Williams, 1985).

Whilst this statement from Gavin remains true even today, it is still very doubtful that the government would have the capacity or budgetary discipline required to operate a marketing board structure effectively. Instead of this very backwards looking policy, this paper is of the firm view that it is better to introduce PPPs to resolve the problems existing within the current arrangement. The government should focus on de-risking the sector to allow for additional private sector investments across the entire agriculture value chain. Also government should be responsible for setting policies and standards through its various organs in the sector, and should leave the fixing of prices to market forces.

Public Private Partnerships to the Rescue?

PPPs can be defined as the relationship between public sector agencies and private sector entities under which the responsibility for any or all of the combination of designing, financing, construction, management and operation of public infrastructure, utilities and other investments that were traditionally undertaken by the public sector are contractually shared and jointly undertaken by both the public and private sector, usually in proportion to the kind of risks each party can best carry (Nwangwu, 2013).

Agriculture PPPs may then specifically be defined as "a formalized partnership between public institutions and private partners designed to address sustainable agricultural development objectives, where the public benefits anticipated from the partnership are clearly defined, investment contributions and risks are shared, and active roles exist for all parties throughout the PPP lifecycle" (FAO, 2016). The most critical success factor for agriculture PPPs is the allocation and mitigation of risks. A party should only be made to assume risks which it is most suited for and willing to shoulder at the most acceptable price (Abrahamson, 1973).

Despite being in wide use in Nigeria, in several infrastructure sectors, most notably in the transport and electric power sectors, there has however been very minimal use of PPPs in the agricultural sector. The reason for this may be attributable to the difficulty in convincing the private sector investors of the viability of the agricultural sector because government policies still speak to agriculture as a social service instead of the business venture that it truly is. Government policies have therefore spectacularly failed to address the unbalanced risk and reward structure in most agriculture projects, therefore alienating the private sector. However, it is a fact that PPPs, if well designed, are suited for solving the problems bedevilling the agriculture sector as they possess the capacity to unlock private sector investment and its superior managerial capabilities to augment the resources of the public sector. They also ensure the sustainability of agriculture projects by ensuring the timely completion and execution of projects by eliminating reliance on the very unreliable government budgetary systems. Indeed for these reasons, a number of countries have adopted PPP-like structures to develop their agriculture sector (Ponnusamy, 2013).

PPPs are well suited to resolving some of the weaknesses that are inherent in the current agricultural produce marketing arrangements. The private sector parties need to be given the right incentives and regulatory backing where required to facilitate agricultural trade. Indeed, private sector can help in ensuring the standardization of produce and thereby galvanize export trade. The popular e-Chaupol initiative in India is a good example of how private sector initiatives can create value for everyone in the agricultural supply chain through private sector initiatives.

e-Chaupol Initiative

A case study that illustrates how the public and private sector can work together to move agricultural marketing forward is the e-Choupal initiative that was designed by ITC Limited of India. ITC Limited's e-Choupal system was designed specifically to tackle some of the challenges that were inherent in the

Indian soya beans supply chain system. The existing system in the Madhya Pradesh region of India at the time, had ensured that the two most important players in the entire supply value chain, the sellers (farmers) and the buyers (ITC), were not making a lot of money from the soya bean trade. Fragmented farms, weak technological infrastructure, lack of access to information and the involvement of intermediaries who prevented critical information about the market reaching the farmers, characterized the soya beans supply chain at the time in the Pradaseh region of India (Parwez, 2013). The intermediaries on the other hand used the information for their own benefits. The middle men could sometimes even buy on credit from the farmers at very low prices and sell to ITC at higher prices (Parwez, 2013). ITC therefore conceived a system to resolve these issues. ITCs solution was in summary, to design an agricultural supply chain system that was competitive and transparent by making information available to farmers through the use of information technology.

ITC provided computers and internet access across the Madhya Pradesh region, allowing farmers negotiate and sell their produce directly to ITC. The access to the internet provided the farmers with sufficient information with which to negotiate the sale of their produce with ITC, obtain information on good farming practices and place orders for agricultural inputs like seeds and fertilizers. ITC provided kiosks with internet access that was run by a *sanchalek* (who was trained by ITC). The *Sanchalek* bears some of the operating costs for running the service and in return earns a service fee for transactions done through the e-Choupal. The middlemen (*samyojaks*) were not completely eliminated, they were entrusted with manning the warehouse hubs but no longer had the power to negotiate on behalf of ITC. This system was very successful and effective in improving the yields from the farms and reducing transaction costs. This led to increase in the income levels of the farmers and of ITC.

The principles underlining the solutions employed by ITC are capable of being replicated universally, even in Nigeria. However, it may well be that majority of the strategies adopted by the company in achieving these goals would have to be tweaked due to slight differences in culture and economic climates in Nigeria. The interesting aspect of ITC's strategy is that the company did not re-invent the entire supply chain. The company merely leveraged the existing structures in the supply value chain. For instance they used the existing meeting venue (Choupal), effectively tapping into the core principles of the system that was based on interaction, knowledge sharing and trust. ITC also continued to retain the services of the Choupal head as a *Sanchalak* and ensured that he made money out of the value chain. The company even retained and converted the erstwhile Commission Agent under the *mandi* system with the title of *Samyojak* and gave him a more

visible and value-add role by basically utilizing his good knowledge of the terrain. Information sharing with the farmers was very critical for the success of the e-Choupal initiative. It gave ITC access and control over the quality of the product it sourced as the company was now able to share information regarding the best practices for sowing, irrigation and harvesting. This improved the company's earning power in the international market.

The farmers were also beneficiaries as their income also improved. One interesting strategy was the creation of ITC hubs and the different value add services that were provided there. The hubs helped provide comfort and convenience to the farmers by providing goods and services to satisfy the ancillary needs of the farmers while they were transacting their primary businesses with ITC. For instance, ITC was able to sell products like herbicides, fertilizers, lanterns and soya oil to farmers. They were also able to render advisory services in the form of scientific tests and fertilizer use. This along with the utilization of the IT platform was instrumental in creating additional income for all the actors across the value chain. For instance, the *Sanchalak* and the *Samyojak* all benefitted from the additional revenues created from these additional sales. Once a level of trust was built between ITC and the farmers, it was very easy to now introduce other services to the farmers at minimal or no cost using the already established channels.

An aspect of ITC's approach which requires collaboration between the private and public sectors is the enactment of relevant legislations to support the innovation. ITC took the lead in this instance and was able to influence the amendment of legislations required to make the process work effectively. This is not easy and requires a lot of patience and severance. In other words, it is difficult to manage political and bureaucratic risks but not impossible. In the e-Choupal case, the existing legislation, which was supposed to protect the farmers, had become a hindrance to them and had enabled the creation of the *mandi* as a monopoly for price discovery. ITC was able to influence the amendment of the legislation to allow transactions outside of the *mandis* in India ostensibly because of their strong networks and influence in India.

In summary therefore, with partnership between the public and private sectors, private actors are capable of bringing in innovation that will solve Nigeria's produce marketing problems. The problems that exists in the Nigerian produce supply chain that has led to the clamour for the reintroduction of the marketing boards can easily be solved. Nigeria can borrow from the e-Choupal system with minor adjustments. These adjustments will take into consideration the different social, legal and economic peculiarities existing in Nigeria.

For example, the basic principles, which enabled the migration of the e-Choupal, supply chain system to different crops and regions within India would work in Nigeria. These principles are: By ensuring that the origin or source of the commodity is traceable, ITC was able to guarantee product quality and standards. This effectively resolves the standardization problem that is fuelling the clamour for the reintroduction of the marketing boards.

Secondly, by being able to match the quality and quantity of the farmers' production to consumer demand, ITC was able to create efficiency and wealth for everyone in the value chain. This will help increase the earning potential of the farmers. Also by helping facilitate the creation of an electronic market place, the company was able to improve price discovery for market participants. This is a better idea than having monopolistic marketing boards fix prices. These principles proved that it is possible, if not easier for companies to make money whilst creating value for society. In this case, e-Choupal system was a force of economic and social change within the villages in India. This can be the same in Nigeria.

Conclusion

The clamour for the re-introduction of the marketing board model to help resolve some of the problems confronting Nigeria's agricultural produce marketing is driven mostly by frustration with the current system, which has not really worked. This paper argued that instead of the reintroduction of marketing boards, that the country should look towards PPPs to help solve all the exiting problems.

The use of PPP for agricultural marketing in Nigeria will lead to a shared responsibility amongst the public and private sector partners to encourage the provision of input supplies to farmers to increase yield, deployment of technology to reduce transaction costs, pooling of resources and the sharing of risks and rewards between the public and private sector partners. These are effectively solutions to most of the problems that are presently bedevilling Nigeria's agriculture supply chain.

The paper uses the famous e-Choupal case study to show that Nigeria's problems in this area are not unique and that the country does not have not resort to the outdated marketing board system to resolve them. Instead, it is proposed that the country ought to tap into the innovation of private sector partners to improve the agriculture supply chain.

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Extraction of Lipase from Germinated Peanut Seeds (*Arachis hypogaea* Linn) and Study of Its Activity on Coconut Oil (*Cocos nucifera* Linn)

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Abstract: Virgin Coconut Oil is the riches source of MCFA (90 %). Therefore, the main aim of this work is to prepare MCFAs from Virgin Coconut Oil and to assess its antimicrobial properties. Medium-chain fatty acids are produced by enzymes in which the specificity of the thioesterase component differs from normal, i.e. the chain-elongation cycle is terminated prematurely. Isolate the lipase enzyme (EC3.1.1.3) from radicals of peanuts seeds and to determine the enzymic properties of the purified enzyme. The optimum condition of germinated peanut seed extraction of lipase was determined to be pH, 4; reaction temperature, 40 °C; substrate amount, 2 g; and reaction time, 60 min when using olive oil as substrate. Under this condition, enzyme activity was found to be 180 EU mL^{-1}. Virgin coconut oil (VCO) was extracted from freshly harvested mature coconut by cold press method and the yield was found to be 21 %. When lipase enzyme was used (32.6 % wt.). According to the GC-MS analysis, lauric acid content of F-II (35.82 % total MCFAs) was observed. MCFAs of VCO have strongly antimicrobacterial activity against gram negative and gram positive bacteria (*P. morgani*, *S. flexneri*, *V. cholerae* 0139 and *S. auereus*).
Keywords: Antimicrobial activity, Lipase enzyme, GC-MS.

Introduction

Coconut oil is one of the primary natural products produced from dry fruit (copra) of coconut (*Cocos nucifera* Linn). Different methods produce different types of coconut oil that their properties are little varied. Desiccated coconut and coconut cream contained about 69 % of coconut oil. Full coconut milk is approximately 24 % of oil. Coconut oil is a colourless to pale brownish yellow oil with a melting point ranging from 23 to 26 °C. It does not become rancid (oxidation) easily. Coconut oil was once mistakenly believed to be unhealthy because of its high saturated fat content, it is now known that the fat in coconut oil is a unique and different from most all other fats and possesses many health giving properties. It is now gaining long overdue recognition as a nutritious health food. Virgin coconut oil (VCO) seemed to be the purest form of coconut oil, water white in colour, contains natural vitamin E and with very low free fatty acid content and low peroxide value. It has a mild to intense fresh coconut aroma.

VCO may be defined as the naturally processed, chemically-free and additive-free product from fresh coconut meat or its derivative (coconut milk and coconut milk residue) which has not undergone any further chemical processing after extraction. Coconut oil is a fat consisting of about 90% saturated fat. The oil contains predominantly medium chain triglycerides, with roughly 92% saturated fatty acids, 6% mono unsaturated fatty acids, and 2% polyunsaturated fatty acids.

Approximately 50% of the fatty acids in coconut fat are lauric acid and 5-9% are capric acid. Although the vast majority of fats and oils in diets are composed of long-chain fatty acids (LCFA), the saturated fatty acids in coconut oil are predominately medium-chain fatty acids. As metabolism of fatty acids depends on their size, physiological effects of MCFA in coconut oil are distinctly different from those of LCFA. The biosynthesis of saturated fatty acids requires a primer molecule, usually acetic acid in the form of its Coenzyme A ester, and a chain extender, malonyl-CoA. Lipases are widespread in nature and have been found in animals, higher plants and microorganisms [12].

As a first step, both the primer and extender substrates are attached to acyl carrier protein (ACP). A sequence of reactions follows in which the chain is extended and butanoate is formed. First, 3-oxobutanoate is formed by a reaction catalysed by β–ketoacyl-ACP synthetase, this is reduced to 3-hydroxy-butanoate by β–ketoacyl-ACP reductase, which is in turn dehydrated to trans-2-butenoate by β–hydroxyacyl-ACP hydratase before it is reduced to butanoate by enoyl-ACP reductase. The process then continues with the addition of a further six units of malonyl-ACP by successive cycles of these reactions until palmityl-ACP is formed. At this point, a thioesterase removes the fatty acyl product as the free acid (with the mammalian enzyme), and it must be converted to the CoA-ester before it can enter into the various biosynthetic pathways for the production of specific lipids. Medium-chain fatty acids are produced by enzymes in which the specificity of the thioesterase component differs from normal, i.e. the chain-elongation cycle is terminated prematurely.

Certain fatty acids (FAs) (e.g., medium-chain saturates) and their derivatives (e.g., monoglycerides, MGs) can have adverse effects on various microorganisms such as bacteria, yeast, fungi and enveloped viruses [8]. The antimicrobial effects of the FAs and MGs are additive, and total concentration is critical for inactivating viruses [4, 5]. The FAs and MGs produce their killing/inactivating effect by lysing the plasma membrane lipid bilayer [6, 7]. Although FAs and MGs are active; diglycerides and triglycerides are found to be inactive. Of the saturated fatty acids, lauric acid has greater antimicrobial activity than caprylic acid, capric acid or myristic acid.

Monocaprin has also been shown to have antiviral effects against HIV and is being tested for antiviral effects against herpes simplex and for antibacterial effects against Chlamydia and other sexually transmitted bacteria. Some recent investigations have described enzyme-catalyzed esterification as an attractive method for synthesis of MG [15]. Lipase breaks down fats into sn-2 MG and fatty acids. Used in reverse, it can catalyze the esterification of glycerol with fatty acids. The ambient to moderate temperatures used in this process minimize the potential side reactions and may allow the preparation of sn-1 MG.

Potential problems with the process are high cost and denaturation of the enzyme as well as slow reaction times. For reactions that carried out in both aqueous and non aqueous media, lipases stand out due to their ability to utilize a wide spectrum of substrates, high stability towards extremes of temperature, pH and organic solvents, and chemo-, regio-and enantioselectivity [10, 11]. Although most enzymatic syntheses are performed in presence of organic non-polar solvents [3], reactions that performed in solvent-free systems have several advantages such as volumetric productivities higher than in organic medium and the possibility that the remaining substrates can be easily separated from the products and readily recycled.

Materials and Methods

Collection of Sample

Coconuts (*Cocosnucifera Linn*) were collected for the present research. The sample was collected from Mingalar Taung Nyunt Township, Yangon Division Myanmar. The collected sample was identified in Department of Botany, University of Yangon.

Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed [14].Germinated Peanut Seeds Lipase was used. All other chemicals and reagents used were of analytical grade.

Isolation of Lipase Enzyme from Seedlings of Peanut (*Arachis hypogaea* Linn)

Peanut seeds (1000 g) were placed in wet sand and kept at room temperature. After 2 days radicals (50 g) were taken and ground gently with a mortar and pestle and then mixed with grinding medium (300 mL) and placed in a shaker-water bath (130 Rev) for 90 min at room temperature. The solution mixture was

filtered through a filter paper. The filtrate was centrifuged at 5000 rpm for 20 min. The filtrate (200mL) was then collected by decantation and ammonium sulphate (101.00 g) was added into it. After standing for some minutes, the mixture was centrifuged. Upper layer was collected by decantation and centrifuged again to precipitate the enzyme. The enzyme pellet (4.5 g) thus obtained was collected and dried at room temperature and then stored in a bottle at 4 °C.

Determination of Lipase Activity by pH–Stat Method

Olive oil (2 g), 0.05 M acetate buffer at pH 5.6 (9 mL), 0.1 M CaCl2 (1 mL) and enzyme solution (1 mL) were placed into a 150 mL beaker. Enzymatic hydrolysis was carried out in shaker-water bath (130 Rev) at room temperature for 60 min. At end of the reaction, ethyl alcohol (40 mL) was added and an amount of liberated fatty acid was titrated with 0.05 M KOH, adjusted to 8.0 by a pH meter. A blank titration was carried out by the same procedure except enzyme solution (1 mL) was replaced by distilled water (1 mL).

Determination of Optimal pH on Lipase Catalyzed Reaction using Olive Oil

Appropriate pH adjusted acetate buffer (9 mL) was pipetted into a beaker containing 1 M $CaCl_2$ (1 mL) and olive oil (2 g). By adding prepared enzyme solution (1 mL), the enzymatic hydrolysis was allowed to start. The beaker was placed in a shaker-water bath (130 Rev) at room temperature. After 60 min, the reaction was interrupted by adding of ethyl alcohol (40 mL) and titrated with 0.05 M KOH.A blank solution was prepared by carrying out the procedure as described above except that 1 mL of distilled water was used instead of 1 mL enzyme solution.

Determination of Optimal Reaction Temperature on Lipase Catalyzed Reaction using Olive Oil

Experiment was carried out by the same procedure describe in previous sections. Reaction temperature was varied as 25, 30, 40, 50 and 60 °C while keeping other parameters at constant as: pH, 4; reaction time, 60 min; and amount of substrate, olive oil, 2 g.

Determination of Optimal Substrate Concentration on Lipase Catalyzed Reaction using Olive Oil

Acetate buffer adjusted pH at 4 (9 mL) was pipetted into a beaker containing 1 M $CaCl_2$ (1 mL) and 1.0g of olive oil. Enzyme solution (1 mL) was added and the container was placed in a shaker-water bath (130 Rev) at 40 °C. After 60 min, the reaction was interrupted by adding of ethyl alcohol (40 mL) and titrated with 0.05 M KOH.A blank solution was prepared by carrying out the procedure as described above except that 1 mL of distilled water was used instead of 1 mL

enzyme solution. The above procedure was carried out by using different amount of olive oil as: 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 g.

Determination of Optimal Reaction Time on Lipase Catalyzed Reaction using Olive Oil

Experiment was carried out by the same procedure describe in previous sections. Reaction time was varied as 15, 30, 45, 60, 75, 90 and 105 min while maintaining other variables as: pH at 4, reaction temperature at 40 °C and amount of substrate, olive oil, 2 g.

Preparation of Virgin Coconut Oil

Extraction of Virgin Coconut Oil was observed by Cold Pressed Method. Mature coconuts were de husked the shell with a sharp knife or a scraper. The shredded meat was pressed at room temperature to get coconut milk and filtered through a cheese cloth to remove the impurities. The coconut milk was separated into four layer parts by centrifugal method [2]. Virgin coconut oil at the top, cream layer at the second, skim milk in third and shredded meat (solid) at the bottom were collected. Water like color of virgin coconut oil was separated from top layer by decanting.

Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in Preliminary Phytochemical analysis were performed in extraction of Virgin Coconut Oil. Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloid, α-amino acids, flavonoids, phenolic compounds, glycosides, and saponins as described by standard procedure.

Enzymatic Hydrolysis of Virgin Coconut Oil

Virgin coconut oil (100 g) was taken in a 250 ml stoppered Erlenmeyer flask and water (60 % by weight of neutral glycerides) containing germinated peanut lipase powder was added. The mixture was magnetically stirred at 35 ± 2 °C. The degree of hydrolysis was monitored by determining the amount of free fatty acid liberated by titration method during hydrolysis time. After reaction completed, the oil layer and water layer containing enzyme and glycerol were separated by centrifugation.

Isolation of Short Chain Fatty Acid

The hydrolysate (100 mL) was subjected to steam distillation under atmospheric pressure. All parts of glass distillation equipment including steam inlet, steam outlet and thermometer pocket adapter were fitted well. The steam carried volatile fatty acids and was collected as the distillate (FI).

Isolation of Medium Chain Fatty Acids from Residual Fatty Acids (FII)

The residual fatty acids from the steam distillation were fractionally distilled. The distillate that collected at (100-140 °C) under 4 mm Hg pressure mainly contained medium chain fatty acids (MCFAs).The distillate as fractions (FII) and residual fractions (LCFA) (R) were then weighed and the yields were calculated on the basis of VCO.

Test Organism

The antibacterial activity of the test samples (VCO, FA fractions and glycerides) was determined by the agar disc diffusion technique. The microorganisms used for testing antimicrobial activity were Gram positive (*Staphylococcus aureus, Shigella flexneri, Vibrio cholerae O139*), Gram negative (*Proteus morganic*)

Preparation of inoculum

A few colonies of the organism to be tested were inoculated into the triple sugar iron agar and incubated at 37 °C for 24 hours. A few colonies of the organism from triple sugar iron agar were introduced into the triplicate soy broth and incubated for 3 hours at 37 °C to obtain the bacterial suspension of moderate cloudiness. This contained approximately 105 to 107 organisms per m L.

Antimicrobial Activity Testing

The antibacterial activity of the test samples (VCO, FA fractions) was determined by the agar disc diffusion technique. Screening was done by the use of impregnated paper disc (6 mm). These discs were sterilized by autoclaving and followed by heating at 60 °C for 1 hour. It was then impregnated with concentrated sample (20 µg/disc) and allowed to dry at 42 °C in an oven. The bacterial suspension from triplicate soy broth was streaked evenly in three plane onto only the surface of the triplicate soy agar plates with sterile cotton swab. After the inoculums had allowed drying for minutes, the dried discs impregnated with test sample were placed on the agar with a flamed forceps and gently pressed down to ensure proper contact. A control discs impregnated with solvent only and with clinical drug (tetracycline) were also included. After inoculation, the plates were incubated immediately or within 30 minutes. After overnight incubation at 37°C, the diameters of inhibition zone including 6 mm discs were measured by dial calipers.

Results and Discussion

Lipases activity present in food reserve tissues of growing seedling and especially in those which contains large amount of triacylglycerols and its activity in plants seeds increases rapidly after germination. Lipases have preference to hydrolyze glycerides to glycerol and fatty acids. In this work, the lipase enzyme was

extracted from the radical of 2 days old peanut seedling. Extraction was carried out by using the grinding medium. Partial purification of lipase was done by precipitation with ammonium sulphate.

The activity of an enzyme is mainly effected by the pH of its medium and each enzyme acts best at a particular pH called the optimum pH at which its action is maximal [9]. In this work, effect of pH on the activity of peanut enzyme was investigated by varying the pH of reaction medium (from 2 to 8) while keeping other parameter at constant as: concentration of substrate, 2.0 g olive oil; reaction temperature at 40 °C; and reaction time, 60 min. pH of the reaction medium was adjusted by using different acetate buffers. Table 3.4 reveals the effect of pH on the activity of enzyme. Optimum pH for peanut lipase was found to be 4 (Table 2, Figure 1).

The rate of an enzyme-catalyzed reaction first increase with the increase in temperature and then decreases by thermal denaturation of enzyme protein [13]. For each enzyme, there is a certain temperature called the optimum temperature at which the enzyme activity is maximum and the activity progressively falls both above and below this temperature. The optimum temperature of each enzyme is largely dependent the incubation time and the nature of the incubation medium. The dependence of enzyme activity on reaction temperature was shown in (Table 3, Figure 2).

When raising the temperature from 25 °C to 40 °C, the activity gradually increased and reached maximum value at 40 °C. When the temperature increased beyond this temperature, the activity was rapidly declined. To study such effect, other variables were kept at constant as: concentration of substrate, 2.0 g olive oil; pH at 4.0; and reaction time, 60 min. As the concentration of substrate strongly influences the enzymatic reaction, it was studied in this work. The substrate concentration was changed as shown in (Table 4, Figure 3) while maintaining other parameters at pH, 4; reaction temperature, 40 ˚C; and reaction time, 60 min. It can be seen that at initial stages increasing the concentration of substrate enhanced the enzyme activity and reached its maximum value.

After this concentration, increased in substrate concentration did not effect the activity of enzyme. Reaction time is also an important factor in the determination of enzyme activity [1]. In this work (section 2.6), the action of the lipase on olive oil substrate was studied in acetate buffer of pH 4. The amount of fatty acid liberated during the various reaction time intervals of 15, 30, 45, 60, 75, 90, 105 min were determined by pH-stat method. The velocity of lipase-catalyzed reaction vs reaction time is shown in (Figure 4, Table 5).

Virgin coconut oil was extracted from freshly harvested mature coconut by cold press method. It is a unique production process; from harvesting of coconut final up to VCO, is within 24 hours and this important factor ensures optimal retention of the coconuts natural flavor, natural vitamin E and micronutrient. No heat was used in this process and no fermentation occurred during the process.

High process temperature and bacterial contamination of the coconut meat before oil extraction cause the yellow colour of the coconut oil. Therefore, for the coconut oil to be categorized as virgin, its color should be water white. The yield % of VCO in this work was found to be 21 %.Hydrolysis of VCO provided free fatty acids and glycerol. The radicals of peanut seedlings lipase was used in the hydrolysis of VCO. During enzymic hydrolysis, formation of FFA monitored by titration method was shown in Table 6. At 11 hr 63.1% of FFA can be provided. The liberation of FFA formed against hydrolysis time in Figure 5.

The hydrolyzed oil after removal of glycerol layer was subjected to steam distillation. Steam volatile fraction (F-I) was found to be rich in SCFAs, C 8:0 and C 10:0. Remaining oil was then fractional distilled under reduced pressure to provide fraction II (F-II) that was rich in MCFAs especially lauric acid. Residual oil (R) consisted mainly of LCFAs. The yields of all isolated fractions were calculated on the basis of VCO and reported in Table 7.

Table 1. Enzyme Activity of Peanut Lipase (Ep) at Different Purification Stages

No.	Purification Steps	Lipase Activity (μmol min^{-1} mL^{-1})
1.	Crude enzyme extract	120
2.	After treated with $(NH_4)_2SO_4$	165
3.	Enzyme solution*	180
* 0.2 g/100 mL		

Table 2. Effect of pH on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

pH	Lipase Activity (EU mL^{-1})
2	100
3	145
4	180
5	160
6	120
7	95
Amount of olive oil, 2.0 g; temp., 40 °C; and time, 60 min.	

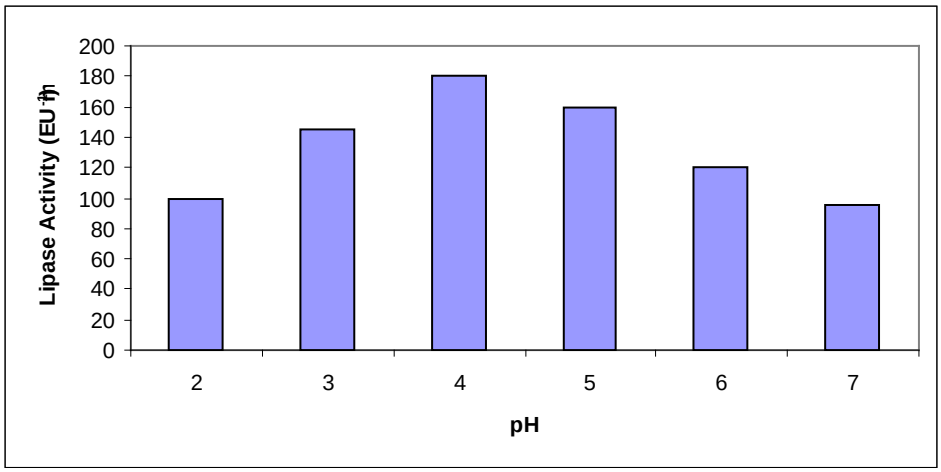


Figure 1. Effect of pH on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Table 3. Effect of Reaction Temperature on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Temperature (˚C)	Lipase activity (EU mL^{-1})
25	120
30	160
40	180
50	150
60	50
Amount of olive oil, 2.0 g; pH, 4; and time, 60 min.	

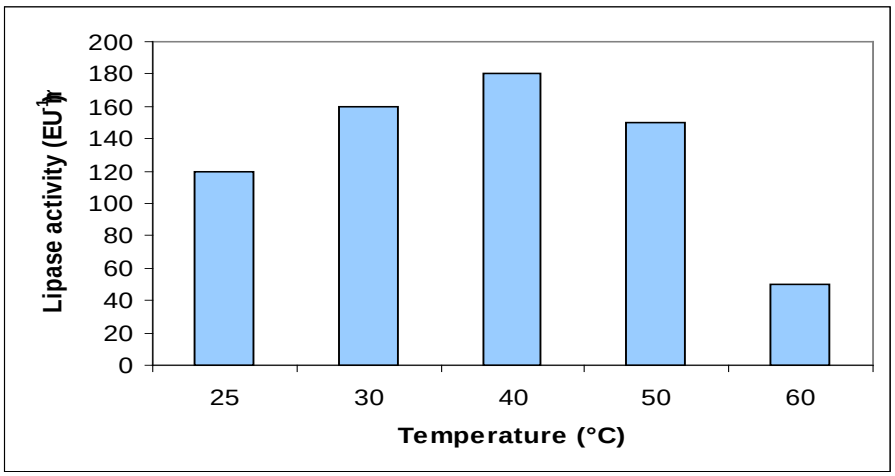


Figure 2. Effect of Reaction Temperature on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Table 4. Effect of Amount of Substrate on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Substrate (g)	Activity (EU mL^{-1})
1.0	100
1.2	115
1.4	140
1.6	160
1.8	175
2.0	180
2.2	180
pH, 4; temp., 40 °C ; and time, 60 min.	

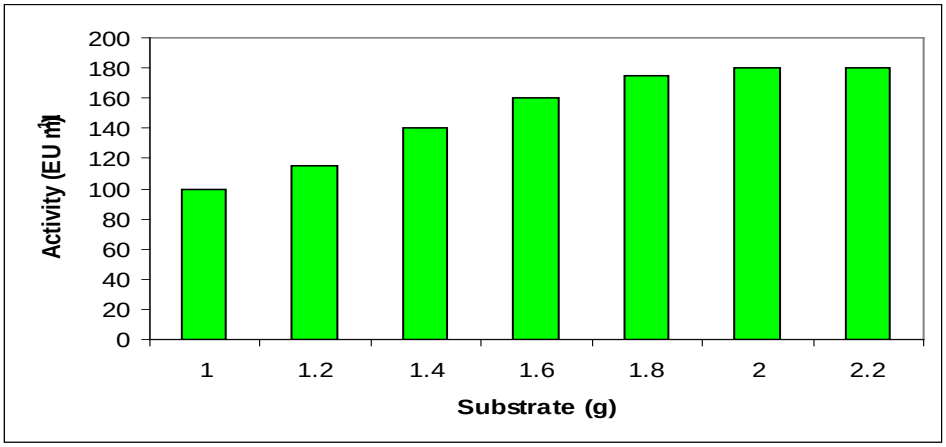


Figure 3. Effect of Amount of Substrate on Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Table 5. Effect of Reaction Time on Velocity of Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Reaction time (min)	Fatty Acid Concentration (M)	Velocity x 10^3 (M min-1)
15	0.1622	10.8133
30	0.1638	5.4600
45	0.1648	3.6622
60	0.1800	3.0000
75	0.1656	2.2080
90	0.1640	1.8222
105	0.1600	1.5238
Amount of olive oil, 2.0 g; pH, 4; and temp., 40 °C.		

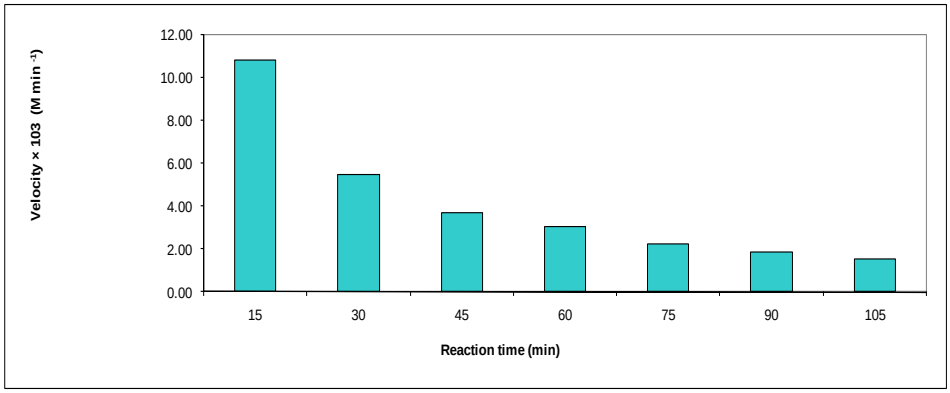


Figure 4. Effect of Reaction Time on Velocity of Peanut Lipase Catalyzed Reaction using Olive Oil as Substrate

Table 6. Free Fatty Acids (FFA) Formed From Virgin Coconut oil by Enzymatic hydrolysis of peanut enzyme

Reaction Time (hours)	% of F.F.A
1	8.64
2	22.44
3	26.84
4	29.16
5	32.4
6	35.88
7	40.13
8	48.51
9	53.11
10	60.38
11	63.1

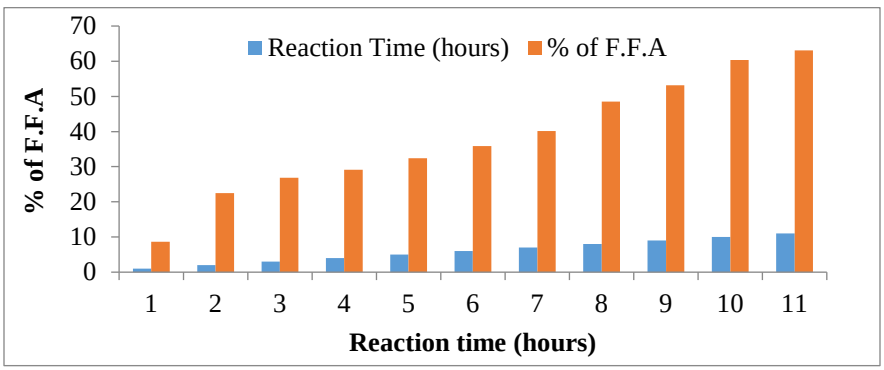


Figure 5. % of FFA Vs enzymic hydrolysis time of coconut oil by using lipase enzyme

GC of F-I (p) (steam volatile fraction isolated from hydrolyzed VCO catalyzed by Ep) also provided major peak at 17.99, 19.38, 21.01, 23.26, 23.60 and 24.94 min. Both GC revealed the presence of C 8:0, C 10:0, C 12:0, and C 14:0 fatty acids in these fractions.

Both spectra provided one outstanding major peak at 22.03 (21.01) min which was identified as methyl ester of lauric acid. GC of F-II(p) (distillate isolated from fractional distillation of hydrolyzed VCO catalyzed by Ep) that also provides five peak at Rt (min) 17.15, 19.29, 22.32, 24.76, and 24.94 min.

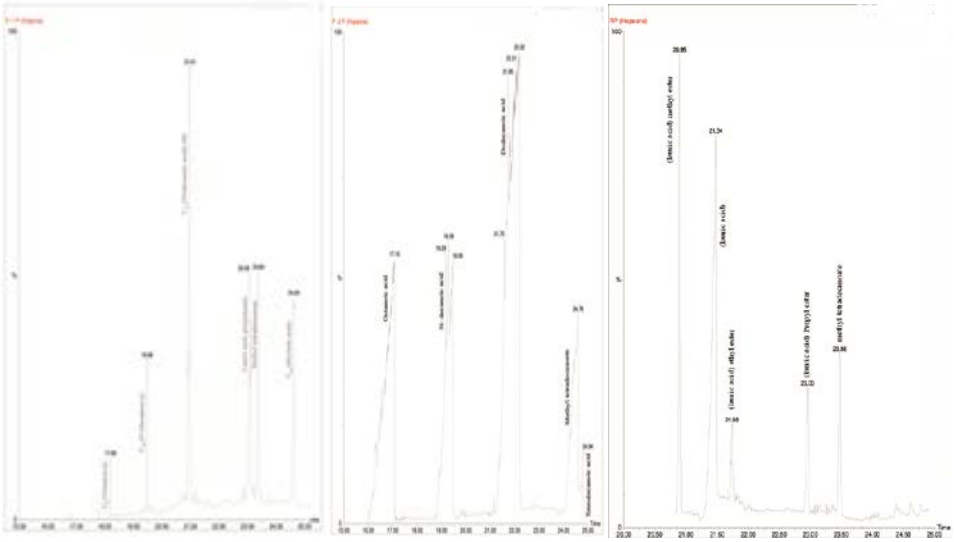
Peak corresponds to lauric acid was observed at 22.32 with 35.82 % relative concentration. In addition, peak corresponds to capric acid was observed at 19.29 (35.82 %). Myristic acid, C 14:0, was also found at 24.76 (15.57 %). GC of fraction R(p) (the residue after removal of F-I(p) and F-II(p) provided peaks at Rt (min): 20.95 (lauric acid methyl ester), 21.54 (lauric acid), 21.80 (lauric acid ethyl ester), 23.03 (lauric acid propyl ester), and 23.56 (myristic acid methyl ester) (Figure 6, 7 and 8).

The Antibacterial activity of different fatty acid fractions derived from VCO were determined by agar plate dilution method, on four species of bacteria such of *S. aureus, P. morganii, S. flexneri* and *V. cholerae* 0139.

The diameter of inhibition zone was strongly depended on the concentration of samples as shown in Table 11 and figure 9.

Table 7. Yield of Isolated Free Fatty Acid Fractions from Enzymatic Hydrolyzed Coconut Oil by using Lipases

Isolated Fatty Acid Fractions	**Yield (%) of F.F.A**
Fraction I (rich in SCFAs)	1.8
Fraction II (rich in MCFAs)	32.6
Residue (LCFAs and non-volatile matter)	65.6
SCFAs = short chain fatty acids MCFAs = medium chain fatty acids LCFAs = long chain fatty acids	



(6) (7) (8)

Figure 6. Gas chromatogram showing fatty acid profile of fraction F-I (p) (isolated as steam distillate from hydrolyzed VCO catalyzed by Ep)
Figure 7. Gas chromatogram showing fatty acid profile of fraction F-II (p) (isolated from fractional distillation of hydrolyzed VCO catalyzed by Ep)
Figure 8. Gas chromatogram showing fatty acid profile of residue R (p) after removal of F-I (p) and F-II (p) from fractional distillation of hydrolyzed VCO (catalyzed by Ep)

Table 8. Fatty Acid Profile of Steam Distillate isolated from Enzymatic Hydrolyzed Virgin Coconut Oil (according to GCMS)

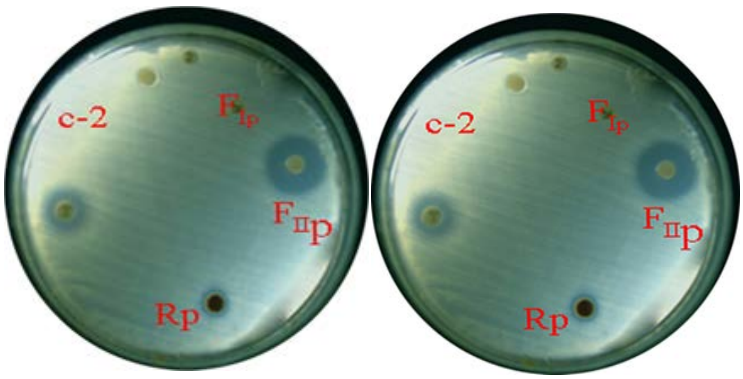
R_t (min)	Identification of Fatty Acid	Formula	% Area F-I(p)
17.99	Octanoic acid	$C_8H_{16}O_2$	4.73
19.38	Decanoic acid	$C_{10}H_{20}O_2$	11.83
21.01	Dodecanoic acid, methyl ester (lauric) methyl ester	$C_{13}H_{26}O_2$	33.72
23.26	Dodecanoic acid, propyl ester (lauric) propyl ester	$C_{15}H_{30}O_2$	11.5
23.60	Tetradecanoic acid, methyl ester (mystric) methyl ester	$C_{15}H_{30}O_2$	11.8
24.94	Tetradecanoic acid	$C_{14}H_{28}O_2$	10.3

Table 9. Fatty Acid Profile of Fractional Distillate isolated from Enzymatic Hydrolyzed Virgin Coconut Oil (according to GCMS)

R_t (min)	Identification of Fatty Acid	Formula	% Area F-II(p)
17.15	Octanoic acid	$C_8H_{16}O_2$	21.49
19.29	Decanoic acid	$C_{10}H_{20}O_2$	22.42
22.32	Dodecanoic acid (lauric acid)	$C_{12}H_{26}O_2$	35.82
24.76	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	15.57
24.94	Tetradecaonic acid	$C_{14}H_{28}O_2$	4.67

Table 10. Fatty Acid Profile of Residue after Removal of F-I and F-II from Enzymatic Hydrolyzed Virgin Coconut Oil (according to GCMS)

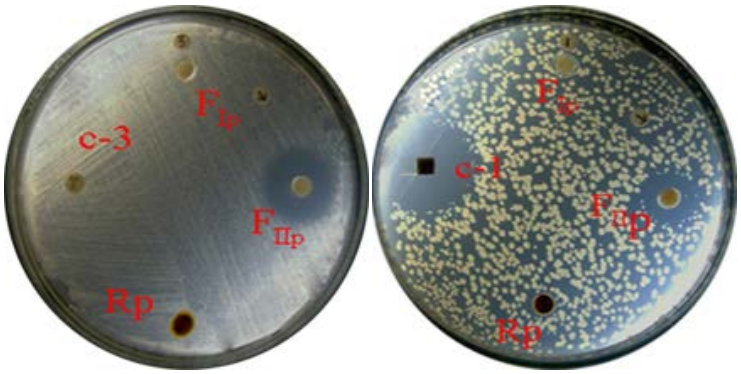
R_t (min)	Identification of Fatty Acid	Formula	% Area Rp
20.95	Dodecanoic acid, methyl ester (lauric acid) methyl ester	$C_{13}H_{26}O_2$	38.83
21.54	Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$	31.06
21.80	Dodecanoic acid, ethyl ester (lauric acid) ethyl ester	$C_{14}H_{28}O_2$	31.45
23.03	Dodecanoic acid, propyl ester (lauric acid) propyl ester	$C_{15}H_{30}O_2$	10.43
23.56	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	13.34



Staphylococcus aureus ***Proteus morgani***

F-I(p) Steam volatile FAs isolated from hydrolysate of VCO; hydrolysis of VCO was carried out by using lipase isolated from seedlings of peanut,
F-II(p) Distillate collected at 100-140 °C and under 4 mm Hg pressure after removal of F-I(p)
R(p) Residual FAs after removal of F-I(p) and F-II(p)

Control C-1 = Ofloxacin,
Control C-2 = Ciprofloracin,
Control C-3 = Tetracycline



Shigella flexneri ***Vibrio cholerae O139***

Figure 9. Photograph showing antimicrobial activity of different fatty acid fractions (disc diameter 6mm)

Table 11. Antimicrobial Activity of Different Fatty Acid Fractions (Disc diameter 6mm)

No	Bacterial Strain	Diameter of Inhibition Zone (mm)		
		F-I$_{(p)}$,	**F-II$_{(p)}$**	**R$_{(p)}$**
1	*Staphylococcus aureus*	16	20	9
2	*Proteus morganic*	14	21	-
3	*Shigella flexneri*	16	-	-
4	*Vibrio cholerae* 0139	-	15	-

Conclusion

The yield of virgin coconut oil (VCO) extracted from freshly harvested mature coconut by cold press method and the yield was 21 %.Fatty acid profile of VCO was caproic acid (C 6:0), 3.75 %; caprylic (C 8:0), 20.83 %; capric acid, (C 10:0), 18.32 %; lauric acid (C 12:0), 33.75 %; and myristic acid (C 14:0), 23.19 %.The optimum condition for lipase Enzyme was isolated from peanut seedlings was: pH, 4; reaction temp., 40 °C; substrate amount, 2 g; and reaction time, 60 min when using olive oil as substrate.

Enzyme activity under optimal condition was 180 EU mL^{-1}.The yield of MCFs isolated from hydrolysate of VCO was dependent on the nature of enzyme used during hydrolysis. When ES was used [F-II(p)] (32.6 % wt.). Lauric acid content of F-II(p) (35.82 % total MCFAs).*S. aureus, P. morganii, and S. flexneri* were found to be sensitive to test samples. All samples except VCO strongly inhibited *S. aureus* with inhibition zone diameter ranging 16-20 mm. F-II(p) showed maximum zone of inhibition against S. aureous (20mm) whereas FI was (16mm). These samples also effective against Gram negative bacteria as, *P. morganii* (14mm) in F_I and (21mm) in FII(p) *was* observed. *S. flexneri* was 16 mm in (F I)inhibition zone diameter. V. cholerae 0139) was shows only in FII(p). Fatty acids and glycerides especially monoglycerides of medium chain fatty acids of coconut oil have strongly anti-bacterial activity against Gram negative bacteria (*P. morganii, S. flexneri and V. cholerae 0139)* and Gram positive bacteria *(S. aureus).*

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Attitudes of Secondary School Teachers Based On Their Age Group towards Inclusive Education in Visakhapatnam District

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Abstract
The article concerns the use of inclusive education for learning and assessment of special needs children and how one can affect the other in either positive or negative ways. The present study assess the statements developed on four dimensions namely concept of inclusive education, organizational structure, classroom management, identification and assessment of children with special need, teaching and training methodologies. A total of 100 prospective secondary school teachers were examined in order to investigate the effect of age group towards inclusive education. In this study the questionnaire "attitude scale to assess the attitude" was used to collect the data from prospective teachers. There is no significant difference between the attitudes of teachers based on their age group towards inclusive education in secondary schools of Visakhapatnam district.
Keywords: Attitudes, secondary schools, classroom management, learning.

Introduction

Inclusive education is the social-cultural approach system used to facilitate students learning abilities during the process of change in education. Inclusive education happens when children with and without disabilities participate and learn together in the same classes. All students attend and are welcomed by their neighborhood school in age-appropriate, regular classes and are support to learn, contribute and participate in all aspects of the life of the school. Children with disabilities form one of the largest groups that are still outside the fold of the general education system, the CWSN scheme provides an opportunity for children with disabilities, who have completed eight years of elementary education to continue their education in regular schools at the secondary level in inclusive environment.

Planning for inclusive education at secondary level is to reduce the gap in the enrolment, retention, completion rates and achievement levels of children with respect to gender and socially advantaged groups. Formation of attitudes and positive attitudes facilitate to develop better competencies in the individuals Teachers are perceived to be an integral component in the implementation of inclusive education (Haskell, 2000). According to Parasuram (2006) teacher's attitude is one of the most important variables in the education of children with disabilities.

Method

Design of the study

The present study is a descriptive study conducted using the survey method. Descriptive research describes what is, describing, recording, analyzing and interpreting conditions that exist. Teachers were selected basing on the step wise simple random sampling technique.

Objective of the study

To study the attitude of teachers based on their age group towards inclusive education in secondary schools of Visakhapatnam District.

Hypothesis

There is no significance difference between the attitudes of teachers based on their age group towards inclusive education in secondary schools of Visakhapatnam District.

Results and Discussion

Table 1. Analysis of Variance (ANOVA) - Results on the perceptions of teachers based on their age group towards Inclusive Education in Visakhapatnam District

Area	Age	N	Mean	Groups	Sum of Squares	df	Mean Square	F-value	p-value
Concept of Inclusive Education	20 to 25	8	41.00	Between Groups	270.23	2	90.08	3.54*	0.04
	25 to 35	23	39.13	Within Groups	5626.52	97	58.61		
	Above 35	69	43.83	Total	5896.75	99			
Organization Structure and Classroom Management	20 to 25	7	39.00	Between Groups	18.72	2	6.24	**0.59**^{Ns}	0.62
	25 to 35	23	40.61	Within Groups	1009.39	97	10.51		
	Above 35	69	40.17	Total	1028.11	99			
Identification and	20 to 25	7	24.00	Between Groups	26.80	2	8.93	0.94^{NS}	0.42

Assessment	25 to 35	23	24.48	Within Groups	909.04	97	9.47		
	Above 35	69	25.26	Total	935.84	99			
Teaching and Training Method	20 to 25	7	21.29	Between Groups	4.36	2	1.45	0.43 NS	0.74
	25 to 35	23	21.57	Within Groups	328.39	97	3.42		
	Above 35	69	21.74	Total	332.75	99			
Overall Response	20 to 25	7	126.29	Between Groups	513.97	2	171.32	4.07 *	0.01
	25 to 35	23	125.78	Within Groups	4039.34	97	42.08		
	Above 35	69	130.00	Total	4553.31	99			

**Significant at 0.01, *Significant at 0.05 level and NS: Not Significant

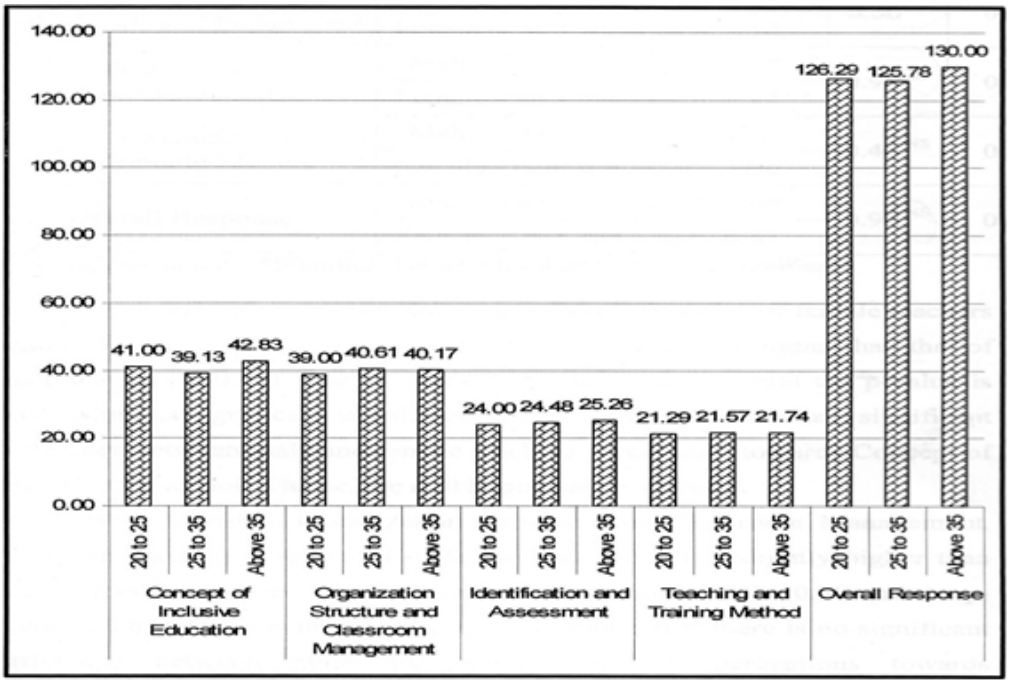
Table 1 revealed that, the ANOVA results on the perceptions of teachers with respect to Concept of Inclusive Education basing on their age group between groups and within groups, the df values are 2 and 97 and sum of squares are 270.23 and 5626.52 and mean squares are 90.08 and 58.61 respectively. The F-value is found to be 3.54 and the p-value is 0.04, which is significant at 0.05 levels. This shows that there is a significant difference among teachers perceptions based on their age group towards Concept of Inclusive Education. Hence, the null hypothesis is rejected. With regard to Organization Structure and Classroom Management, the ANOVA results on the perceptions of teachers basing on their age group between groups and within groups, the df values are 2 and 97 and sum of squares are 18.72 and 1009.39 and mean squares are 6.24 and 10.51 respectively. The F-value is found to be 0.59 and the p-value is 0.62, which is not significant. This shows that there is no significant difference among teachers perceptions based on their age group towards Organization Structure and Classroom Management. Hence, the null hypothesis is accepted.

With regard to Identification and Assessment, the ANOVA results on the perceptions of teachers basing on their age group between groups and within groups, the df values are 2 and 97 and sum of squares are 26.80 and 909.04 and mean squares are 8.93 and 9.47 respectively. The F-value is found to be 0.94 and the p-value is 0.42, which is not significant. This shows that there is no significant difference among teachers perceptions based on their age group towards Identification and Assessment. Hence, the null hypothesis is accepted.

With regard to Teaching and Training Method, the ANOVA results on the perceptions of teachers basing on their age group between groups and within groups, the df values are 2 and 97 and sum of squares are 4.36 and 328.39 and mean squares are 1.45 and 3.42 respectively.

The F-value is found to be 0.43 and the p-value is 0.74, which is not significant. This shows that there is no significant difference among teachers perceptions based on their age group towards Teaching and Training Method. Hence, the null hypothesis is accepted.

With regard to overall perceptions towards Inclusive Education, the ANOVA results on the perceptions of teachers basing on their age group between groups and within groups, the df values are 2 and 97 and sum of squares are 513.97 and 4039.34 and mean squares are 171.32 and 42.08 respectively. The F-value is found to be 4.07 and the p-value is 0.01, which is significant at 0.05 levels. This shows that there is a significant difference among teachers overall perceptions based on their age group towards Inclusive Education. Hence, the null hypothesis is rejected.



Graph 1. Mean comparison between the perceptions of teachers based on their age group towards Inclusive Education in Visakhapatnam District

Conclusion

Teachers expressed high perceptions in the aspects of concept of inclusive education, organization structure and classroom management, identification and assessment, teaching and training method and overall perception towards inclusive education in Visakhapatnam district. According to their age group, above 35 years age group teachers expressed high perception with respect to

concept of inclusive education and overall perceptions towards inclusive education in Visakhapatnam district than that of 20 to 25 to 35 age group teachers.

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