# STUDIES ON BILIRUBIN CLEARANCES OF METHANOLIC LEAF OF VELVET BEANS (*Mucuna pruriens*) ON CCl4 INDUCED ALBINO RATS

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**Abstract:** This study investigated the bilirubin clearance and the hepatoprotective properties of the methanolic extract of *Mucuna pruriens* leaves in Carbon Tetrachloride ($CCl_4$) induced albino rats. $CCl_4$ was injected into the albino rats to induce jaundice; the rats were given oral dose (20, 40 and 60 mg/kg body weight) of methanolic extract of *M. pruriens* simultaneously. The effect of this treatment on liver enzymes, total bilirubin, total albumin, total protein, urea, creatinine and cholesterol were evaluated. The administration of $CCl_4$ alone significantly ($P<0.05$) increased activities of serum, ALT, AST, ALP, GGT, total bilirubin, creatinine, cholesterol. The concentration of albumin and total protein were significantly reduced ($P<0.05$). Simultaneous treatment of $CCl_4$ injection and oral administration of 20, 40 and 60 mg/kg body weight of the methanolic extract of *M. pruriens* significantly reversed ($P<0.05$) these changes in the serum. The above results suggest that the extract may probably possess component that has anti jaundice properties.

**Keywords:** Bilirubin, carbon tetrachloride, hepatoprotective, Jaundice, *Mucuna pruriens*

## Introduction

Jaundice is a clinical symptom presenting as a yellowish pigmentation of the skin, the conjunctiva, and other mucous membranes due to elevated serum bilirubin. Bilirubin is the yellow breakdown product of the non-protein haeme ring of haemoglobin, cytochromes, catalase, peroxidase and tryptophan pyrrolase (Dennery *et al.*, 2001). It is formed during breakdown of senescent erythrocytes in the spleen, bone marrow, and in hepatic Kupffer cells, and is released into the plasma. Concentration of bilirubin in blood plasma is usually below 1.2 mg/mL.

In the plasma, bilirubin is bound to albumin and transported to the liver for further metabolism (Arias *et al.*, 2011). In the liver, unconjugated bilirubin is metabolized into water-soluble conjugated bilirubin. Unconjugated bilirubin is toxic to many cell types, intracellular organelles and physiological processes. Bilirubin inhibits DNA synthesis and ATPase activity of brain mitochondria, and uncouples oxidative phosphorylation (Hansen, 2000; Wennberg, 2000; Dennery, 2001). Increased rate of bilirubin production, albumin deficiency, low activity of the bilirubin-conjugating enzyme UDP-glucuronyltransferase, and disorders of the liver can increase the concentration of unconjugated bilirubin (Sherlock and Dooley, 2008). When bilirubin in plasma exceeds 2.5 mg/dL, bilirubin diffuses into body tissues, causing jaundice (VanDeursen *et al*., 2010; Arias *et al*., 2011). The conjunctiva of the eye is one the first tissues to change colour as bilirubin levels rise giving the usually white sclera a yellow appearance. Urine is usually dark in colour (Sherlock and Dooley, 2008).

In general, there are various treatments for jaundice, depending on the underlying cause. For instance, in treating pre-hepatic jaundice, the objective is to prevent the- rapid breakdown of red blood cells that cause bilirubin levels to build up in the blood. In cases of infections: such as malaria, use of medication for the treatment of the underlying infection, is usually recommended and, for genetic blood disorders, such as sickle cell anaemia or thalassaemia, blood transfusions may be required to replace the red blood cells. Gilbert's syndrome does not usually require treatment because the jaundice associated with the condition is not particularly serious and does not pose a serious threat to health (Scriver *et al*., 2000). In recent years, the growing demand for herbal products has been known to be a very good source of alternative treatment traded across countries. The use of herbs dates back to the early man who used herbs in their raw and cooked forms to keep fit. Since then, herbs such as *Mucuna pruriens* and others are known and generally accepted by many nations as the first art of treatment available to man (Baladrin *et al*., 1985; Yakubu *et al*., 2016).

*Mucuna pruriens*, a tropical legume, is one of the most popular medicinal plant that is traditionally used in India; and a constituent of more than 200 indigenous drug formulations (Sathiyanarayanan and Arulmonzhi, 2007; Kavitha, 2014). *M. pruriens* seed is a natural source of the amino acid L-3,4-dihydroxy phenyl alanine (L-DOPA); the direct precursor to the neuro transmitter dopamine which is used widely in treatment of Parkinson's disease (PD). Serotonin, oxitriptan, nicotine, N,N-DMT, and bufotenine are the other chemicals found in *M. pruriens* in the addition to L-DOPA (Spencer *et al*., 1995; 1996; ). According to Ancient Ayurvedic literature, *Mucuna* is used as a potent aphrodisiac (Amin *et al*., 1996), geriatric tonic and vermifuge. In addition, *Mucuna* is also grown as food crop, ornamental plant, living mulch and green manure crop.

Traditionally, The Yoruba tribe of Nigeria, have these seed for the treatment of various human and veterinary disease such as fever, constipation, menstruation disorder, oedema, tuberculosis and for the management of sickle cell anaemia (Guerranti *et al*., 2001; Kavitha and Thangamani, 2014). However, there is still limited scientific information on the treatment of jaundice with *M. pruriens.*

In the absence of reliable hepatoprotective drugs in orthodox medicine, a large number of alternative medicines are recommended for the treatment of liver disorders and are very often claimed to offer significant relief. A number of plants have been shown to possess hepatoprotective properties by improving the antioxidant status (Akhtar *et al*., 1990; Gupta *et al*., 1997; Ahmed and Beg, 2001; Ajit *et al*., 2010). An example of such a plant is *M. pruriens.*

In view of *M. pruriens* being a rich source of natural anti-oxidants and its numerous uses in alternative medicine, there is a need to conduct more studies on the plant. The primary objective of this investigation was to evaluate the possibility of the extracts of *M. pruriens* in enhancing the bilirubin clearance from the serum when its level is elevated. Also to examine the hepatoprotective effect of the crude methanolic extract of *M. pruriens* using carbon tetrachloride induced rats as experimental models.

## Materials and Methods

***Plant samples***

*Mucuna pruriens* leaves were obtained from Mushin Market Lagos, identified and authenticated at the herbarium section of the Department of Botany, University of Lagos.

***Experimental animals***

The twenty five Australian wistar albino rats (*Ratus norvegicus*) of both sexes weighing between 120 – 180 g used for this study were obtained from the College of Medicine Animal Care Unit, University of Lagos, Idi-Araba, Nigeria. The rats were maintained on standard laboratory feed and tap water (*ad libitum*). This procedure was performed according to the rules in Nigeria governing the use of laboratory animals (Carlson *et al*., 2004) as acceptable internationally.

***Chemicals and reagents***

Carbon tetrachloride ($CCl_4$) used is a product of Sigma, United Kingdom. The alkaline phosphatase kit is a product of Teco Diagnostic Limited, Canada, Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase kits are products of Randox Laboratories Limited, U.K and the Artesunate tablets used is a product of Mekophar Chemical Pharmaceutical Joint-Stock Company Chi Minh City, Vietnam. All other chemicals used were of analytical grade and obtained from BDH London.

***Preparation of plant materials***

The *M. pruriens* leaves were washed in tap water and rinsed in sterile distilled $H_2O$. The leaves were sun-dried for about a week, blended to fine powder with an electric blender, weighed and stored in containers at room temperature until when required for analyses. The *M. pruriens* was extracted with cold methanol and hot water. These were prepared using the method as described by Oyagade *et al*. (1999). The residues obtained were reconstituted in 95% methanol at stock concentration of 0.2 g/ml. The extract was then stored in the refrigerator at 4 ± 2$^oC$ until when needed (Omojasola, 2004).

***Experimental design***

The rats were randomly allotted into five groups of five rats each and categorized as follows:

Group A received 1 ml of distilled water and were tagged the **control group**

Group B received 0.5 mg/kg body weight $CCl_4$ and were tagged the **untreated group**

Group C received 0.5 mg/kg body weight $CCl_4$ and 20 mg/kg weight of the *M. pruriens* leaves extract simultaneously

Group D received 0.5 mg/kg body weight $CCl_4$ and 40 mg/kg body weight of the *M. pruriens l*eaves extract simultaneously

Group E received 0.5 mg/kg body weight $CCl_4$ and 60 mg/kg body weight of the *M. pruriens* leaves extract simultaneously.

Administration of all materials was by oral gavage and treatment lasted for seven days.

***Phytochemical screening***

Phytochemical analysis of methanolic extracts of *M. pruriens* was carried out as described by Odebiyi and Sofowora (1978).

***Biochemical studies***

The procedure as described by Reitmann and Frankel (1957) was employed for the assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and guanine glutamyltransferase (GGT) activities while that of bilirubin and albumin concentration in the serum was done using the method described by Malloy and Evelyn (1937) and Rodkey (1965), respectively. The total protein concentration was estimated in the serum using the method as described by Plummer (1978). The Jaffe's method using the alkaline picrate method was usedfor determination of serum creatinine while the Beckman Coulter AU system was used for the determination of the serum urea.

***Cholesterol***

Total cholesterol was determined using a simple colorimetric assay that measures the amount of cholesterol in serum (Shahjahan *et al.*, 2004).

**Creatinine:** The Jaffe's method using the alkaline picrate method was used for determination of serum creatinine.

**Serum Urea:** The Beckman Coulter AU system was used for the determination of the serum urea

**Calculation:** For serum SI units (mmol/L), multiply the results by 0.357

***Statistical analysis***

The statistical methods used in analyzing the data include mean, standard deviation and T-test for equality of means. The analysis was performed using Microsoft excel software. Mean differences were considered statistically significantly ($P<0.05$).

***Histopathological analysis***

Histopathological analysis was carried out as described by Rolls (2008). The tissues were fixed in 10% formalin for 24 h,cut into thin slices with a scalpel blade and placed in embeddings of labeled cassettes. The tissues were cut into slices of about 0.3 - 0.5 cm thickness in order to ensure proper penetration of processing reagents.This process was followed by dehydration with 70% alcohol (1 h), 90% alcohol (1 h), 90% alcohol (1 h), 90% alcohol (2 h) Absolute alcohol 100% (2 h) and Absolute alcohol 100% (3 h). After dehydration, it is essential to treat tissue with a regent that mixes with both alcohol and paraffin wax which in turn is removed in the process of wax. This process is called cleaning and the reagent used for this is xylene.

***Wax impregnation***

This process removes the cleaning agent from the tissue and allows it to be permeated by the molten paraffin wax (impregnation reagent) which was subsequently allowed to harden to produce a block from which sections were cut.

***Microtomy***

Fine sections of the tissue were obtained using a rotary microtome. The cut sections were then detached from the knife with a Carmel hair brush, placed on a glass slide containing 20% alcohol, and then floated out. Floating out involves gently lowering the strip of sections from the slide on to the surface of water which was 5 – 10°C below the melting point of the wax. The section would float, expand slightly and become flatted. The slide was dipped in the water obliquely and used in picking the section onto it after which it was placed on a hot plate for the surrounding wax to melt off the tissue (Rolls, 2008).

***Staining***

The slides were stained after 20 min according to the haemotoxylin and Eosin Staining Technique as described by Rolls (2008).

## Results and Discussion

Table 1 shows the results obtained from the phytochemical screening of the aqueous and methanolic extracts of *M. pruriens* leaves. It was revealed that tannin, terpenoid, cardiac glycoside, phenol, alkaloid and reducing sugar were detected in the aqueous and methanolic extracts while flavonoid, steroid and phlobatanin were detected in the aqueous extract only. Flavonoid, phenol and alkaloid were more abundant in the aqueous extract than in the methanolic extract, excluding tannin which was more abundant in the methanolic extract than in the aqueous extract in Table 2.

**Table 1: Qualitative phytochemical screening of aqueous and methanolic extracts of *M. pruriens* leaves**

| Phytochemical component | Aqueous | Methanolic |
|---|---|---|
| Tannin | + | + |
| Flavonoid | – | + |
| Phenol | + | + |
| Phlobatanin | - | + |
| Cardiac glycoside | + | + |
| Alkaloid | + | + |
| Reducing sugar | + | + |
| Terpenoid | + | + |
| Steroid | - | + |
| Anthaquinone | - | - |
| Saponin | - | - |

Key: (+) = Present, (-) = Absent

**Table 2: Quantitative phytochemical screening of aqueous and methanolic extracts of *M. pruriens* leaves**

| Phytochemicals present | Aqueous | Methanolic |
|---|---|---|
| Flavonoid (mg/100g) | 31.11 ± 0.23 | 24.66 ± 0.32 |
| Phenol (mg/100g) | 63.65 ± 0.16 | 15.17 ± 0.11 |
| Alkaloid (mg/100g) | 180.35 ± 0.18 | 127.78 ± 0.48 |
| Tannin (mg/100g) | 25.88 ± 0.04 | 50.36 ± 0.13 |

**Table 3: Effects of methanolic extracts of *M. pruriens* leaves on the body weight of the albino rats after 7 days of administration**

| | Initial weight (g) | Final weight (g) |
|---|---|---|
| Group A | 155.60 ± 4.20 | 155.61 ± 4.21 |
| Group B | 152.40 ± 3.21 | 127.80 ± 3.20 |
| Group C | 150.71 ± 3.90 | 159.10 ± 2.80 |
| Group D | 156.80 ± 2.90 | 166.33 ± 3.10 |
| Group E | 163.31 ± 3.20 | 174.31 ± 2.70 |

Values are means of four replicates ± S.D; Values with different superscripts across a row are significantly different (P<0.05); Group A received orally, 1 ml of distilled water daily for 7 days; Group B received 0.5 mg/kg body weight $CCl_4$ for 7 days; Group C received 0.5 mg/kg body weight $CCl_4$ and 20 mg/kg body weight of the *M. pruriens* leaves extract (orally) simultaneously for 7 days; Group D received 0.5 mg/kg body weight $CCl_4$ and 40 mg/kg body weight of the *M. pruriens* leaves extract (orally) simultaneously for 7 days; Group E received 0.5 mg/kg body weight $CCl_4$ and 60 mg/kg body weight of the *M. pruriens* leaves extract (orally) simultaneously for 7 day

In Table 3, there was a significant (P<0.05) increase in the final body weight of the rats that were treated with different doses of methanolic extracts (Groups C, D, and E) when compared with the untreated jaundice induced rats (Group B) which experienced reduction in weight. The concentration of the urea and creatinine is shown in Table 4. The concentration level of urea and creatinine in the serum of rats treated with 40, 60 mg/kg (Group D and E) was significantly increased (P<0.05) when compared to group A. The activity of total bilirubin and con bilirubin was significantly reduced (P<0.05) after the 7 days administration of the dose 20, 40 and 60 mg/kg when compare to group A

The Lipid profile of cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein in the serum of the rats is revealed in Table 5 the concentration level in cholesterol and triglycerides in the serum of the rats treated with 20 and 40 mg/kg (group C and D) was significantly increased (P<0.05) when compared to group A. while the concentration level in HDL and LDL was significantly reduced in treated dose 60 mg/kg when compared to group A. The enzyme activities of AST, ALP and ALT in the serum of the rats are revealed in Table 6. The ALP and ALT in the serum of the rats was significantly reduced (P<0.05) in all the treatment groups (Group C, D and E) when compared to group A after the 7 days administration of the doses 20, 40 and 60 mg/kg, respectively. The activity of AST was significantly increased (P<0.05) after the 7 days administration of the dose 60 mg/kg when compared to group A.

Table 7 shows the concentration of total protein and albumin in the serum of the albino rats. The concentration of the total protein level was significantly reduced (P<0.05) in the group treated with 20 and 60 mg/kg dosage when compared to group A. The concentration level of albumin in group D significantly increased (P<0.05) when compared to group A after the 7 days administration of the methanolic extracts of *M. pruriens leaves*. Table 8 shows the concentration of cation. The concentration level in cation in the serum of the rats treated with 40 and 60 mg/kg was significantly increased (P<0.05) when compared to group A.

**Table 4: Effects of administration of the methanolic extracts of *M. pruriens* leaves on the concentration of urea, creatinine, total bilirubin and con bilirubin in the serum of Jaundice induced albino rats**

| | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| UREA | 34.00±4.04 | 33.00±2.30 | 31.66±2.60 | 45.00±1.15*#α | 38.33±2.02#αβ |
| CREAT | 1.30±0.35 | 1.23±0.14 | 1.13±0.14 | 1.86±0.08#α | 1.70±0.20#α |
| TBIL | 0.93±0.03 | 0.80±0.05* | 0.83±0.08 | 0.86±0.03* | 0.80±0.05* |
| ConBIL | 0.56±0.03 | 0.43±0.03* | 0.53±0.08 | 0.53±0.03# | 0.46±0.06* |

**=P<0.05 when compared with group A; #=P<0.05 when compared with group B; α=P<0.05 when compared with group C; β=P<0.05 when compared with group D;* Values are means of four replicates ± S.D; Values with different superscripts across a row are significantly different (P<0.05); Group A received orally, 1ml of distilled water daily for 7 days; Group B received 0.5 mg/kg body weight $CCl_4$ for 7 days; Group C received 0.5mg/kg body weight $CCl_4$ and 20 mg/kg body weight of the *M. pruriens* Leaves extract (orally) simultaneously for 7 days; Group D received 0.5mg/kg body weight $CCl_4$ and 40mg/kg body weight of the *M. pruriens*, Leaves extract (orally) simultaneously for 7 days; Group E received 0.5mg/kg body weight CCL4 and 60mg/kg body weight of the *M. pruriens* leaves extract (orally) simultaneously for 7days.

**Table 5: Effects of administration of the methanolic extracts of *M. pruriens* leaves on the concentration of cholesterol, triglycerides, HDL and LDL in the serum of Jaundice induced albino rats**

| | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| CHOL | 95.33±2.90 | 94.00±2.88 | 109.33±5.20*# | 102.33±7.21 | 88.33±3.48*#αβ |
| HDL | 29.33±1.45 | 27.33±1.45 | 32.67±1.20*# | 31.00±2.64# | 26.00±1.52*αβs |
| TG | 65.00±7.0 | 69.00±4.58 | 78.67±2.40*# | 65.33±3.75α | 57.00±1.52*#αβ |
| LDL | 51.66±6.91 | 56.57±3.99 | 63.81±2.44*# | 51.24±2.60#α | 45.18±0.91*#αβ |

**=P<0.05 when compared with group A; #=P<0.05 when compared with group B; α=P<0.05 when compared with group C; β=P<0.05 when compared with group D;* Values are means of four replicates ± S.D; Values with different superscript across a row are significantly different (P<0.05)

**Table 6: Effects of administration of the methanolic extracts of *M. pruriens* leaves on the concentration of AST, ALP and ALT in the serum of Jaundice induced albino rats**

| | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| AST | 59.33±4.33 | 57.00±5.00 | 63.67±2.40* | 63.00±2.00* | 66.00±3.78# |
| ALT | 33.33±2.33 | 31.66±3.71 | 36.67±1.45 | 34.33±1.45 | 38.00±2.08*# |
| ALP | 59.33±0.33 | 59.00±0.57 | 58.67±0.66 | 60.33±0.88α | 59.33±0.33 |

**=P<0.05 when compared with group A; #=P<0.05 when compared with group B; α=P<0.05 when compared with group C; β=P<0.05 when compared with group E;* Values are means of four replicates ± S.D; Values with different superscripts across a row are significantly different (P<0.05)

**Table 7: Effects of administration of the methanolic extracts of *M. pruriens* leaves on the concentration of total protein and albumin in the serum of Jaundice Induced Albino Rats**

| | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| TP | 6.00±0.11 | 6.30±0.15* | 5.83±0.08*# | 5.93±0.14*# | 5.90±0.05# |
| ALB | 4.06±0.12 | 4.16±0.03 | 3.97±0.14# | 4.13±0.08 | 4.03±0.08 |

**=P<0.05 when compared with group A; #=P<0.05 when compared with group B; α=P<0.05 when compared with group C; β=P<0.05 when compared with group D;* values are means of four replicates ± S.D; Values with different superscripts across a row are significantly different (P<0.05)

**Table 8: Effects of administration of the methanolic extracts of *M. pruriens* leaveson the concentration of cation in the serum of Jaundice induced albino rats**

| | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| Ca | 9.90±0.11 | 10.40±0.11* | 9.23±0.52*# | 10.36±0.14*α | 10.10±0.15α |

**=P<0.05 when compared with group A; #=P<0.05 when compared with group B; α=P<0.05 when compared with group C; β=P<0.05 when compared with group D;* Values are means of four replicates ± S.D; Values with different superscripts across a row are significantly different (P<0.05)

**Histological Structures of Liver Tissues of Albino Rats**

**Plate 1:** Showing normal hepatocyte (Control group)

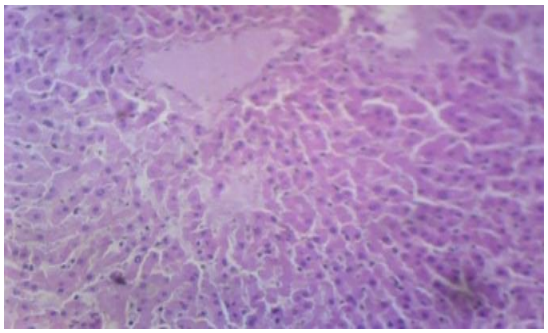

**Plate 2:** Showing mild deformations in the hepatocytes of the $CCl_4$ +60 mg/kg b.wt extract

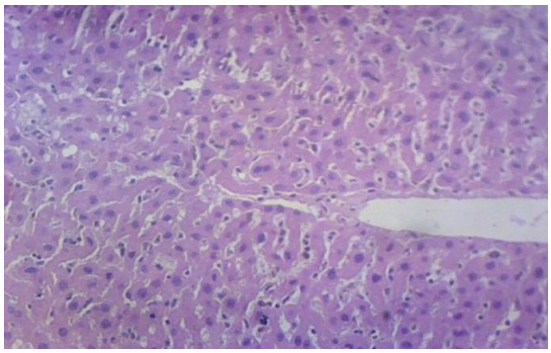

**Plate 3:** Showing mild deformations in the hepatocytes of the $CCl_4$ group + 40 mg/kg b.wt extract group

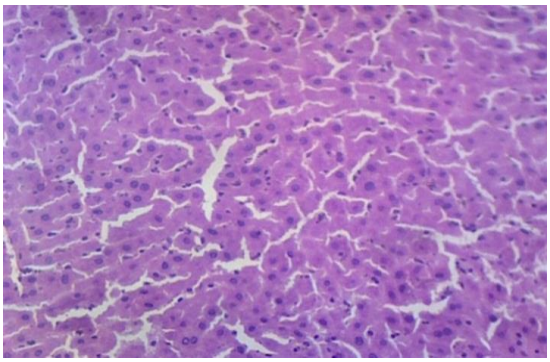

**Plate 4:** Showing mild deformations in the hepatocytes of the $CCl_4$ + 20 mg/kg b.wt extract group

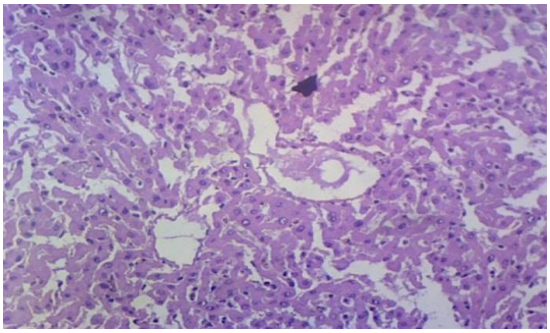

**Plate 5:** Showing deformations in the hepatocytes of the liver

Jaundice is a very important health condition as it is responsible for most liver malfunction, it results from imbalance between bilirubin (a yellowish bile pigment: a reddish yellow bile pigment that is an intermediate product of the breakdown of haemoglobin in the liver) production and excretion. It is the appearance of yellow pigmentation in the skin, sclera and mucous membranes (Dennery *et al*., 2001; Arias *et al*., 2011). Under normal circumstances, only a tiny amount of urobilinogen if any is excreted in the urine, but when the liver's function is impaired or when biliary drainage is blocked , some of the conjugated bilirubin leaks out of the hepatocytes, and appears in the urine, turning it dark amber.

The combined increase in the levels of Total cholesterol, Triglyceride and Low density lipoprotein both at low and medium doses (Table 5) is an indication of anti-cardio protective properties of the treatment employed in this study but at the high dose the levels were reduced close to the control levels. These lipids have been shown by various studies to promote/induce the pathogenesis of cardiovascular diseases such as arteriosclerosis, hypertension and heart failure (Vicenova *et al.,* 2009) while there was a corresponding increase in the levels of High density lipoproteins, which is a strong indication of a beneficial consequence (Tackett *et al.,* 2005). This coupled effect points to the fact that the treatment has no negative effect on cardiovascular functions. Increased concentration of low density lipoprotein (LDL) cholesterol or decreased levels of high density lipoprotein (HDL) cholesterol are important risk factors for coronary atherosclerosis. However, an independent association of triglycerides (TG) with atherosclerosis is uncertain (Beatriz *et al.,* 2011). On the other hand, the treatment could have induced systemic hypotension/bradycardia in the animals, which is another index of lowered lipid profile (Seifert *et al*., 1994; Nermeen *et al*., 2013). The treatment with high dose resulted in a significant improvement in the lipid profile (Table 5). This finding is similar to previous report by Hamid *et al.* (2011) that the underlying mechanism by which cholesterol is lowered may be due to a decrease in cholesterol absorption from the intestine, by binding with bile acids within the intestine and increasing bile acids excretion (Wu *et al*., 1999; Binu, 2009) or by decreasing the cholesterol biosynthesis especially by decreasing the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA reductase) activity, a key enzyme of cholesterol biosynthesis (Sharma *et al*., 2003) and/or by reducing the NADPH required for fatty acids and cholesterol synthesis (Lorenzetti *et al*., 1998; Misra and Wagner, 2007).

The significant increase of serum TG level in the low and medium doses is another important finding; recent studies have also shown that triglycerides are independently related to coronary heart disease (Hamid *et al.,* 2011). The observed hyper-triglyceridemic effect may be due to an increase of fatty acids synthesis (Bopanna *et al*., 1997), enhanced anabolism of LDL, deactivation of LCAT and tissue lipases and/or activation of acetyl-CoA carboxylase (Spina and Cohen, 1988; Lee *et al*., 2005) and production of triglycerides precursors such as acetyl-CoA and glycerol phosphate. Compared to Silymaril, *Arachis* oil and vitamin E, Methanolic extract of *Argemone Mexican apapavericaoe* provided a better protective effect as regards the lipid profile.

Also in this study, the levels of alkaline amino transferase, (ALT), AST and ALP of the treated rats which increased marginally when compared to the levels of these enzymes in the control groups indicates that there is a damage in the liver because of increase in the blood. ALT and AST are enzymes involved in amino acid metabolism and used as a marker in liver diseases (Kottai *et al*., 2010; Ajit and Das, 2010). These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Wu *et al*., 1999). Therefore, the increment of the activities of AST and ALT, in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993), and this is an indication on the hepatotoxic effect of $CCl_4$.

Aspartate transaminase (AST) is an enzyme that helps metabolize alanine, an amino acid. AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage or disease. The result obtained from (Table 6) indicates that there is an increase in the alanine metabolism in the liver indicating a hepato-protective effect. Alanine transaminase (ALT) is an enzyme found in the liver that helps the body to metabolize proteins. When the liver is damaged, ALT is released into the bloodstream and levels increase. The graded reduction in the blood levels at the medium and high doses is an indication that the hepatic protein metabolism/synthesis is greatly enhanced by the treatment.

Alkaline phosphatase (ALP) is an enzyme in the liver, bile ducts and bone. Higher than normal levels of ALP may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases. The dose dependently reduction in the blood levels (Table 6) is an indication that there was no impairment in the biliary system or bone damages which could result in a reduction in red blood cell synthesis. The present study shows exposure to $CCl_4$ causes an increase in the serum concentrations/values of AST, ALP and ALT. This is similar to a report in another study by Pourgholam *et al.* (2006) they studied the effect of different sub-lethal concentrations of diazinon on grass carp after 45 days and found that levels of ALT, ALP and AST were lower than control.

Similar changes were also observed in *R. frisii* male brood stocks by Luscova *et al*. (2002). They examined the effect of diazinon on carp and showed that Na and K levels were higher and AST, ALP and ALT levels were lower in common carp after being exposed to insecticides. Treatment with medium and high doses resulted in a mild improvement in the liver enzymes status. Goutam *et al.* (2013) has previously shown that there was a significant improvement in the liver enzyme status on the administration following treatment with Methanolic extract of *Argemone Mexican apapavericaoe*, but this study did not result in a significant improvement. This could be due to the fact that the metabolic function of the liver had been improved following oral treatment with the treatment, the treatment with the ginger oil seems to produce a protective effect on the animal, and this is not only due to the acute nature of the study.

In this study also, plasma samples were analyzed for their bilirubin, urea and creatinine levels. The results obtained in this study also showed that the levels of plasma bilirubin significantly reduced in the treated groups compared to the control (Table 4). The decrease in serum bilirubin (hypo-bilirubinemia) could have resulted from an increase in liver uptake, conjugation or reduced bilirubin production from haemolysis (Dennery *et al*., 2001). In addition, the reduction in serum bilirubin indicates liver adaptation as confirmed by the changes in the activities of liver enzymes (AST and ALT). Thus, the reduced level of bilirubin observed in animals in the treated groups when compared with the control group could be attributed to protection over the initial liver damage caused by $CCl_4$ that the animals are recovering from. The results obtained from this study also indicated that blood urea nitrogen was significantly increased in the treated groups of animals compared to the controls.

From the result obtained, there is a clear indication that the plasma creatinine level was significantly increased at medium and high doses as compared to the control. These findings reveal that there is strong relationship of blood sugar level with creatinine level. The rise in glycemia, involves changes in carbohydrate metabolism and secondarily of lipids and proteins, leading to a loss or degradation of structural proteins due to hepatotoxicity (Wu *et al.,* 1999). Although high levels of creatinine indicate several disturbances in kidney, but also High-serum creatinine level is a marker of muscle wastage. Creatinine (Cr) participates in metabolic reactions within cells and eventually is catabolized in the muscles creating creatinine which is then excreted by the kidney in urine. When Cr is being stored, it is converted to the high energy

form of phosphocreatinine (PCr) which acts as a high-energy reserve in a coupled reaction in which energy derived from donating phosphate is used to regenerate the compound ATP. Since Cr is a critical component of maintaining cellular energy homeostasis, a decrease in creatinine levels, will further contribute to low energy levels in the cells (even in the cells of the brain). The implication of this is that it could serve as a substrate or oxidative stress.

The results from the histological slides helps to buttress the biochemical findings on the hepatotoxicity of $CCl_4$ and the protective capacity of the graded dose of the extract *M. pruriens* when compared with the standard drug used in this study. This confirmed that the hepatoprotective effect of this extract is only relevant at the high dose of 60 mg/kg body weight.

**Conclusion**

It can be concluded that *M. pruriens* possesses the phytochemicals that can aid bilirubin clearance from the serum. These phytochemicals also have hepatoprotective properties which enable them to protect the liver against oxidative injuries. It may therefore be recommended as part of the diet for jaundiced individual and also can be used for the management of jaundice in the future.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.

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