



## **Ameliorative Effect of Nutmeg (*Myristica fragrans*) Supplement against Acetaminophen - Induced Hepato-Renal Toxicity in Wistar Rats**

**Chukwunyere Ogechi Abaekwume<sup>1\*</sup> and Hope Delesi Kagbo<sup>2</sup>**

<sup>1</sup>*Department of Animal and Environmental Biology, University of Port Harcourt, Nigeria.*

<sup>2</sup>*Department of Pharmacology, University of Port Harcourt, Nigeria.*

### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author COA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HDK managed the analyses of the study. Authors COA and HDK managed the literature searches. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Recently, many drugs have been developed and used for the treatment of hepatic and renal diseases. Nutmeg apart from being utilized as kitchen spices in Nigeria has also been used for healing and medicinal purposes.

**Aims:** In the present study, nutmeg supplement was evaluated against Acetaminophen (APAP) induced hepato-renal-toxicity.

**Study Design:** Twenty (20) adult wistar rats weighing 185-220 were obtained from animal house of the department of Pharmacology University of Port Harcourt were divided into four groups having 5 rats each (n=5).

**Methodology:** The animals were divided into four groups of 5 rats each: Group A (normal control) were administered distilled water, group B (negative control) received a single dose of acetaminophen (1000 mg/kg) for two days. Group C received 500 mg/kg body weight of nutmeg supplement one hour before receiving 1000 mg/kg acetaminophen, while group D received acetaminophen (1000 mg/kg) only on day 1 and 2 and the drug extracts on day 3-7. All dosage was

\*Corresponding author: E-mail: [chuogeson@yahoo.com](mailto:chuogeson@yahoo.com);

dissolved in distilled water orally. The experiment lasted for seven days. Twenty four hours after drugs administrations the animals in each group were anaesthetized. Blood samples were collected and animals sacrificed, liver and kidney tissues removed for various histopathological, biochemistry, antioxidant and haematological examinations using standard procedures. Statistical analysis was done using ANOVA and Tukey poc-hoc Test.

**Results:** Administration of nutmeg supplement orally effectively restrained APAP-induced alterations in the activities of hepatic (48.60-38.00) and renal markers and Antioxidant enzymes in liver (0.43-0.53). The hepatic and renal architecture of APAP administered rats showed distorted liver and kidney tissues, hepatic vacuolations, destroyed glomerular and renal tubules. Nutmeg+APAP as well as APAP+ Nutmeg administrations were able to ameliorate the effects of APAP administration.

**Conclusion:** The result indicated that APAP overdosed is hepato-renal- toxic and Nutmeg supplement possessed hepato-prote-curative properties as well as renal-prote-curative properties against APAP-induced damage in rats.

*Keywords: Acetaminophen; antioxidant enzymes; biochemistry and hepato-renal-protective agents.*

## ABBREVIATION

APAP: Acetaminophen

## 1. INTRODUCTION

Drug inducement is not a new thing in our society today. It has been the problem encountered intentionally and unintentionally in our day to day use of drugs [1]. Toxicology is defined as the study of adverse physicochemical effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects (Abubakar et al. 2018).

Acetaminophen toxicity accounts for 70,000 hospitalizations each year [2]. The liver is the most indispensable organs in the mammalian body and performs all important functions that affect all body system. They are concurrent with metabolic process in the body [3]. Acetaminophen (APAP) also known as paracetamol is a widely studied and most commonly used drug for the treatment of mild pain and reduces fever. It is a widely used over the counter analgesic [4]. It is a safe drug when given in remedial doses but it's over dose is fairly common since it has narrow therapeutic index. APAP over dose is the most common cause of hepatic and renal damage in both humans and experimental animals after viral hepatitis [4,5]. N-acetyl – P benzoninoneimine (NAPQI) is one the metabolites of acetaminophen which is responsible for its toxicity [4]. Over dose of acetaminophen causes release of NAPQ1 which triggered decrease in glutathione by 70%. The excessive amounts of NAPQ1 bind to hepatocytes causing cellular toxicity, which is

manifested as hepatic necrosis (Obu et al., 2012; Juma et al., 2015).

The liver possesses detoxifying property, support homeostasis and regulating energy balances [6]. All the chemical substances injected in the body through food are absorbed through the gastrointestinal tract (GIT) and are eliminated by liver. The injection of these chemicals can lead to tissues damage [2].

Kidney is the second core organ found in animal body. They are pair of bean-shaped organs found on the lower part of the abdominal cavity, below the ribs and below the stomach (in the renal system) [7]. They help in blood filtration, maintenance of acid-base balances, excretion i.e. ultra- filtration, filtering waste materials from food, medications and toxic substances filtering [1]. Because of all these basic functions the kidney performs and the toxins they encounter the kidney are prone to various problems.

Regularly taking of certain over the counter medication such as non steroidal anti-inflammatory drugs, can cause liver and kidney damage over time [8].

Human reliance on medicinal plants to alleviate diseases had been since ancient time. Medicinal plants had been used and applied in treatment for over thousands of years ago. Herbal drugs are major constituents of various alternative systems of medicinal used worldwide [9]. Recently, many drugs have been developed and used for the treatment of hepatic and renal diseases. Several herbal medicines had been found to possess antibody and antiviral properties [10]. However these drugs are harmful

and have side effects such as insomnia, vomiting, constipation and depression [6]. They are used in ethno-medicine to treat different ailments [11,12]. *Myristica fragrans* Houtt (nutmeg) is a hard brown seed from the nutmeg tree. It is indigenous to Banda Islands of Indonesia but also grown in the Caribbean especially in Grenada [13,14]. It belongs to the family myristicaceae. It is cultivated for two species derived from its, nutmeg and mace. Studies have shown that apart from being used as kitchen spices has also been used for healing and medicinal purposes, potent brain booster, increasing circulation and allowing better concentration, promotes digestion, assists in oral health, works as a detoxifier, treats insomnia, relieves pain, cure cancer and also acts as a powerful antioxidant [14,15,16]. Others also had been reported to possess some phytochemical properties which had hepatoprotective, nephroprotective and improvement to these liver and kidney damages. The most effective usage of medicinal drugs in ameliorating or militating liver and kidney damages caused by Acetaminophen excessive intake has not been reported by few researchers [17]. Base on this, the study is aimed as to ascertain the ameliorative effects of nutmeg on the hepatorenal damages caused by acetaminophen toxicity.

## 2. MATERIALS AND METHODS

### 2.1 Procurement of Animals

Twenty (20) healthy adult male wistar rats weighing 185-220 kg were obtained from the animal house of the Department of Pharmacology of the University of Port Harcourt.

The rats were maintained under 12 hour's light-dark cycle. Rats were housed in polypropylene cages and fed with finishers' marsh. They were allowed free access to regular tap water *ad libitum*.

Rats were acclimatized for one week before the start of the study. Handling of experimental animals was in accordance with the National Institute of Health Guide for care and use of Laboratory Animals.

The animals were divided into four groups of 5 rats each: After 24 hours, group A which serves as normal control group were administered with distilled water for 7 days, group B (Acetaminophen group) which serves as

negative control group received a single dose of acetaminophen (APAP) (1000 mg/kg) dissolved in 5 ml of distilled water orally for two days. Group three which serves as prophylactic group were administered with 500 mg/kg body weight of nutmeg dissolves in distilled water one hour before the administration of 1000 mg/kg acetaminophen dissolves in distilled water, group while group D (curative group) received the acetaminophen (APAP) (1000 mg/kg) dissolved in 5ml of distilled water orally only on day 1 and 2 and the drug extracts on day 3-7. The weights of the animals were taken on day 1, 4 and 7 and dosages adjusted according to the changes in body weight. The experiment lasted for seven days. This is according to the method described by [18] with slight modification. Twenty four hours after drugs administrations the animals in each group were anaesthetized. Blood samples were collected. After blood collection, animals were sacrificed, liver and kidney tissues removed and serum collected for histopathological tests hours, liver and kidney biochemistry, antioxidant enzymes and haematological examinations using standard procedures. Histological examinations of the liver and kidney were done by fixing in formaldehyde and hematoxylin staining. Statistical analysis was done using ANOVA and Tukey poc-hoc Test.

### 2.2 Experimental Design

Twenty (20) adult male wistar rats were divided into four groups having 5 rats each (n=5). The supplement powders of the medicinal drugs were dissolved in distilled water.

The animals were fasted for twenty four hours, prior to the experiment under standard laboratory condition, but were allowed free access to water *ad libitum*. After 24 hours, group A which serves as positive control received distilled water (5 ml/kg) orally for 7 days. Group B which serves as negative control group received a single dose of acetaminophen (APAP) (1000 mg/kg) dissolved in 5 ml of distilled water orally for two days. Group C were given *Myristica fragrans* (nutmeg) powdered supplement dissolved in distilled water (500 mg/kg) were administered orally daily one hour before administering acetaminophen powder dissolve in distilled water for 7 days. Twenty four hours after drugs administrations the animals in each group were sacrificed [5,6] these groups are called prophylactic groups. Group D which is curative groups was administered with Acetaminophen only on day 1 and 2 and the drug supplement from day 3 to day 7 according to the

method used by [18] with slight modification. Slight modification is administering of acetaminophen drug one hour after extracts administration. The weight of the animals was measured on the first, fourth, and seventh day of drugs and extracts administration respectively and dosages adjusted according to change in body weight.

### **2.3 Biochemical Evaluation**

All the rats were fasted for 12 hours at the end of the treatment period. The blood was collected by cardiac puncture using sterile disposable syringes under mild chloroform anesthesia. Sera were separated out by centrifuging at 3000 rPm for 10 minutes to get serum for Biochemical parameters study. The animals were then sacrificed by cervical and homogenized for histopathological studies.

### **2.4 Liver and Kidney Enzymes Assessment**

Using the standard liver enzymes Kidney biochemical assessment method, the serum collected was assayed. Liver enzymes assayed are; Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Aspartate Aminotransferase (AST), Bilirubin, Total Protein, and Albumin levels. Kidney enzyme assessments like Creatinine, Blood Urea Nitrogen (BUN) and cholesterol. Blood Electrolyte such as sodium, potassium, chlorides was measured using the Hitachi 902, Automatic Chemical Analyzer.

### **2.5 Histopathology**

The liver tissue were dissected out and fixed in the 10% formalin, dehydrated in gradual ethanol cleared in Xylene and embedded in paraffin wax sectioned (50<sup>#</sup> m) with a rotary microtome and were stained with Haemotoxylin and eosine (H&E).

The liver sections were evaluated histologically with light microscope with camera attached to it. The liver sections were scored and evaluated to the severity of the hepatic injury.

The kidney was freed from connective tissue coverings and gently removed, weighed and examined microscopically 3-5 mm<sup>2</sup> thick pieces were excised from the organ and fixed in 10%

formations solutions, dehydration in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin. 50<sup>#</sup> m thick sections were obtained and subsequently stained with eosine and haematoxylin and PAS and examined under light microscope [5].

### **2.6 Haematological Examination**

The haematological parameters such as Red Blood Cell (RBC) count, White Blood Cells (WBC), Platelets (PLT), Packed Cell Volume (PCV), Haemoglobin, Neutrophils, Lymphocytes, Eosinophil and Monocytes were calculated by using automated haematology analyzers.

### **2.7 Antioxidant Studies / Biomarkers**

Hepatic and Renal Antioxidant: GSH (Glutathione), SOD (Superoxide Dismutase), MDA (Malondialdehyde) and Catalase (CATA) were determined using method described by [18] in [19] with slight modification.

### **2.8 Statistical Analysis**

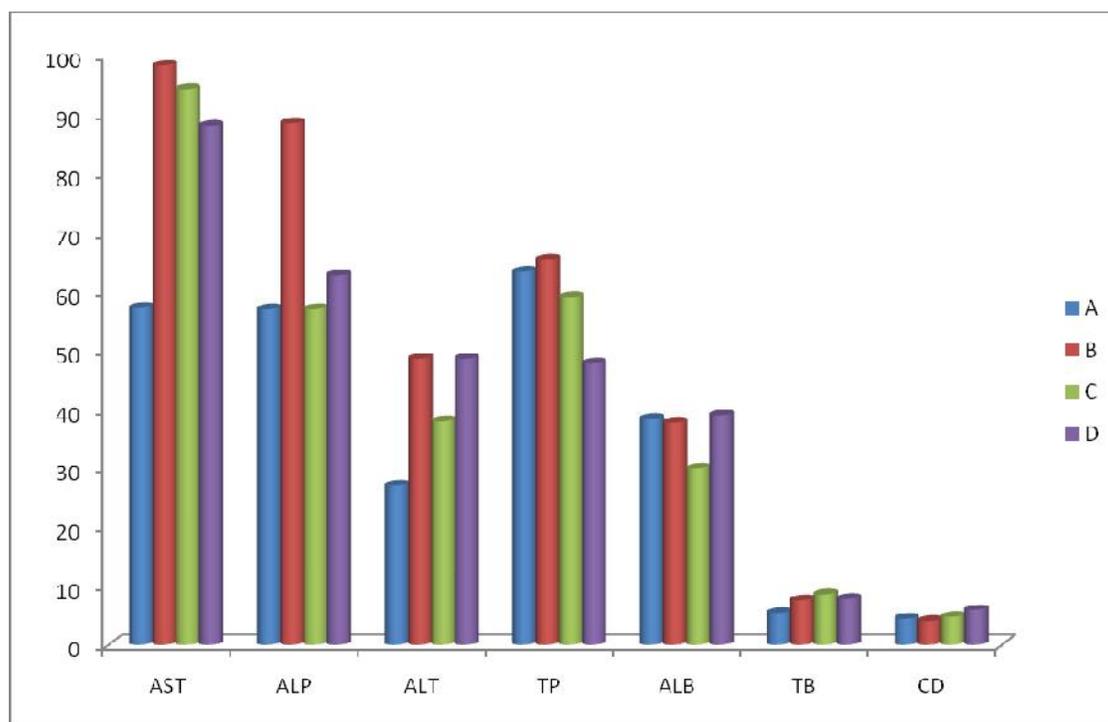
Statistical Analysis was conducted using Statistical package for Social Sciences (SPSS) version 16.0 (Chicago IL, USA). The mean value of data collected was represented as means, standard error of mean (S.E.M). The data were analyzed using one way analysis of Variance (ANOVA) and the difference between the groups was determined using Tukey's Post Hoc test. Statistical significant was set at the  $P < 0.05$  levels.

## **3. RESULTS AND DISCUSSION**

### **3.1 Effects of Extracts on Biochemical Parameters**

#### **3.1.1 Effect on the liver enzymes**

In Fig. 1, there was significant ( $P < 0.01$ ) elevation in the levels of AST, ALP, ALT levels on the liver enzymes of the group administered with APAP. In Group C (Nutmeg+APAP), there was significant ( $P < 0.01$ ) rise in the level of AST, ALP and TB. In group D (APAP +Nutmeg), there was significant ( $P < 0.01$ ) rise in the levels of AST, ALP, ALT and significant ( $P < 0.05$ ) elevation in TB compared to control group A. There was significant ( $P < 0.05$ ) reduction in AST compared to Group B.



**Fig. 1. Effect on the liver enzymes**

### 3.1.2 Effect on the kidney biochemistry

Table 1 show that, there was significant ( $P < 0.05$ ) elevation in creatinine level on the liver enzymes of the group administered with APAP.

In group C (Nutmeg+APAP) there was significant ( $P < 0.01$ ) increase in creatinine level in comparison with control group A.

### 3.2 Effects of Different Extracts on the Hepatic Anti-Oxidant Parameter

#### 3.2.1 Effects on the liver antioxidant parameter as shown in Table 2

APAP Administration causes a significant ( $P < 0.01$ ) rise in hepatic CATA. Liver antioxidants of rats treated with Nutmeg+APAP extract shows significant ( $P < 0.01$ ) reduction in hepatic CATA and GSH. In group D (APAP + Nutmeg), there was significant ( $P < 0.01$ ) reduction in hepatic GSH compared to control group A.

Table 3 shows the effects of drugs administration on renal antioxidant. Group treated with APAP showed significant ( $P < 0.01$ ) reduction in the level of renal CATA and renal GSH, levels respectively compared to Group A. Groups treated with Nutmeg+APAP and APAP +Nutmeg, showed

significant ( $P < 0.01$ ) reduction in renal CATA and renal GSH, as compared to group A.

#### 3.2.2 Effects on haematological parameters: The effects is shown in Table 4

Rats treated under curative use (APAP+Nutmeg), showed significant ( $P < 0.01$ ) reduction in the serum PCV count, HB and RBC count levels compared to group A.

The kidney of the control group in Plate 1 (A1b), showed histologically normal kidney and renal tubules. Kidney section of group B in Plate 2 (B1b) showed kidney sections with severe distortion of the renal tubules, and shrinkage of glomeruli. Group C and Group D kidney sections in Plate 3 and 4 (D1b, H2b)) showed distorted kidney with distorted renal tubules (RT) and glomeruli (G) with preserved glomerular tufts.

The histological investigation of the liver tissue as illustrated in plate 5-8 (A2b-D2b) showed normal control group with good hepatocytes with central vein (CV) containing blood cells as well as normal hepatic sinusoids. Liver tissue of APAP treated rats groups indicated distorted tissue and deteriorated cords of hepatocytes, sinusoids, central vein and portal triad.

**Table 1. Effects of extract on kidney biochemistry**

Groups	Sodium	Potassium	Urea	Creatinine	Cholesterol	Chloride
A	113.80±4.15	5.42±0.27	2.74±0.13	98.40±4.70	42.00±2.78	26.20±3.31
B	142.20±8.38	7.58±10.44	4.68±0.22	130.00±6.16*	49.20±1.24	26.80±1.02
C	118.50±9.87	4.95±0.78	17.13±11.97	146.75±4.96**	41.75±2.14	25.50±1.71
D	125.40±9.14	5.00±0.76	3.84±0.27	113.80±3.40	48.00±1.41	28.40±0.75

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with Group A. \*p<0.05, \*\*p<0.01 vs Group B. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test

**Table 2. Effects on liver biomarkers**

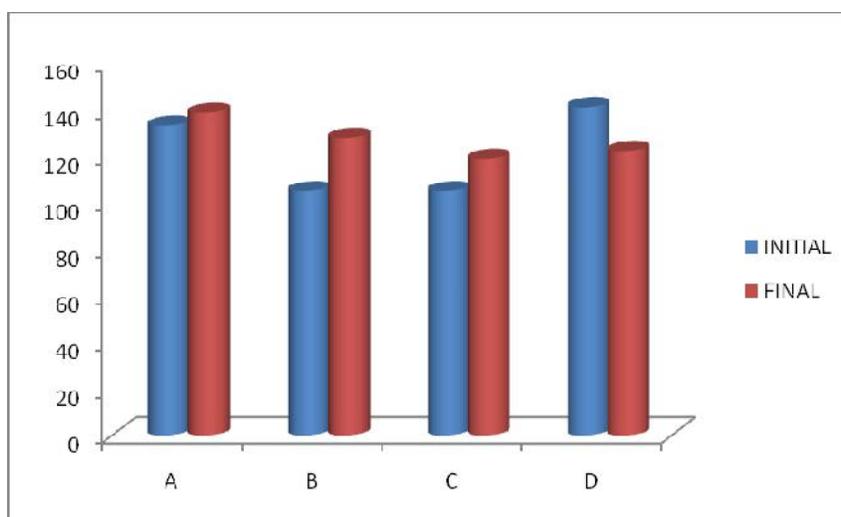
Groups	CATA	SOD	MDA	GSH
A	1.83±0.53	0.56±0.12	0.39±0.09	1.46±0.09
B	0.43±0.08**	0.57±0.05	0.34±0.05	0.29±0.03**
C	0.53±0.11**	0.38±0.10	0.53±0.07	0.36±0.02**
D	1.15±0.14	0.76±0.07	0.34±0.09	0.42±0.02**

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with Group A. \*p<0.05, \*\*p<0.01 vs Group B. P: Statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test

**Table 3. Effects of extract on kidney biomarkers**

Groups	CATA	SOD	MDA	GSH
A	1.32±0.11	0.31±0.05	0.70±0.05	1.11±0.06
B	0.64±0.09**	0.37±0.07	0.57±0.08	0.39±0.01**
C	0.51±0.15**	0.41±0.16	0.56±0.16	0.45±0.03**
D	0.46±0.08**	0.34±0.06	0.75±0.04	0.41±0.03**

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with Group A. \*p<0.05, \*\*p<0.01 vs Group B. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test



**Fig. 2. Effects of the extracts on the mean body weight of rats**

There were no significant ( $P>0.05$ ) different in the body weight of the rats in all the groups as compared to group A and B (Fig. 2)

**Table 4. Effect of the nutmeg extracts on haematological parameter**

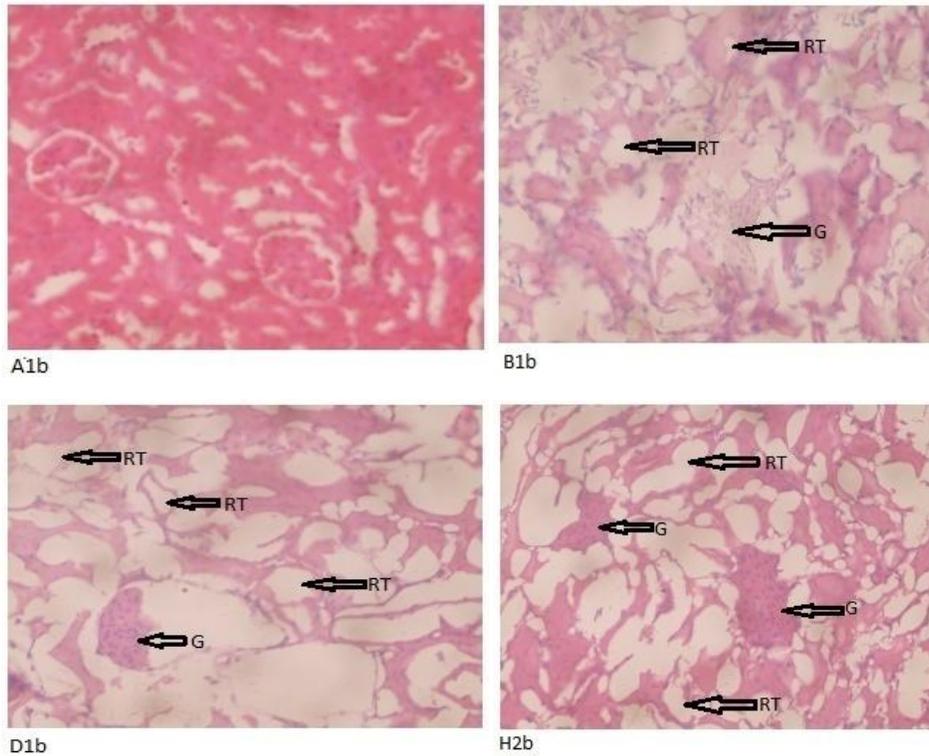
<b>Groups</b>	<b>PVC (%)</b>	<b>HB (g/dl)</b>	<b>RBC (<math>\times 10^{12/l}</math>)</b>	<b>WBC</b>	<b>PLT</b>	<b>N (%)</b>	<b>L (%)</b>	<b>E (%)</b>	<b>M (%)</b>
A	41.20±0.74	13.68±0.25	6.04±0.14	8.62±0.55	248.60±11.81	33.00±11.81	66.00±1.87	3.00±0.32	8.00±0.55
B	33.40±1.03	11.12±0.33	4.87±0.20	7.52±0.35	235.00±8.64	21.80±1.36	66.00±1.38	4.20±0.37	8.00±0.55
C	35.75±2.84	11.87±0.93	5.33±0.49	7.33±1.57	221.00±16.11	26.00±1.68	62.50±1.04	3.75±0.48	7.75±1.32
D	29.20±1.24**	9.68±0.43**	4.13±0.11**	10.76±0.73	217.00±6.40	30.20±1.56	58.00±2.55	3.60±0.51	8.20±1.36

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with Group A. \* $p < 0.05$ , \*\* $p < 0.01$  vs Group B. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test

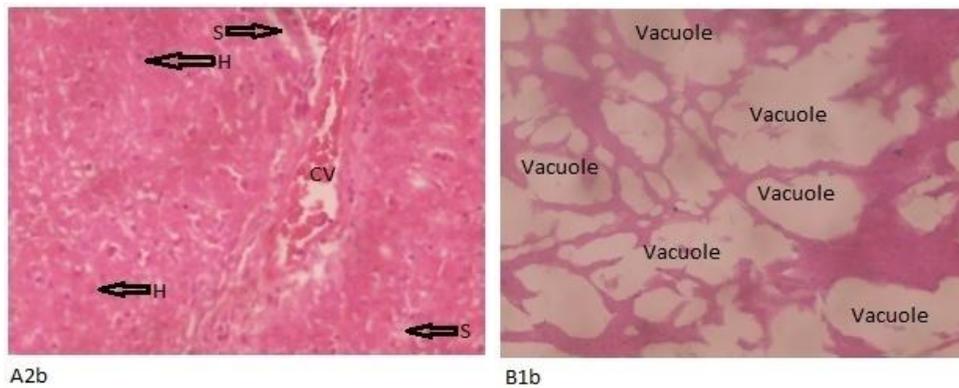
**Table 5. Effects on initial and final effects on the mean body weight of rats**

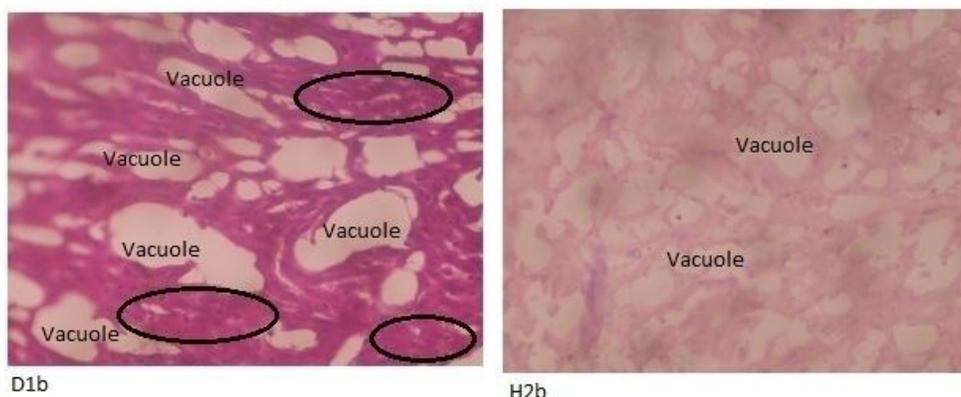
Groups	Body weight		
	Initial body weight	Final body weight	Percentage change in body weight (%)
A	133.60±6.61	139.00±9.34	4.0
B	105.00±4.28	128.00±4.62	2.2
C	105.00±4.38	119.00±9.79	13.3
D	141.40±15.50	122.40±6.88	13.4

Values are given as mean ± SEM for 5 rats in each group; experimental groups are compared with Group A. No significant difference compared to Group A at  $p < 0.05$ . P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test



**Plate 1-4. Photomicrograph (x400) showing histological slides of the Kidneys**  
 A1b- Normal control, B1b- Acetaminophen, D1b- nutmeg+Apap, H2b- Apap+nutmeg





**Plate 5-8. Histological results Plate 5-8 (Aib-H2b) showing the liver of rats treated with APAP and nutmeg powdered supplements**

Liver histology of the group that received Nutmeg+APAP and (APAP+ Nutmeg) treated rats group as illustrated in Plate 7 and Plate 8 showed histological distorted tissues, generalized vacuolations with patchy areas of normal hepatocytes encircled.

### 3.3 Discussion

Subsequent upon acetaminophen intake, greater percentage of the drug is metabolized by sulphation and glucuronidation to unreactive metabolites [17,20,21,22,23]. Overdosed of Acetaminophen, cause large amount of APAP to generate cytochrome P450 enzymes in rats leading in the formation of reactive metabolite NAPQI, thereby resulting to the saturation of the hepatic glucuronide and sulphate then increase the P450 sulphate conjugation pathway triggering the oxidation pathway [24,25], resulting to subsequent depletion in detoxification ability of the liver intracellular GSH and mitochondrial proteins, thereby inducing mitochondrial oxidant stress and dysfunction; this results in nuclear DNA fragmentation and necrotic cell death [26,27,28,29]. Therefore, increased level of free NAPQI mediates oxidative damage and thus enhances cellular injuries and organ dysfunction [30].

Intracellular glutathione is protective in nature and once it is depleted, hepatic and renal damage may occur [31].

In the present study, the ability of nutmeg supplement drug to protect or cure drug- induced hepto-renal toxicity was assessed. Double dosages of acetaminophen were used to induce liver and kidney damage.

In our work, acetaminophen administration resulted in rise of ALP, AST and ALT, TB and TB levels [32].

In this work, high level of AST and ALT was shown which indicated that AST and ALT were released into circulation indicating liver damage. Acetaminophen overdose or abuse results in permanent obliteration of liver cells in turn resulting in alarming and absolute high elevation in serum level of enzymes ALT, ALP, and AST [33]. This is in agreement with [18,34,35,36] who reported that paracetamol intoxication produced a significant increase in AST, ALT ALP. In our work, APAP administration (1000 mg/kg) caused acute liver injury in rats, characterized by an increase in serum activity of transaminase and phosphatase (AST, ALT and ALP). When there is damage of cell, cytoplasmic transaminase will be released thereby leading to the damage of the liver structure cohesion, because these are normally located on the cytoplasm, mitochondrial and microsome released into the circulation after cellular damage [37] or due to the alteration to the cell membrane permeability, increase anabolism and reduction in breaking down of aminotransferase. This is also in agreement with [38,39] who found out that serum levels of both ALT and AST were elevated almost four folds in acetaminophen treated group in relation to control. Decrease in serum, plasma level of total protein, albumin is also the evidence of chronic liver damage. Similar to our work, [26] reported that acute acetaminophen toxicity induced remarkable elevation on plasma ALT, AST and ALP action and significantly decrease in plasma level of total protein and albumin of rats. It is also in agreement with [40] who revealed that acetaminophen induced toxic injury of the liver of

rats as seen by significant decrease in albumin level. This indicated the decrease in capacity of hepatic to synthesize protein and consequently liver weight.

Significant reduction in the level of AST, ALP, ALT, TP and ALB I both use indicated present of ameliorative properties indicating that Nutmeg reduced the damage induced by APAP and hence avoids leakage of marker enzymes. This is in consistent with [37] who reported administration of nutmeg to the acetaminophen induced toxicity was able to reduced the levels of elevated liver enzymes, ALP, ALT and AST indicating antioxidants exposing hepatoprotective effects of nutmeg seen on its ability to mitigating the toxic effect on the liver. This could be due to the scavenging property of nutmeg extract showing the present of antioxidant such as alkaloids, flavonoid and myristicin [41].

Administration of APAP to the rats in our result reviewed that acetaminophen caused elevation in serum blood urea nitrogen and serum creatinine level indicating kidney damage. Our findings were also in line with an earlier reported work of [42] that sera levels of creatinine and urea in kidney diseases will rise because the rate of production exceeds the rate of clearance due to the defect in kidney function and these elevated state of urea and creatinine had been considered as index of assessing nephrotoxicity, supporting to this, the disturbance of the renal functions was further reflected on its faulty re-absorptive power of albumin and protein in plasma levels, which led to their facade in high quantities in the urine and thus decreased in sera levels [35]. In addition, attenuation of total protein and albumin associated with improved serum urea and creatinine was cons signifying a further indication of kidney glomerular damage after APAP injection [43]. Thus, significant rise in creatinine level in the present study established renal damage in rats following APAP over dosed. Although, it is clear from earlier medical data that elevations in the urea and creatinine plasma levels have been demonstrated to be reflects succession of renal disease in a range of animal models and man [44].

Yoon et al. [45] reported improvement ALT, ALP and AST enzymes, insignificant different in creatinine, urea (improvement in kidney function), lipid peroxidation, reduce the formation of cholesterol preventing tissue damage.

The concentration of the electrolyte on the serum when acetaminophen is administered only had

no significant related effect as compared to Control A. This is in disagreement with [17] which reported significant rise in blood electrolytes on administration of acetaminophen. This may be due to differences drug duration. Antioxidant is any substance that delays, prevents, removes or inhibits oxidation of substrate when present at low concentrations compared with those of an oxidisable substrate [46]. It protects tissue against oxidative stress which accounted for its hepato-reno-protective activity.

Administration of doses of APAP produced remarkably depletion in the hepatic glutathione, significant effects on the antioxidant enzyme, superoxide dismutase with significant reduction in catalase. Decreases in the antioxidant indicated increased generation of ROS a reactive oxygen species (ROS) thereby creating an oxidative stress in the liver MDA production which means enhancement of lipid peroxidation leading to tissue damage and failure of the antioxidant defense mechanism which resulted to, an excess modulation of free radical and depletion of GSH and SOD resulting to liver necrosis. This is in contrarily with [5,47] who reported elevated MDA in Acetaminophen is as a result of GSH depletion leading to oxidative stress and lipid peroxidation. This can also be as a result of duration of the dose which caused alteration of the organ.

Rats pretreated with APAP + nutmeg extract showed significant reduction in hepatic CATA, hepatic GSH but no significant effect on hepatic SOD and hepatic MDA compared to A but no significant elevation in CATA and GSH when compared to group B. In curative (APAP + Nutmeg) use, however produced high rise in hepatic CATA and non significant increase in hepatic GSH compared to APAP group. Hepatic CATA is closer to the normal control indicating free radical scavenging property The result is in agreement with what was previously reported by [37] that nutmeg was found to possessed myristicin which is an antioxidant and hepatoprotective compound suppressing lipid peroxidation in the liver by trapping free radical ROS directly.

Treatment of rats with nutmeg showed reduction in renal CATA and renal GSH in both use compared to A. whereas there was no significant changes in both uses compared to B, thus indicating lack of improvement to kidney damage. This is in disagreement with [14] which reported improvement on renal antioxidant. The

discrepancy in result may be due to difference on duration of the drug dosage administration.

Haematological parameters evaluation is usually done as to ascertain the adverse effects of any foreign molecule in the blood of the rats under investigation [48].

There was insignificant statistical effect on haematological parameter in APAP treated rats serum compared to control A. This result disagreed with [42] who reported significant decrease in RBC, Hb, PCV in APAP treated rats compared to control. This may be due to different dosages. Differently from our result, [49] also reported decrease in PCV and RBC counts relative to control. The report also recorded decrease in WBC causing deleterious effect on the blood chemistry. The discrepancy from our result could be as a result of different in doses, route and duration of drug administration.

There was no haematological related treatment effect on APAP + Nutmeg group in prophylactic use but in curative use there was significant reduction in serum Levels of HB and RBC and significant rise in PCV compared to control A this may be due to imbalance in antioxidant activities in the blood cells.

The hepatic tissues of the experimental animals are illustrated histologically in the photomicrographs presented in Plates 5-8 (ai-di). Plates 1-4 illustrated histologically normal liver showing: Hepatocytes (H) that are histologically good. There was present of central vein (CV) containing blood cells, hepatic sinusoids; that are histologically normal. In the APAP treated group, the liver tissue as illustrated in Plates 5-8 showed distorted and destroyed liver with vacuolation. This is in consistent with [18] who reported ballooning and degeneration as well as sinusoidal congestion of the liver 24 hours after APAP administration. This also agreed with [35] who reported that in paracetamol treated group, there was distortion of liver architecture; there were also vacuolation of hepatocytes, infiltration with inflammatory cells.

Hepa-architecture of the group administered with nutmeg after APAP (Nutmeg+APAP) intoxication in prophylactic as shown in Plates 1-4 showed patchy area of normal hepatocytes unlike in curative use (APAP+Nutmeg) which produced generalized vacuolations and microvesicular steatosis. In agreement with [37] which reported much healthier tissues than APAP

group with apoptotic hepatic and slight vacuolations of hepatocytes after acetaminophen and nutmeg administration. This is also in line with [48] who reported to countable alteration of the liver after nutmeg administration, thus indicating the hepato-protective and curative effects of nutmeg.

Acetaminophen overdose generated reactive oxygen species which triggered oxidative stress which is scavenge by antioxidant found in the drugs used as hepato-renal protective and hepato-curative agents. The above results and discussion suggested that liver damage was caused by oral administrating of 1000 mg/kg double doses of acetaminophen in wistar rats and oral administrations of medicinal drugs supplements resulted in ameliorating the damage caused by APAP administration for seven days.

The photomicrographs of the renal tissues of the experimental animals when illustrated histologically in Plates 5-8 (A1b- H2b) The kidney of the control groups shows glomerular surrounded with bowman capsules and renal tubules of control groups as illustrated by A1b-A2b (Plate 1).

Hence, in kidney sections APAP as illustrated in Plate 2 caused severe changes in renal cells, distorted renal tubules and glomerular. This is similar to [5] who reported toxic effects of APAP on the kidney. This report also agreed with [42] who reported that kidney sections of APAP treated group caused severe alterations in renal cells. [17] also reported disorganized glomerulus, dilated and inflammatory tubules.

Histopathology of the kidney of rats treated with nutmeg (Nutmeg+APAP) as illustrated in Plate 3 reviewed preserved glomeruli tuft in both uses. Curatively, (APAP+nutmeg) produced distorted renal tubules with distorted glomeruli and preserved tuft. Distorted renal and glomerular is in consistent with Eweka and Eweka [42] who reported that administration of nutmeg (500 mg/kg) produced deleterious effects on the kidney of adult wistar rats at higher dose. This may be due to dose dependant. The preserve tuft may be added advantage since it means the glomeruli are not shrunken.

#### **4. CONCLUSION**

It is concluded that Nutmeg possesses hepatorenal-prote-curative property which improved the damage caused by

Acetaminophen-induced hepato-renal-toxicity thus it's used in traditional medicine. There is therefore need for further research as to validate the reliability of the findings.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Shana K. The mammalian kidney: How nephrons perform osmoregulation. Biology Gatech. Ed.; 2016.
2. Ozougwu JC. Physiology of the liver. International Journal of Research in Pharmaceutical Sciences and Biosciences. 2017;4(8):13-24.
3. Venkatesan SP, Deecaraman M, Vijayalakshmi M, Sakthivelan SM. Sub-acute toxicity studies of acetaminophen in Sprague rats. Biology and Pharmaceutical Bulletin. 2014;37(7):1184-1190.
4. Lee WW. Acetaminophen (APAP) hepatotoxicity isn't it time to go away? Journal of Hepatology; 2017. (Pubmed PMID 28734939).
5. Mahmoud MF, Diaai AA, Ahmed F. Evaluation of the efficacy of ginger, Arabic gum and Boswellia in acute and chronic renal failure. Renal Failure. 2012;34:73-82. DOI:http://doi.org/10.3109/0886022x.2011.623563
6. Michael MC. Essential biology for senior secondary schools. Eds. Lagos Nigeria; Tonad Publishers Ltd. 2018;262-265,294-300.
7. Mazhar F, Akram S. Acute renal damage in acetaminophen poisoning. Journal of Young Pharmacology. 2016;8(4):505-506.
8. Ozougwu JC, Eyo J. Hepatoprotective effects of *Allium cepa* (onions) against paracetamol induced liver damage in rats. African Journal of Biotechnology. 2014;13(26):2679-2688.
9. Dkhil M, Abdel-Moneim AE, Hafiz T, Mubarak M, Walid F, Al-Quraishy S, et al. *Myristica fragrans* kernels prevent paracetamol-induced hepatotoxicity by inducing anti-apoptotic genes and Nrf2/Ho-1 pathway. International Journal of Molecular Sciences. 2019;20(4):993.
10. Dulger G. Herbal drugs and drug interactions. Marmara Pharmaceutical Journal. 2012;16:9-22.
11. Hauwa SB, Baba G, Yusuf H. Haematological and histological changes induced on the selected organs of (wistar) rats by leaf extracts from herbs of ethno medical applications. Nigerian Journal of Chemical Research. 2013;18:48-57.
12. Moncel B. What is nutmeg? www.the spruceeats.com; 2017.
13. Aboelhassan DM, Hafiz NA, Darwish HR, Shabana ME, Eshak MG, Hassanane MM, et al. Enhancing effects of *Moringa oleifera* leaf extract on carcinogenic Aflatoxin B1-induced genetic alterations, haematotoxicity and histological changes in liver and kidney of rats. J ISISN. 2013;15(2):814-833.
14. Wang X, Wu Q, Liu A, Anadon A, Rodriguez JC, Martinez-Larranaga ML, et al. Paracetamol: Overdosed induced oxidative stress toxicity, metabolism and protective effect of various compounds *in vivo* and *in vitro*. Drug Metabolism Reviews; 2017. (Pubmed PMID 28766385).
15. Hartanto S, Ko HS, Jee HS, Kang UJ, Seb ST, Ohh SJ. Effect of dietary nutmeg on heat stress tolerance-related parameters in Korean. J. Anim Physiol a Nutr. 103(4). DOI: 10.1111/JPN.13113
16. Karthivashan G, Kura AU, Arulselvan P, Norhaszalina MI, Fakurazi S. The modulatory effect of *Moringa oleifera* leaf extract on endogenous antioxidant system and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. Peer Journal Do. 2016;10:7717, peerj. 2127.
17. Mathew C, Nair C, Shenoy T, Varghese J. Preventive and curative effects of *Acalypha indica* on acetaminophen induced hepatotoxicity. International Journal of Green Pharmacy. 2011;49-54.
18. Abdel-Azim SA, Abdel-Rahem MT, Said MM, Khattab A. Protective effect of *Moringa peregrina* leaves extract on acetaminophen-induced liver toxicity in albino rats. AJICAM. 2017;14(2):206-216.
19. Toussaint K, Yang XC, Zielinski MA, Reigle KI, Sacavage SD. What do we (not)

- know about how paracetamol (acetaminophen) works? Journal of Clinical Pharmacy and Therapeutics. 2011;35:617-638.
20. Singh SM, Kushwah SR, Kumar. Acetaminophen (Paracetamol) drug overdosed, toxic effect on human health. International Journal of Recent Scientific Research; 2017
  21. El Badwi SM, Al Tayib OA. Assessment of ameliorative effects of aqueous extracts of *Moringa oleifera* on acetaminophen-induced nephrotoxicity in rats. JOSR (LOSR-JHSS). 2016;21(9):1-7.
  22. Abdel-Azeem AS, Hegazu AM, Abraham K, Mahmud A, El-Sayed H. Hepatoprotective, antioxidant and ameliorative effects of ginger (*Zinger officinale* Roscoe) and vitamin E in acetaminophen treated rats. J. Die Suppl. 2013;10(3):195-209.
  23. Hamilton S, Terentyer D. Altered intracellular calcium homeostasis and arrhythmogenesis in the aged heart. International Journal of Molecular Sciences. 2019;20(2386):1-22.
  24. Reddy TS, Shama KP, Nirmala P, Shastry CS. Biochemical studies on hepato and nephroprotective effect of Butterfly Tree (*Bauhinia purpurea* Linn) against acetaminophen – induced toxicity. International Journal of Research in Ayurveda and Pharmacy. 2012;3(3):455-460.
  25. Reddy UT, Lakshmi M, Kumar S, Kumar D. Protective effect of *Abutilon indicum* L. (Malvaceae) against acetaminophen induced nephrotoxicity in rats. Journal of Life Science. 2013;1:40-43.
  26. Swaroopa M. Effect of ginger extract consumption on renal function during ethanol withdrawal induced-stress. IJIR, Engineering and Technology. 2013;6412-6418.
  27. Mammucari CA, Raffelo A, Reane DV, Gherardi G, Rhizzuto AR. Mitochondrial calcium. European Journal of Physiology. 2018;470(8):1165-1179.
  28. Al-Qudah MMA, Haddad MA, EL-Qudah JMF. The effects of aqueous ginger extract on pancreas histology and on blood glucose in normal and alloxan monohydrate-induced diabetic rats. Bio Res. 2016;27:350-356.  
Available: [https://www.researchgate.net/publication/301649907\\_The\\_effects\\_of\\_aqueous](https://www.researchgate.net/publication/301649907_The_effects_of_aqueous)
  29. Al-Qudah MMA, Al-Ramanneth ED, Haddad MA, Al-Abadi A. The effects of aqueous ginger extract on kidney and lungs of diabetic rats. IJB. 2018;10(4):23-28.
  30. Subramaya S, Venkataraman B, Meeran MFN, Goyal SN, Patil CR, Shreesh O. Therapeutic potential of plants and plant derived phytochemicals against acetaminophen induced liver injury. International Journal of Molecular Sciences. 2019;19:3776-3918.
  31. Masoud RE. Hepatoprotective effect of curcumin versus silymarin on paracetamol induced hepatotoxicity in rats. Intern Jour of Pharmacol and Biol Sciences. 2017;8(2):134-141.
  32. Mohammad SI, Mustafa IA, Abdulqader SZ. Ameliorative effects of aqueous extract of *Zingiber officinale* on the cadmium-induced liver and kidney injury in female rats. Jordan Journal of Biological Sciences. 2013;6(3):231-234.
  33. Pathan MM, Khan MA, Moregaonkar SD, Somkuwar AP, Gaikwad NZ. Amelioration of paracetamol-induced nephrotoxicity by *Maytenus emarginata* in male wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(4):471-474.
  34. Paliwal R, Sharma V, Precheta S, Yadav S, Sharma S. Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA induced renal carcinogenesis in swiss albino mice. Biology and Medicine. 2011;3(2):27-35.
  35. Sharifudin S, Fakurazi M, Hidayat M, Hairuszah M, Moklas P, Arulselvan. Therapeutic potential of *Moringa oleifera* extracts against acetaminophen-induced hepatotoxicity in rats. Pharmaceutical Biology. 2013;51:279-88.
  36. Hassanem NHM. Protective effect of nutmeg and Rosemary on oxidative stress in hypercholesterolemia rats. International Journal of Nutrition and Food Sciences. 2015;4(4):465-476.
  37. Salehi B, Martorelli M, Albiser JC, Sureda A, Martins, Maurya PKM, Sharifirad M, et al. Antioxidant: Positive or negative actors? Biomolecules. 2018;8:124.
  38. Ali MD, Naseer MA, Mirza MA, Alam MS. Haematological, biochemical and histopathological study of the plant extract of *Terminalia chebula* Rentz, *Aloe vera* Linn and *Tamarindus indica* Linn on animal model. A comparative study. National

- Journal of Physi, Pharm and Pharmacol. 2019;9(3):268-274.
39. Oyedeji KO, Bolrinwa AF, Ojeniran SS. Effects of Paracetamol (APAP) on haematological and reproductive parameters in male albino rats. International Journal of Pharmaceutical Sciences Review and Research. 2013;20(2):296-300.
40. Bachri MS, Yullaris S, Sari AK. Effects of subchronic administration of nutmeg (*Myristica fragrans* Houtt) ethanolic extract to haematological parameters in rats. IOP Publishing Ltd. 2017;289.
41. Slaoui M, Fiette L. Histopathology procedures: From tissue sampling to histopathological evaluation. Methods in Molecular Biology (Clifton N J). 2011;691:69-82.
42. Eweka AO, Eweka A. Histological effects of oral administrations of nutmeg on the kidneys of adult wistar rats. North American Journal of Medical Sciences. 2010;2(4): 189-192.
43. Mustapha RO. The easier approach to pharmacology for all health professionals. Akins Media Prints 3<sup>rd</sup> eds., Kwara; 2012.
44. Farrell SE and Miller MA. How are renal function test used in the work up of acetaminophen toxicity/poisoning; 2020. Available:www.medscape.com
45. Yoon E, Babar A, Choudhary M, Kutnev M, Pysopoulos N. Acetaminophen induced hepatotoxicity. A comprehensive update. J, Clen, Transl Heptatol. 2016; 4:131-142.
46. Ugwuona FU, Onweluzo JC, Ojmelukwe PC. African nutmeg (*Monodora myristica*) Posseses Antioxidant Properties and can preserve African bread-fruit (*Treculia Africana*) seed and flour. ACTA Sci Nutr Health. 2019;3(6):41-50.
47. Yadav A, Kumari R, Yadav A, Mishra JP. Antioxidants and its functions in human body. A review; 2016. Available:www.researchgate.net
48. Kadiri HE and Okoro IO. The Antioxidant effect of aqueous extract of *Monodora myristica* on cyanide induced Hepatic and Renal Toxicity in Rats. Nig. J. Pure an Appl Sci. 2018;31(2):8.
49. Ahmed S, Abdelhaud F, Abdalla O. Effects of rosemary oil against hemato-biochemical alterations and renal oxidative damage induced by paracetamol in rats. Annals of Veterinary and Animal Science (AVAS). 2019;6(5).

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