Assessment of Ameliorative Role of *Citrullus vulgaris* (Schrad) Seeds on Caffeine-induced Hepatic and Renal dysfunction in Male Wistar Rats

Abstract

Caffeine is the most widely consumed psychoactive drug in the world. It isoften marketed for its physical and cognitive performance benefits. Unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world. The ingestionof potentially toxic amounts of caffeine in the forms of energy drinks,over-the-counter supplements, addiction or use of anhydrous caffeine products places individuals at risk for accidental overdose. An overdose of this drug is not without its attendant consequences on the liver and the Kidney. This study therefore aimed at evaluating the possible hepatoprotective and nephroprotective effects of *Citrullus vulgaris* seed extracts on caffeine induced toxicity. Thirty (30) male Wistar rats were divided into five groups. They were induced with caffeine (100 mg/kg)and treated with graded doses (100, 300 and 500 mg/ kg bwt.) of aqueous seeds extract of *C. vulgaris* for 21 days. The plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined and the concentrations of total protein, albumin, total bilirubin, creatinine and urea were estimated. Electrolytes (sodium, calcium, potassium and magnesium ion)were also estimated. Plasma lipid profiling (total cholesterol, triacylglyceride, high density lipoprotein (HDL), Low density lipoprotein (LDL) was carried out and the liver and kidney of the rats were examined for histopathological changes. The results showed that administration of aqueous seeds extract of *Citrullus vulgaris* restored the levels of the plasma enzymes, blood proteins, urea and creatinineas well as the levels of electrolytes close to normal control levels which were significantly altered by caffeine intoxication, Photomicrographs sections of the liver and kidney showed that the aqueous seeds extract of *Citrullus vulgaris* was able to repair damage to organs caused by caffeine-intoxication. The study concluded that the aqueous seeds extract of *C. vulgaris* possesses ameliorative potential against hepatic and renal damage that arises from caffeine-intoxication.

Keywords: *Citrullus vulgaris*, caffeine,hepatoprotective, nephroprotective, ameliorative potential

1. INTRODUCTION

"The liver is a major organ in the human body and is a frequent target for a number of toxicants" [1]. "As the metabolic center of the body, the liverperforms important functions such as bile secretion, bilirubin metabolism, nutritional metabolism, regulation of essential hormones, producing immune agents to control infections, metabolism of foreign molecules (exogenous chemical and endogenous chemical)and is principally responsible for the detoxification of compounds that have the potential to induce oxidative stress. This crucial role makes the liver vulnerable to injury. Liver diseases are problems worldwide, and the conventional drugs used in the treatment of liver diseases are sometimes inadequate and with serious adverse

effects. Thus, interest and effort have shifted toward medicinal plants as new sources of hepatoprotective agents" [2].

"The kidney is a vital organ of the body that carries out important roles such as excretion of waste products, electrolytes balance in the body, control blood pressure, stimulate the production of red blood cells, regulate the balance of water and metabolites in the body and maintain acid-base balance in the blood. Most drugs are excreted by the kidneys, making them susceptible to damages by the use of drugs that exceed the therapeutic dosage" [3].

"Caffeine (chemically known as 1,3,7- trimethylxanthine) is a white crystalline xanthine alkaloid, which is bitter in taste" [4, 5]."It is one of the bioactive compounds that can be self-administered in its natural or synthetic form"[6, 7]."Caffeine is naturally found in tea leaves, cocoa, beans,nuts such as kola nut and bitter kola, and fruits of more than sixty plants"[5, 6, 8]."Caffeine extract is used as additives in the production of soft drinks, coffee, and tea and in some processed foods"[9,10]."Synthetic and plant-based sources of caffeine such as Caffeine-containing supplementsmarketed as diet aids, performance enhancers or energy boosters, may causetoxicity, particularly with concomitant energy drink use"[11]."Given its known stimulant effects, it is commonly used to enhance mental or physical performance, in pursuit of performance benefits such as improved physical endurance, weight loss, cognitive alertness, and reduction of perceived fatigue"[12]. "Whilegenerally safe when consumed in moderation, caffeine may be tempting to be consumed in excess, which increases its risk of significant toxicity". [5]

*"Citrullus vulgaris*seeds which are neglected food parts have been given more attention recently in order to recover valuable compounds and recycle into the food chain in an economic and sustainable way. The juice or pulp from watermelon is considered as the edible portion but rind and seeds are discarded as major solid wastes"[13]. "The fruit has numerous small black seeds embedded in the middle of the flesh. The seeds have sweet and nutritious kernels. Several studies have shown that seeds of *Cucurbitaceae* species are potential sources of nutrients such as protein, minerals and lipids as well as ingredients for native medicine"[14].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1.Plant Material

Fresh fruit of *Citrullus vulgaris* was purchased from Sabo market in Ile-Ife, Osun state, Nigeria. The fruit of *Citrullus vulgaris* (watermelon) was cut opened, after which the seeds were removed, washed and shade dried.

The dried seeds were blended to powder form using Kanchan 750 watts high performance blender and kept in air tight container for further use.

2.1.2. Experimental Animals

Thirty (30) male Wistar rats weighing between (120-150g) were procured from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept in plastic cages in aventilated room under controlled laboratory conditions ofnormal light–dark cycle (12 h light/dark) and temperature(25 + 2_C). The animals had access to food and water *adlibitum.*The animals were allowed to acclimatize to standard laboratory condition for a period of two weeks prior to commencement of administration.All animals in each group were weighed before and after the experiment and the percentage weight gain calculated.

2.2 Methods

2.2.1. Preparation of Aqueous Seed Extract of *Citrullus vulgaris*

Powdered seed (150g) of *C. vulgaris*was soaked in 1.5 litres of distilled water (1:10 w/v) in a refrigerator for 48 h. After 48 h, the filtrate was collected using a Whatman filter paper (No. 1),and the residue was re-extracted with distilled water until the filtrate became colourless.The filtrates were combined and evaporated to dryness under reduced pressure using a rotary evaporator. The extract was scrapped, weighed and stored at room temperature in an air tight container for further use.

2.2.2. Experimental Design

The rats were randomized into five groups of six animals each. Group 1 animals received standard feed and drinking water *ad libitum* and served as the normal control group.Group 2 animals received caffeine solution (100 mg/kg bwt) and drinking water *ad libitum*.Group 3 animals received aqueous seeds extract of *C. vulgaris* (100 mg/kg bwt) after administration of caffeine solution (100 mg/kg bwt). Group 4animals received aqueous seeds extract of *C. vulgaris* (300 mg/kg bwt) after administration of caffeine solution (100 mg/kg bwt). While Group 5 animals received aqueous seeds extract of *C. vulgaris* (500 mg/kg bwt) after administration of caffeine solution (100 mg/kg bwt). This treatment lasted for 3 weeks after which the animals were fasted overnight and euthanized by cervical dislocation.Blood was collected by cardiac puncture into well-labelled heparinized sample tubes. Plasma was obtained by centrifuging the blood at 4,000 rpm for 15 min and stored for biochemical investigations. The liver and kidney of each animal in the group were excised, decapsulated and perfused in 10% buffered formalin for histological analysis.

2.2.3. Biochemical Analysis

2.2.3.1. Determination of Alanine Aminotransferase Activity (ALT).

The activities of alanine aminotransferase (ALT) in plasma was assayed according to themethod of [15], as outlined in theRandox assay diagnostic test kit. ALT catalyzes the transfer ofan amino group from alanine to a-oxoglutarate. The reactiongives rise to glutamate and pyruvate. The activity of ALT wasthen measured by monitoring the concentration of pyruvatehydrazone formed with 2, 4-Dinitrophenylhydrazine at546 nm. The activity of ALT was determined from the standard ALT activity table provided.

2.2.3.2. Determination of Aspartate Aminotransferase Activity(AST).

The activity of aspartate aminotransferase (AST) in plasma was assayed by the method of[15]. AST catalyzes the transfer of anamino group from aspartate to a-oxoglutarate. The reactiongives rise to glutamate and oxaloacetate. Hence, the activityof AST was measured by monitoring the concentration ofoxaloacetate hydrazone formed with 2, 4-Dinitrophenylhydrazine at 546 nm. The activity of AST wasalso determined from the standard activity table provided.

2.2.3.3. Determination of Alkaline PhosphataseActivity(ALP)

"Alkaline phosphatase (ALP) activity was assayed using themethod" of [16]. "Theenzyme ALP, hydrolyzes a colorless substrate of p-nitrophenylphosphate giving rise to phosphate and phenol a pink colored complex in alkalineenvironment, which can be monitored using a spectrophotometer at 405 nm". [16] The activity of ALP in the sample was calculated using the expression:

ALP activity (U/L) = 2760 x ΔAbsorbance at 405 nm

2.2.3.4. Estimation of Urea

Urea was analysed by urease-Berthlot method [17]based on the principle that in the presence of urease, urea in the serum is hydrolysed to ammonia which is trapped by Berthelot's reaction using analytical kits (Randox Laboratories), and measured spectrophotometrically .

2.2.3.5. Estimation of Creatinine

Creatinine level was estimated using Jaffe's-alkaline picrate method as described by [18]using assay kits. The analysis is based on the principle that creatinine in alkaline solution reacts with picric acid to form a coloured complex whose intensity is directly proportional to the creatinine concentration.

2.2.3.6. Estimation of Total Protein

The total protein concentration was estimated by quantitative assay method of small amount of protein in biological membrane as described by [19]. The analysis is based on the principle that protein reacts with Folin-

Ciocalteu reagent to give a purple/violet coloured complex whose intensity is proportional to the protein concentration.

2.2.3.7. Estimation of Albumin

Albumin concentration was assayed by modified procedure of Pinnell and Northan [20]. The analysis is based on the principle that Albumin reacts specifically with 3,3',5,5'-tetrabromo-m-cresolsulphonephthalein (bromocresol green, BCG) to form a stable blue-green colour (albumin-BCG complex) whose intensity is directly proportional to the albumin concentration in the plasma.

2.2.3.8. Estimation of Bilirubin

Total bilirubin was estimated as described by [21]. This determinationwas carried out based on the principle that bilirubin reacts with diazotised sulphanilic acid to form a blue coloured complex that is measured spectrophotometrically at 560nm

2.2.3.9. Determination of lipid Profile

Plasma lipids including the total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels were estimated using Randox diagnostic kits.

2.2.3.10. Determination of Electrolyte levels

Electrolytes levels (sodium, potassium, magnesium and calcium) were determined according to the instructions on their diagnostic kits purchased from TECO diagnostics, United State of America.

2.2.4. Histopathological Analysis

The liver and kidney of the rats were fixed in 10% formalin and were processed for paraffin sectioning. Sections of 5-6 μm thickness were stained with eosin and hematoxylin for photomicroscopic observation under a LEICA DM750 microscope.

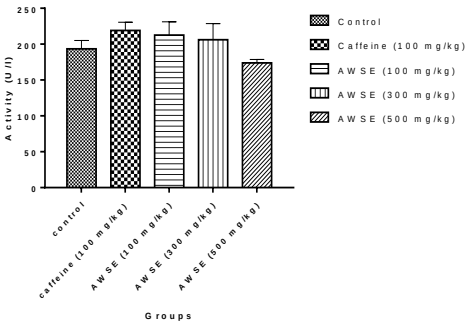
2.2.5. Statistical Analysis

Data were expressed as mean ± SEM. Data of the different groups were compared using one-way analysis of variance (ANOVA) using Graph Pad Prism. Post-hoc test was carried out with a value of $P<0.05$ considered to be statistically significant.

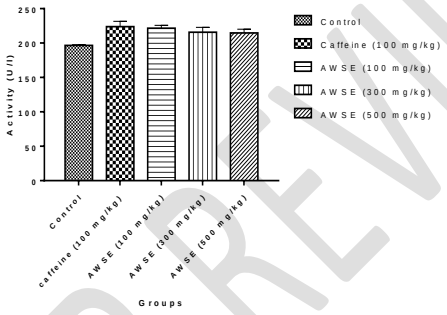
3. RESULTS

3.1. Effect of Aqueous Seeds Extract of *C. vulgaris* on the activities of AST, ALT and ALP in Caffeine-intoxicated Male Wistar rats

Rats administered caffeine showed significant increase in plasma ALT compared to the control C and the *C. vulgaris* treated groups ($p<0.05$). We found a significant increase in the AST levels in the caffeine group relatively to the Control and *C. vulgaris* treated groups ($p<0.001$). Moreover, there was a significant increase in ALP levels in the caffeine treated group when compared to the C group ($P<0.01$). (Figure 1) Administration of caffeine with *C. vulgaris* seed extract significantly decreased plasma AST and ALT levels compared to the caffeine group ($p<0.001$, $p<0.05$ respectively).



A



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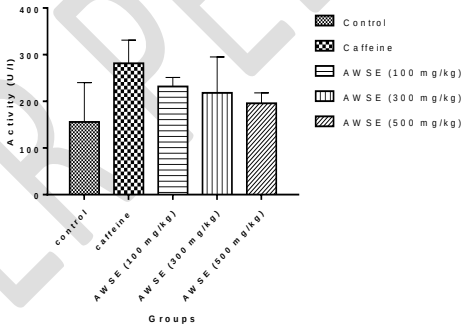


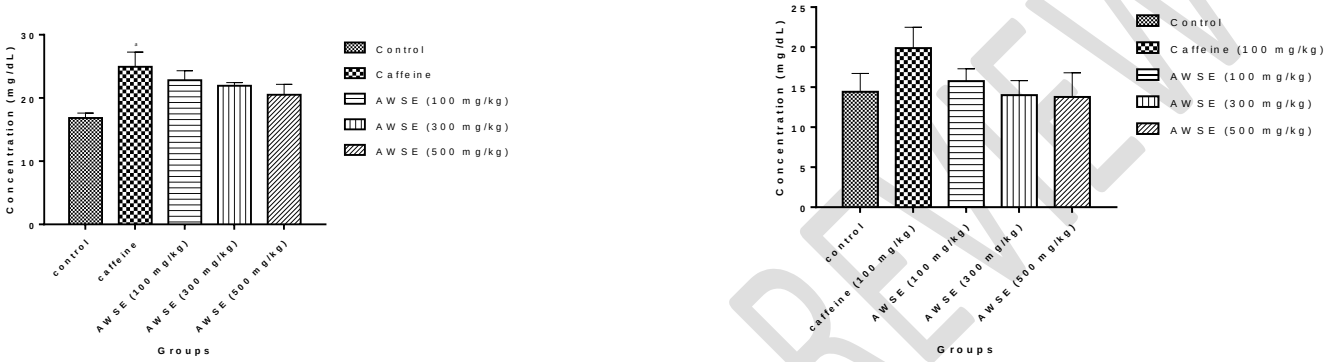
C

Figure 1: Effect of Aqueous Seeds Extract (ASE) of *C. vulgaris and caffeine* on the Plasma AST (A), ALT (B) and Alkaline Phosphatase (ALP) C Activities of Male Wistar Rats

3.2. *Effect of Caffeine and Aqueous Seeds Extract of C. vulgaris on Plasma Urea (A) and Creatinine (B) Levels of Male Wistar Rats*

A significant increase in urea level was observed in the caffeine groupwhen compared to the Control and *C. vulgaris* treated groups ($p<0.001$, $p<0.01$ respectively). Plasma urea level was also significantly lower in the

C. vulgaris treated groups, compared to the caffeine group ($p<0.05$) Fig.2A. The caffeine group exhibited significantly higher plasma creatinine levels compared to the Control and *C. vulgaris* treated groups ($p<0.001$, $p<0.05$ respectively, Fig 2B).

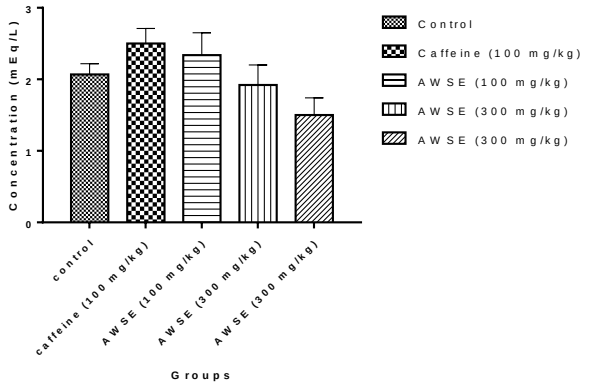


A B

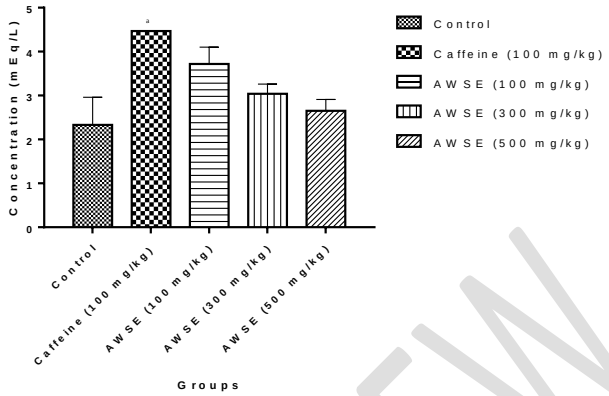
Figure 2: Effect of Caffeine and Aqueous Seeds Extract of *C. vulgaris* on the Plasma Urea (A) and Creatinine (B) levels in the Plasma of Male Wistar Rats. AWSE = Aqueous Watermelon Seeds Extract

3.3. *Effect of caffeine and Aqueous Seeds Extract of C. vulgaris on Electrolytes levels (Sodium, Potassium, Calcium and Magnesium) of Male Wistar Rats*

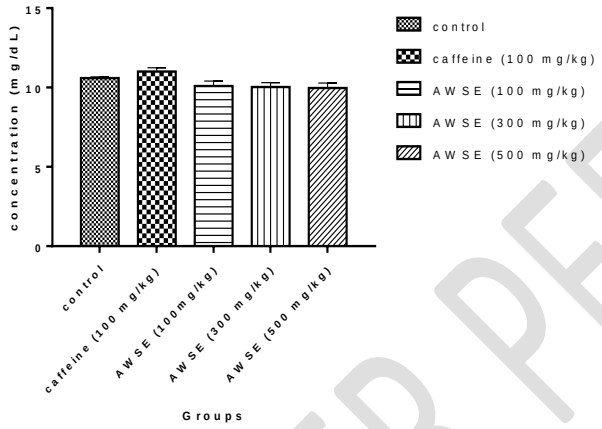
A significant increase was observed in the levels of sodium, potassium, calcium and magnesium in caffeine intoxicated group (group 2) when compared to the Control and *C. vulgaris* treated groups ($p<0.001$, $p<0.01$ respectively) Figure 3. A decrease in the electrolyte level was observed in the groups treated with aqueous seeds extract of *Citrullus vulgaris* when compared to the caffeine-intoxicated group.



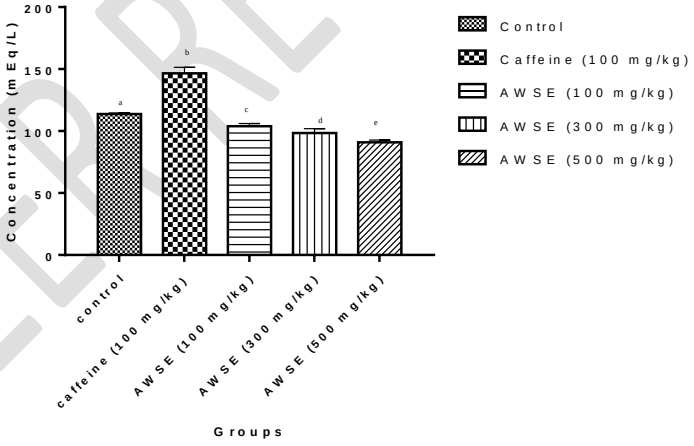
Magnesium



Potassium



Calcium



Sodium

Figure 3: Effect of Caffeine and Aqueous Seeds Extract of *C. vulgaris* on Electrolytes levels: Sodium (A), Potassium (B), Calcium (C) and Magnesium (D) of Male Wistar Rats

3.4. *Effect of Caffeine and Aqueous Seeds Extract of C. vulgaris on Total Protein, Albumin and BilirubinConcentrations in plasma of Male Wistar Rats*

A significant decrease in total protein concentration level was observed in the caffeine groupwhen compared to the Control and *C. vulgaris* treated groups ($p<0.001$, $p<0.01$ respectively). Total protein level was also significantly higher in the *C. vulgaris* treated groups, compared to the caffeine group ($p<0.05$) Figure 3A. The caffeine group exhibited a slightly high albumin concentration which was not significant but a

significantly higher bilirubin levels compared to the Control and *C. vulgaris* treated groups (Figure 3B). These alterations were reversed by the administration of graded doses of *C. vulgaris.*

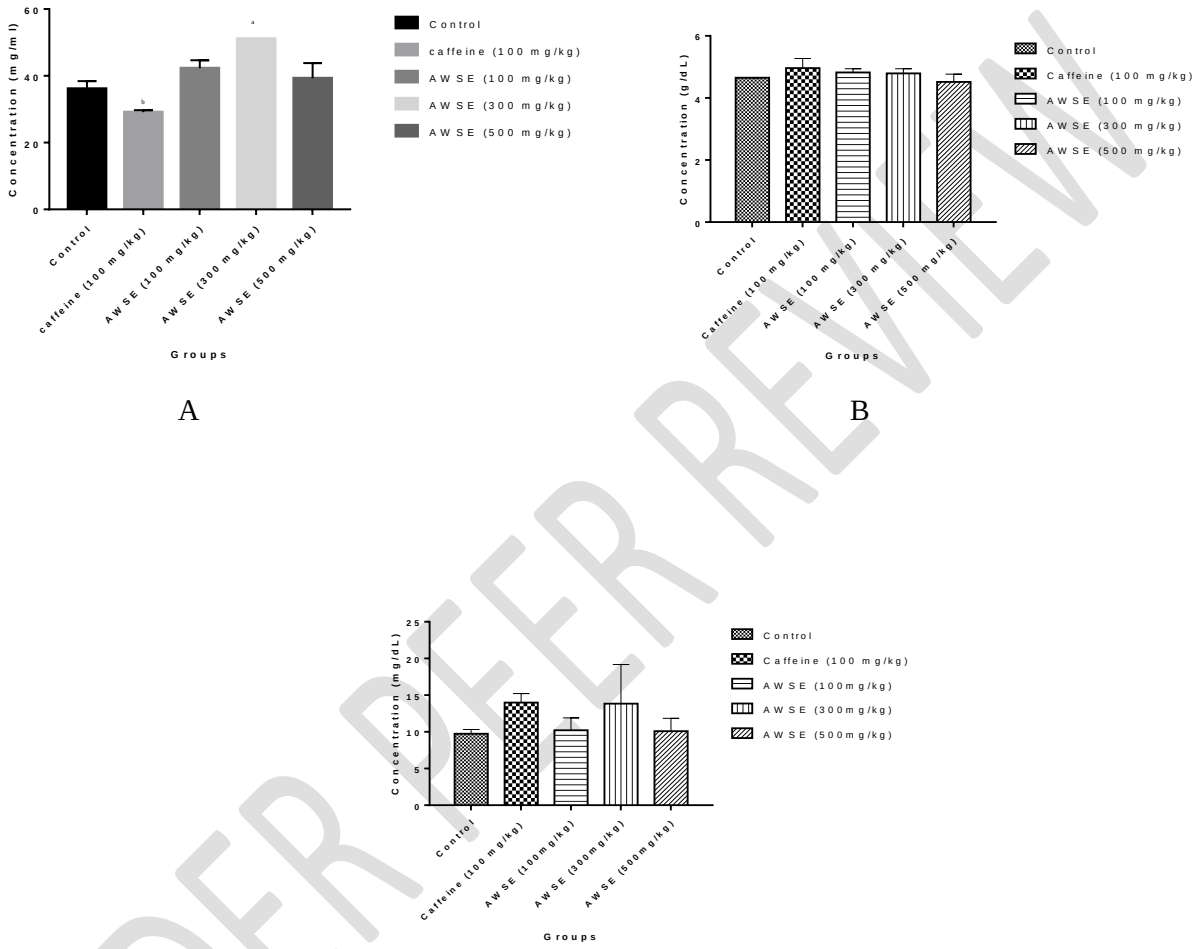


Figure 4: Effect of Caffeine and Aqueous Seeds Extract of *C. vulgaris* on the Total Protein (A), Albumin (B) Bilirubin (C)levels in the Plasma ofMale Wistar Rats. AWSE = Aqueous Watermelon Seeds Extract

3.5. *Effect of Caffeine and Aqueous Seeds Extract of C. vulgaris on Plasma Lipid Profile of Male Wistar Rats*

The total cholesterol, triglyceride and LDL levels were elevated and HDL level was reduced in caffeine intoxicated group (group II). However, reduction was observed in the level of total cholesterol, triglyceride

and LDL of the group treated with aqueous seeds extract of *Citrullus vulgaris* when compared to the caffeine-intoxicated group with the exception of HDL which increased.

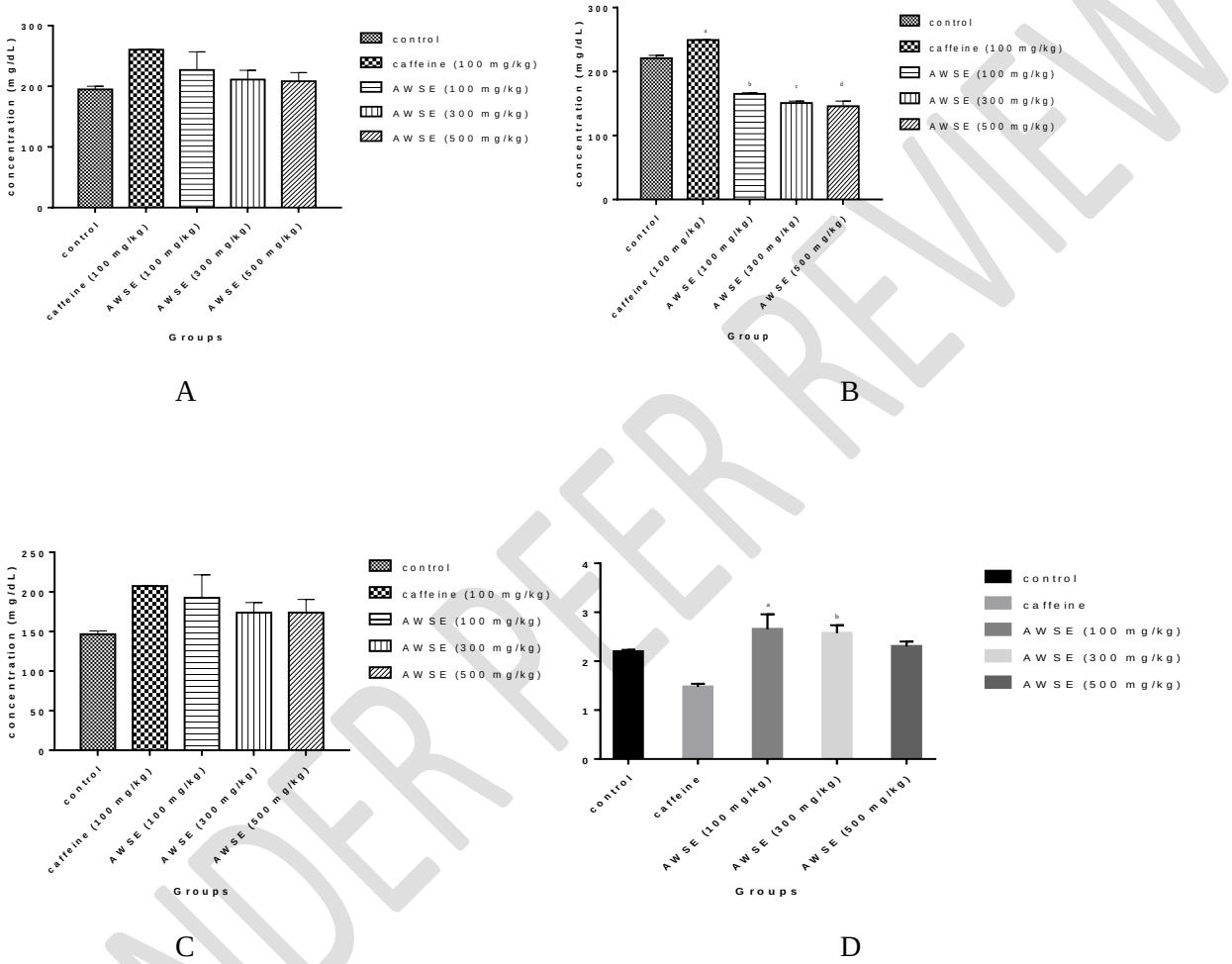
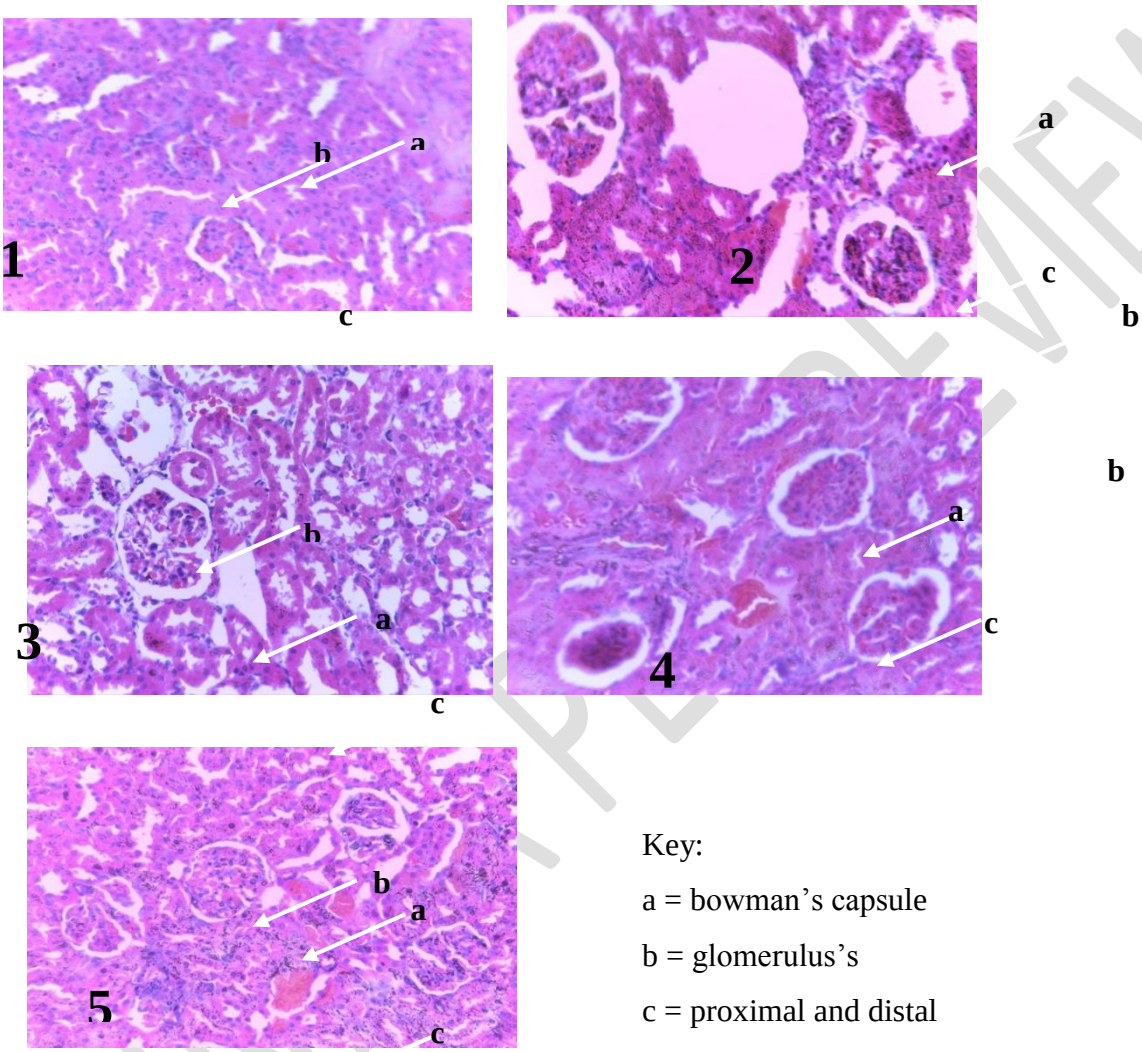


Figure 5: Effect of Caffeine and Aqueous Seeds Extract of *C. vulgaris* on Total Cholesterol (A) Triglyceride (B) Low Density Lipoprotein (C) High density Lipoprotein (D) Levels in the Plasma of Male Wistar Rats
AWSE = Aqueous Watermelon Seeds Extract

3.6. Results of Histopathological Analysis of the Kidney

The results of the histopathological analyses of the kidneys of male wistar rats is presented in plates 1-5. Plate 1(control group) showed the structure of a normal rat kidney where the Bowman's capsules and

the glomeruli were without deformities. On this plate, the endothelial cells which form a single cell layer that lines all blood vessels and regulates exchanges between the bloodstream and the surroundings is present. Plate 2 showed that the caffeine administered to the rats caused deformation and disruption of the kidney tissues. The Bowmans capsules and other parts of the kidney were disintegrated. Scattered coagulative necrosis was also observed due to exposure to toxic effect of caffeine.



Key:

a = bowman's capsule

b = glomerulus's

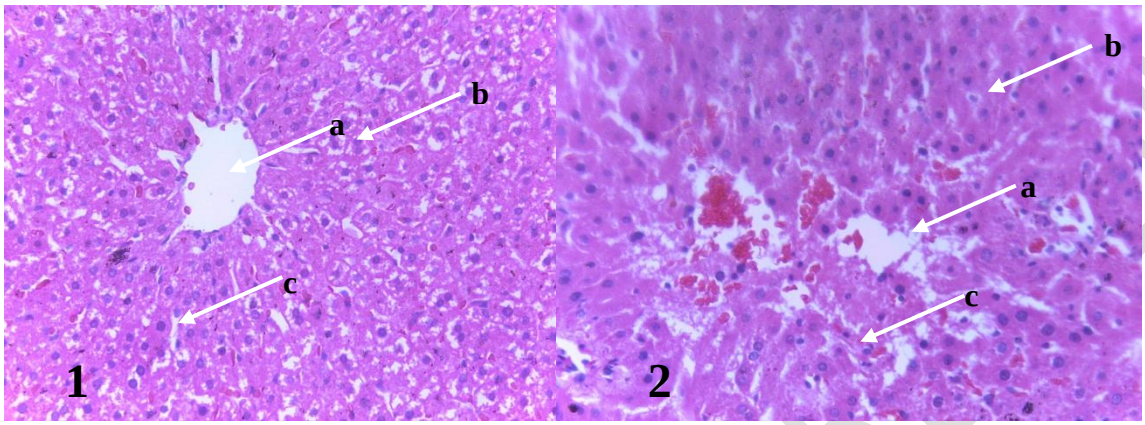
c = proximal and distal tubules

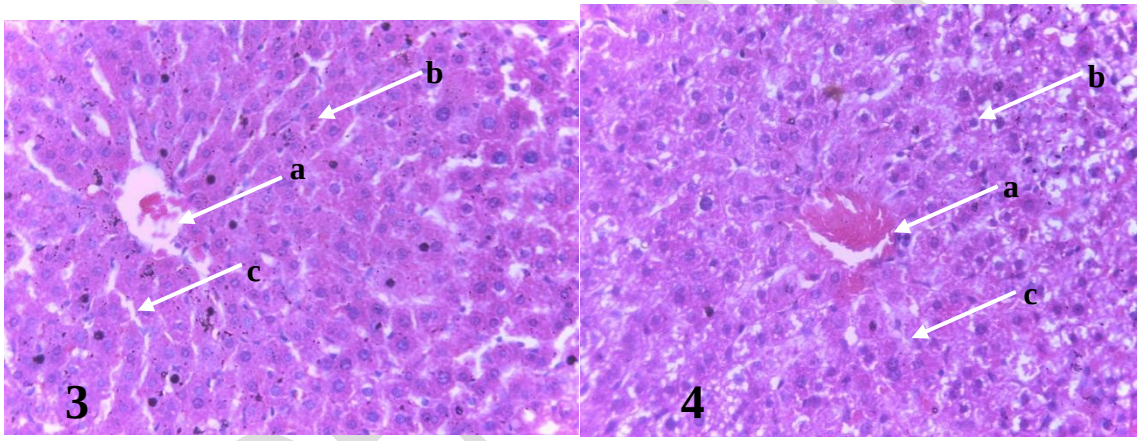
Plate 1: Histopathology of the kidney of various groups (Mg. x400). (1) Kidney of control, (2) Caffeine only (negative) and (3), (4), (5) kidney of rats treated with aqueous seeds extract of *C. vulgaris* at 100, 300 and 500 mg/kg respectively.

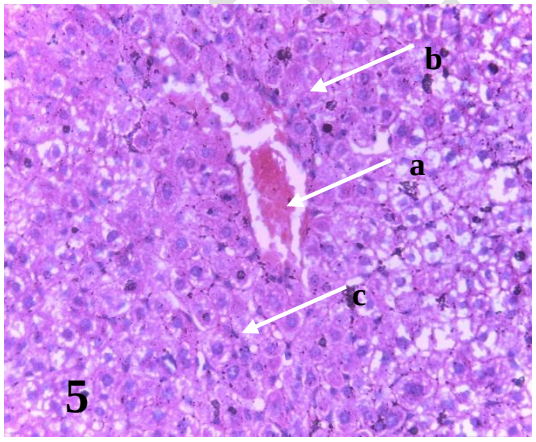
3.7. Results of Histopathological Analysis of the Liver

The histopathogical analysis of the liver of caffeine-intoxicated rats showed a distortion of the liver cells, necrosis, widespread haemorrhage and vacuolation of the liver cells (plate 2). It also showed disruption of hepatocytes, blood vessels and collagen fibers deposition, scattered haemorrage and lesions when compared

with the control (plate 1) where the hepatocytes, central vein, endothelial cells and Kupffer cells are distinct with no haemorrhage. The administration of aqueous extracts of *C. vulgaris*seeds at dosages of 100 mg/kg, 300 mg/kg and 500 mg/kg body weights was able to repair/reverse the tissue damage (plates 3, 4 and 5). The repair was progressive and dose dependent with more of the liver regeneration observed in plate 5.







Keys:

a = hepatocytes

b = central vein

c = endothelial cells and kupffer cells

Plate 2: Histopathology of liver of various groups (Mg. x400). (1) Liver of control, (2) Caffeine only (negative) and (3), (4), (5) Liver of rats treated with aqueous seeds extract of *C. vulgaris* at 100, 300 and 500 mg/kg respectively showing slight improvement in the distribution of collagen fibers in liver parenchyma.

4. DISCUSSION

"Both the liver and the kidney play vital roles in the elimination process of wastes produced in all living organisms. Thereby, any damage in both organs results in metabolic dysfunctions and the accumulation of toxins in the body leading to systemic toxicity and atrophy" [5]. "The absence of potential compounds that can alleviate and protect vital organs from any damage is a major problem, especially in conventional medicine. Thereby it is extremely crucial to find out natural compounds that may protect our vital organs from induced damage and cytotoxicity due to daily misused consumed "[22]"Our result demonstrated the protective effect of aqueous *C. vulgaris* against caffeine-induced hepatotoxicity and nephrotoxicity in male Wister rats. Hepatic marker enzymes (AST, ALT, ALP) are usually elevated in hepatic damage due to the membrane damage.ALT is predominantly present in the cytoplasm of hepatocytes, whereas AST is predominantly present in the mitochondria. Both AST and ALT enzymes are excellent markers of liver damage caused by exposure of liver to toxic substances"[23]. "However, ALT is more specific liver enzyme for diagnostic use when the integrity of the hepatocellular membrane is compromised"[24]. "Tissues damage or excess production of the enzyme is usually associated with the spilling of cell derived enzymes into the plasma"[25]. "The increase transaminase levels in test groups against the control as observed in this study could be linked to consumption of caffeine. The mechanism behind this enzyme leakage into the plasma could be attributed to the peroxidative effect of caffeine in fatty liver tissues in rats"[26]. "ALP is also found in the liver next to the bile ducts and it leaks to the blood stream in a manner similar to that of AST and ALT"[27]. "The loss of these cellular enzymes from the tissues into the blood stream could be attributed to the disruption of the ordered lipid-bilayer of the cell membrane structure, probably by peroxidation of polyunsaturated fatty acids on the plasma membrane, thereby increasing their activities"[28]. "The observed increase in transaminase activities due to caffeine consumption observed in this study is in line with report of other investigators"[29, 30]. The decreasedactivities of the hepatic enzymes were observed in the *C. vulgaris*

treated group suggesting its hepathoprotective role. These results were supported by the histopathological findings of the liver as it showed a better improvement of the hepatic tissue (Figure 2). The dose-dependent reduction in AST, ALT, ALP activities observed in groups treated with aqueous seeds extract of *C. vulgaris*in this study is in line with the report of [31] who reported similar decrease following the administration of 200mg/kg bwt *C. lanatus*seeds.

The liver is the principal organ for amino acid metabolism [32]. "In the present study, a significant decrease in serum total protein concentration was detected in the caffeine treatedgroup when compared with the negative control. Hypoprotenemia may be induced due to hepatocellular injury or inflammation induced by caffeine toxicity which leads to the disturbance of protein biosynthesis"[33]

"Bilirubin is excreted by the liver, and hence any interference with the normal liver functionsaffects its rate of excretion"[34]. Thus, elevated level of bilirubin is used as an index of liver function. High plasma bilirubin observed in this study is an indicator of hepatobiliary injury due to caffeine toxicity which was ameliorated by the administration of *C. vulgaris*.

Liver is the major organ for synthesis of many blood proteins such as albumin [32]. Variation observed in Albumin concentration observed in this study was not significant however, the treatment with *C. vulgaris* restore it to normal values.

The liver plays a critical role in maintaining whole body lipids [35]. "According to this work, caffeine administration resulted in significantincrease in total cholesterol, triglycerides, and low density lipoproteins and significant reduction in HDL.The alteration in these lipid profiles caused by caffeine toxicity showsthat over dose of caffeine could be a predisposing factor to coronary heartdisease (CHD) and could also be an indication of liver damage. The aberrationin lipid profiles was corrected by the administration of *C. vulgaris*.

Urea and creatinine can be used to evaluate renal function"[35]. "Urea is the main end product of protein catabolism"[36]. "Amino acid deamination takes place in the liver, which is also a site of urea cycle where ammonia released is converted into urea and is excreted through urine"[37]. "Urea varies directly with protein intake and inversely with the rate of excretion"[38]. "Renal disease which diminish the glomerular filtration lead to urea retention"[23]. "Creatinine is a waste product of muscle creatine metabolism. Its

retention in the blood is evidence of kidney impairment"[38]. "The observed increase in urea and creatinine levels in test groups when compared to the control suggest that the kidney isunabletoexcretetheseproducts following the administration of caffeine whichindicatesan impairment of the kidney functions"[39]. These effects may be due to the changes in the tubular re-absorption threshold, renal blood flow and the rate of glomerular infiltration[40] thus compromising the renal functions. However, the administration of *C. vulgaris* resulted in ameliorating the effect of caffeine by reducing the urea and creatinine levels. These results were supported by the histopathological findings of the kidney as it showed a better improvement of the renal tissues. Some authors have noted that assessment of levels of excretory metabolites and electrolytes can also be used to evaluate renal functions [35]. Nitrogenous waste accumulation and imbalance ofelectrolytes are key indicators of nephrotoxicity [41]. The levels of sodium, potassium, calcium and magnesium significantly increased in caffeine intoxicated group (group II) when compared to the control. The observed increase in sodium (28.98%) and potassium (91.85%) ions in the caffeine-intoxicated group could meanan effect on both sodium ion and potassium ion pumps respectively and inhibition of Na-KATPase by caffeine,which in turn leads to changes in electrolytes [42]. However, the administration of *C. vulgaris* brought the electrolytes levels back to near normal values in treated rats.

Histopathological examinations of the liver and the kidney revealed hepatocyte and nephrotic damage of variable prevalence and intensities in caffeine group which were reversed by the administration of graded doses of *C. vulgaris*. The rate of regeneration of the liver and the kidney, observed in this study was dose dependent with the highest regeneration in the group treated with highest concentration of *C. vulgaris*. Dose-dependent regeneration of organs has been reported [43, 44, 45]. Several plant extracts have been reported to possess hepathoprotective and nephroprotective abilities [43, 45,47, 48].

C. vulgaris seed is not only rich in minerals and nutrients but also contain phytochemicals such as phenol, tannin, flavonoids, carotenoids, lycopenes [49] which plays critical roles in the maintenance of cell functions and integrity and maintenance of normal cell activities [50].

"C. vulgaris has demonstrated in this study to possess hepatoprotective and nephroprotective ability against caffeine induced toxicity. The possible mechanism by which the *C. vulgaris* exerted its protectiveeffect against caffeine-induced liver and kidney injuryin rats could be accrued to the ability of *C. vulgaris* to sequesterfree radical elements, thereby acting as a potent free radical scavengers and membrane stabilizers. This effect could be linked to the presence of bioflavonoids such as quercetin,rutin, luteolin, and isorhamnetin, and non-phenolic compounds such as the soyasaponin triterpenoid previously identified in *C. vulgaris"*[51]. Generally, flavonoidshave been reported to have protective effects on the liverdue to their antioxidant property [52,53], and membrane stabilization effect [54]. Quercetin in particular identified in *C. vulgaris*[51] has been reported to prevent hepatotoxicity by inducing cytochrome p62 expression and induction of antioxidantresponse [55].

5. CONCLUSION

Observations from this present study revealed that the aqueous seeds extract of *Citrullus vulgaris* possessameliorative effect against caffeine-induced toxicity. However, further research is required to isolate the active compound responsible for the observed ameliorative effect.

ETHICAL CLEARANCE

All animal experiments were conducted according to the institutional principles on the useof laboratory animals and in compliance with the National Institute of Health Guidelines for Careand Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

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