



Effect of Ethanol Leaf Extract of *Annona Squamosa* Linn on the Lipid Profile and Renal Function of Acetaminophen Induced Hepatotoxicity in Wistar Rats

Yakubu Ejeh Ojochenemi¹, Jankada Patience Audu^{2*}, Achilus Francis³, Okoh Jocelyn Cletus⁴, Wanmi, D. A⁵, Larubi Luka⁶, Shitta Nasisi Namuma⁷

^{1,2}Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari. PMB 1020 Katsina-Ala Road, Wukari, Taraba State, Nigeria

^{3,5,6}Department of Science Laboratory Technology Taraba State Polytechnic Suntai, Taraba State, Nigeria

^{4,7}Sechenov University, Moscow Russia.

Corresponding Author: patienceauduj@gmail.com* +2349130035989

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ABSTRACT

This study was aimed at investigating the effect of ethanol leaf extract of *Annona squamosa* on the lipid profile and renal function of acetaminophen induced hepatotoxicity in Wistar rats. Liver damage was induced by oral administration of 2g/kg body weight of acetaminophen for seven days in group 4 and treated for 14 days, groups 3 and 5 were given paracetamol (2g/kg body weight) alongside silymarin and *A. squamosa* leaf extract for 21 days respectively. Group 2 were given acetaminophen for 21 days and left untreated. The results showed a significant ($p < 0.05$) increase in K, Na, Cl⁻, urea and creatinine levels in group 2 compared with group 1 (normal control) and a significant ($p < 0.05$) decrease in all the treated groups compared with group 2 (hepatotoxic group). There was no significant ($p < 0.05$) difference in the level of CHOL in group 2 compared with group 1 but TAG significantly ($p < 0.05$) decreased in groups 3, 5, and 6 compared with the group 2. RBC, HGB, HCT and PLT significantly (< 0.05) decrease in group 2 compared with group 1 but significantly (< 0.05) increased in all the treated groups compared with group 2 while WBC was significantly increased in group 2 compared with group 1. *Annona squamosa* was found to alter the negative effect of paracetamol induced toxicity in all the treated groups which suggest that it could be have a positive effect in treatment of cardiovascular disease, renal dysfunction and may also be useful in haematopoiesis.

Keywords: Lipid Profile, *Annona squamosa*, Kidney Biomarkers, Hematology, Hepatotoxicity

Introduction

The current pharmacopoeia has over a thousand medications that might cause liver damage with various clinical manifestations. Drug-induced liver injury (DILI) can result in serious outcomes such as liver transplantation, patient death, or the withdrawal of medications from the market prior to complete clinical testing, which can result in significant financial losses. The exact cause of DILI is currently unknown in Nigeria, however common over-the-counter (OTC) drugs, including paracetamol (ACM), can often be contributing factors [1]. The development of therapeutic agents from plant-based substances has attracted more attention globally and sparked a new wave of studies on the advantages of herbal medicine as a successful substitute therapy for a range of ailments [2]. The management and treatment of human diseases has significantly raised the value of medicinal plants on a global scale. Due to its price and accessibility, it is the primary method of healthcare delivery for the majority of rural people, especially in several African countries [3]. One of the most popular and frequently used medications for the treatment of pain and fever is acetaminophen (acetaminophen). It holds a special place among medications used to relieve pain [4]. According to Ramachandran and Jaeschke [5], paracetamol is a safe analgesic when used at therapeutic levels, but an overdose can result in substantial toxicity. The oxidative stress caused by an excess of paracetamol is mainly caused by peroxynitrite and superoxide in the mitochondria. Superoxide and nitric oxide react to form the highly reactive peroxynitrite species, which is the main source of oxidative and nitrosative stress [6]. After glutathione conjugation transforms NAPQI into safe metabolites, it is subsequently removed. Glutathione peroxidase activity is 60% reduced by acetaminophen. Higher doses of paracetamol result in a prolonged duration of glutathione depletion, which is dose-dependent [7]. Reactive oxygen species and peroxynitrite excretion is reduced as a result of this glutathione reduction in the mitochondria and cell cytoplasm. Furthermore, in the absence of glutathione, oxidative stress causes the mitochondrial permeability transition pores to open, destroying the membrane potential and preventing the production of ATP [7, 8]. Ultimately, this results in the induction of apoptosis, the disintegration of DNA and cell membranes, severe inflammation, and cell death [9]. Even in the absence of liver impairment, nephrotoxicity—which is less prevalent than hepatotoxicity in paracetamol overdose—may result in acute renal failure and renal tubular damage, which can even be fatal in

people and experimental animals [10]. *Annona squamosa*, often known as the sweetsop or custard apple tree, is a small shrub that is a member of the Annonaceae family and genus *Annona*. According to research reports, the plant contains amino acids, glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, and phenolic chemicals [11].

Material and Methods

Materials: Containers for maceration, surgical scissors, pipettes, beakers, test tubes, test tube racks, spatulas, rotary evaporator RE 100-S, surgical trays, Separating funnel, conical flask, digital analytical weighing balance (Ohaus: PA-1000), aluminium foil, Whatman number 1 filter paper, beakers, Spatula, Sample bottles, Retort stand, plastic funnels, wooden mortar and pestle, masking tape, micro pipette, thermostatic water cabinet, spectrophotometer (UV-Visible light), measuring cylinder, cotton wool, rotary evaporator (SM-5286A), SPIN tissue processor (STP 120 ThermoScientific), MICROM HM340E ThermoScientific, tissue Tek Embedding Centre (SLEE MPS/P2), rat rearing cages measuring with the top covered with wire mesh, label paper, Agile autohaematology analyzer (S-30), Liston classic centrifuge (C2204), Sykam HPLC (S3250 UV/visible detector), and gloves.

Reagents/Chemicals

Aluminum chloride, formalin, chloroform, n-hexane, absolute ethanol, methanol, water, follin-ciocalteu reagent, sodium carbonate, trichloroacetic acid (TCA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), normal saline. The rest of the chemicals were of analytical grade.

Plant Collection and Preparation

Annona squamosa fruits and fresh leaves were collected at Takum, Taraba State. The fruit was stripped of its seeds. After being cleaned with water, the leaf and the seeds were allowed to dry in the shade. They were then ground into a fine powder using a conventional mortar and pestle and stored in a glass jar.

Preparation of Ethanol Extract of *Annona squamosa*

For this procedure, the approach adopted by Yakubu *et al.* [12] was used. 500g of ground leaf and seed samples each were weighed into a plastic container, which was then filled with 2000 mL of ethanol (1:4 w/v). The mixture was let to rest for 72 hours, shaking occasionally, before being filtered through muslin cloth and Whatman No. 1 filter paper. In order to prevent the active components from becoming denatured, the filtrate was concentrated using a hot water bath and a rotary evaporator operating at decreased pressure and 45 degrees Celsius. Before being administered, the concentrate was moved into an airtight container and kept in the refrigerator. Using distilled water, the extract was dissolved prior to administration.

Experimental Animals

Wistar rats of both sexes weighing between 50 g and 200 g were used in this experiment. The animals were housed in cages with saw dust as bedding in animal house facility of Department of Biochemistry, Federal University Wukari. All experiments on laboratory rats were performed according to protocols authorized by animal ethical committee of Department of Biochemistry, Federal University Wukari. The animals were maintained under exposure to a 12 h/12 h light/dark cycle at a room temperature of $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and free access to food in the form of standard pellets and drinks were given ad libitum.

Experimental Design

Twenty-five Wistar rats were randomly selected and divided into five groups of five rats each. Group I served as the normal control and receive only the vehicle (normal saline) at a daily dose of 5 mL/kg body weight throughout the study. Group II were administered acetaminophen (2g/kg body weight) daily for 21 days. Group III were administered acetaminophen (2g/kg body weight) followed by silymarin (100mg/kg body weight) daily for 21 days. Groups IV, were administered acetaminophen (2g/kg body weight) followed by leaf extract (200mg/kg body weight) daily for 21 days. Groups VI were administered acetaminophen (2g/kg body weight) daily for 7 days and leaf extract daily for 14 days. The vehicle and test drugs were orally administered.

Induction of Hepatotoxicity

Hepatotoxicity was induced in the rats by oral administration of paracetamol (acetaminophen) using micro syringe at a dosage of 2g/kg body weight.

Animal Sacrifice and Collection of Blood Samples

After the 21 days, the animals were starved overnight and were weighed before being sacrificed under chloroform anaesthesia. Blood was collected from each of the animal through cardiac puncture using 5 ml disposable syringe, and dispensed into 2 different types of tubes. The 1st part of the blood (dispensed into EDTA anti-coagulant containing sample tube) was used for haematological analysis. The 2nd part of the blood (dispensed into a very cleaned plain tube) was allowed to clot for about 10 min and centrifuged at 4000 rpm for 10 min. The serum was separated from the clot and was used for biochemical analysis.

Estimation of Serum Kidney Biomarkers

For renal function, serum electrolytes (Sodium, Potassium, Chloride), urea and creatinine level were investigated using their respective assay kits.

Assessment of Serum urea

Serum urea was measured using the commercially available kit (Liquicheck AGAPPE Diagnostics LTD), following the GLDH-Urease method [13].

Principle

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green chromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample.



Assessment of Creatinine

The amount of creatinine in serum was estimated using Chemcheck AGAPPE Diagnostics commercial kit according to Tietz method [13] and the absorbance was read at 490nm.

Principle

Creatinine reacts with picric acid to produce a coloured compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration.

Determination of Serum Sodium Ion (Na⁺)

The presence of sodium was evaluated with Spectrum sodium detection kit according to the manufacturer's instructions.

Principle

The present method is based on the reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly concentration of sodium in the serum measured spectrophotometrically at 630nm [14].

Determination of Serum Potassium Ion (K⁺)

The presence of potassium was evaluated with Spectrum potassium detection kit according to the manufacturer's instructions.

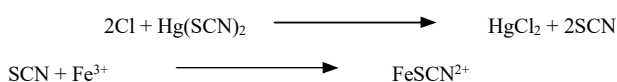
Principle

The amount of potassium was determined using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which was proportional to potassium concentration measured spectrophotometrically at 578nm [15].

Determination of Serum Chloride Ion (Cl⁻)

The presence of chloride ion was evaluated with Agappe Chemchek chloride ion detection kit according to the manufacturer's instructions.

Principle



The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate and subsequent formation of a red ferric thiocyanate complex is measured spectrophotometrically. The intensity of the colour formed is proportional to the chloride ion concentration in the sample [16].

Determination of Lipid profile

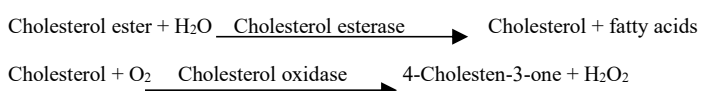
Total cholesterol (TC), high density lipoprotein (HDL) and triglycerides (TAG) were also investigated.

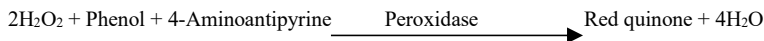
Estimation of Total cholesterol

Measurement of serum total cholesterol was done according to the method of Roeschlaue *et al.* [17] as outlined in Agappe Liquicheck cholesterol assay kit manual.

Principle

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol is then oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide combines with phenol and 4-aminoantipyrine to produce a chromophore (quinoneimine dye) which is measured at a wavelength of 630nm [18]. Enzymatic colorimetric determination of total cholesterol according to the following reactions:





Assessment of HDL Cholesterol

HDL Cholesterol was measured according to the method of Allain *et al.* [19] as outlined in Agappe Liquichek HDL cholesterol assay kit manual.

Principle

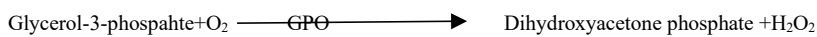
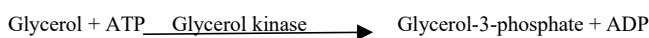
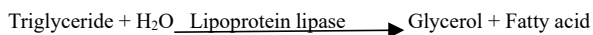
The very low density (VLDL) and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant containing high density lipoproteins (HDL) is used for the determination of HDL cholesterol.

Determination of Triglyceride

The presence of Triglyceride will be evaluated with Agappe Liquichek Triglyceride detection kit according to the manufacturer's instructions.

Principle

Enzymatic determination of triglyceride is based on following reactions:



GPO = Glycerol-3-phosphate Oxidase

Haematological Analysis

The determinations of haematological parameters were carried out using automated haematology analyzer (Agile S30 haematology autoanalyzer V2.0, China). Using whole blood, the total red blood cell (RBC) count, haemoglobin (HB) concentration, packed cell volume (PCV), white blood cell (WBC) count and platelet count were determined.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for this analysis. Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) followed by Duncan multiple comparison test. The results were expressed as Mean \pm standard deviation (SD). (n = 5) with p < 0.05 being statistically significant.

Results and Discussion

Effect of ethanol leaf extract of *Annona squamosa* on kidney biomarkers of acetaminophen induced hepatotoxicity.

Table 2. Result of Kidney Biomarkers Analysis

GROUPS	K (mmol/l)	Na (mEq/l)	Cl ⁻ (mEq/l)	Urea (mg/dL)	Creatinine (mg/dL)
1	4.76 \pm 0.10 ^a	75.64 \pm 2.58 ^a	95.00 \pm 1.44 ^b	24.21 \pm 3.34 ^a	1.60 \pm 0.89 ^a
2	5.27 \pm 0.29 ^b	144.43 \pm 7.00 ^d	100.12 \pm 4.50 ^c	39.27 \pm 3.10 ^c	4.80 \pm 1.10 ^b
3	4.58 \pm 0.33 ^a	115.52 \pm 9.78 ^c	95.53 \pm 0.72 ^b	30.04 \pm 2.83 ^b	2.60 \pm 0.89 ^a
4	4.73 \pm 0.51 ^a	106.77 \pm 9.01 ^c	90.26 \pm 3.17 ^a	33.03 \pm 0.28 ^b	2.60 \pm 2.19 ^a
5	4.76 \pm 0.45 ^a	89.79 \pm 8.46 ^b	93.24 \pm 4.62 ^{ab}	33.13 \pm 0.28 ^b	2.40 \pm 0.89 ^a

Results represent mean \pm standard deviation of group results obtained (n=5). Values with different alphabet as superscripts in the same column are statistically significant (p < 0.05).

1=Normal control, 2= Hepatotoxic control, 3= Standard group, 4= Leaf Protective group, 5= Leaf Ameliorative group.

The table above shows the result of Potassium (K), Sodium Na, Chloride (Cl⁻), Urea and Creatinine. From the result, there was a significant (p < 0.05) increase in the level of K, Na and Cl⁻ in group 2 compared with the normal group (group1). Administration of the standard drug (silymarin) and the *A.*

squamosa leaf resulted in a significant ($p < 0.05$) decrease in the level of K, Na and Cl^- in all the treated groups compared with the group 2 (hepatotoxic group). Also, there was no significant ($p < 0.05$) difference in the level of K all the treated groups compared with the normal group and there was no significant ($p < 0.05$) difference in all the extract treated groups. For sodium, there was a significant ($p < 0.05$) decrease in group 5 (leaf ameliorative) compared with group 4 (leaf protective) and a significant ($p < 0.05$) increase in all the treated groups compared with group 1. Cl showed no significant (< 0.05) difference in groups 3 (silymarin treated group), and 7 compared with the normal group. There was a significant (< 0.05) increase in the level of urea and creatinine in group 2 (hepatotoxic control) compared with group 1 and a significant (< 0.05) decrease in all the treated groups compared to group 2. Also, there was no significant difference between the extract treated groups. A significant number of polyphenolic chemicals, which are antioxidants and may help prevent disorders linked to oxidative stress, are found in *Annona* species [20]. These substances, which are crucial components of plants, have drawn a lot of interest due to their possible antioxidant properties, which give them the ability to scavenge free radicals [21]. PCM is well-considered to be both an antipyretic and a mild analgesic at therapeutic levels. However, excessive dosages of it result in nephrotoxicity and hepatotoxicity in both experimental animals and humans [22].

Hyperkalaemia frequently arises as a consequence of decreased kidney function in patients with acute-on-chronic liver disease, impairing urine K^+ excretion and increasing renal sodium and water absorption. Although the exact reasons behind the development of hyperkalaemia in liver disease patients remain unclear, there is a strong correlation between the elevated risk of hyperkalaemia in patients with advanced cirrhosis and impaired potassium uptake by the liver [23]. Ascites, hyponatraemia, and retention of water and salt are possible outcomes of hyperkalaemia development [24]. The primary by-product of muscle catabolism is creatinine, which the kidneys eliminate. Renal failure is indicated by elevated creatinine levels [25]. In this investigation, it was shown that rats given acetaminophen had significantly higher levels of creatinine and urea. According to Pedraza-Chaverri *et al.* [26], increased amino acid catabolism, reduced renal function, or liver injury are likely the causes of the elevated urea level in hepatotoxic rats. The body typically produces creatinine at a fairly consistent pace, which the kidneys then remove from the blood. Blood levels of creatinine increase when the kidneys' ability to filter substances is compromised [27].

In mammals, urea is the main by-product of protein catabolism and the main means by which harmful ammonia is eliminated from the body. The liver is the primary site of production, while the kidneys secrete it [28]. As shown in this study, the levels of creatinine and plasma urea significantly increased in the hepatotoxic control group (2). Treatment with *A. squamosa* leaf extract reduced the levels of creatinine and plasma urea in both the hepatoprotective and ameliorative groups which is in line with a recent study conducted by Kaleem *et al.* [29]. This study's findings are consistent with [30] in that paracetamol overdose can result in acute kidney damage (AKI) even in the absence of hepatotoxic consequences.

Effect of ethanol leaf extract of *Annona squamosa* on lipid profile of acetaminophen induced hepatotoxicity.

Table 3. Result of Lipid Profile

GROUPS	TC/HDL				
	TAG (mmol/L)	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	
1	96.19±10.94 ^a	201.18±1.61 ^a	141.97±9.88 ^b	153.57±4.70 ^{ab}	1.423±0.11 ^b
2	122.44±11.35 ^b	202.35±1.32 ^a	104.92±4.34 ^a	170.71±5.82 ^c	1.93±0.007 ^c
3	84.38±20.48 ^a	204.71±1.61 ^b	170.74±25.69 ^{bc}	153.65±5.74 ^{ab}	1.220±0.17 ^b
6	89.77±19.40 ^a	202.35±1.32 ^a	202.82±46.48 ^c	143.79±10.57 ^a	1.044±0.26 ^a
7	108.75±24.82 ^{ab}	208.24±1.32 ^a	153.52±18.58 ^b	155.72±7.98 ^b	1.374±0.18 ^b

Results represent mean ± standard deviation of group results obtained (n=5). Values with different alphabet as superscripts in the same column are statistically significant ($p < 0.05$).

1=Normal control, 2= Hepatotoxic control, 3= Standard group, 4= Leaf Protective group, 5= Leaf Ameliorative group.

Table 3 shows the result of lipid profile; triacylglycerol (TAG), cholesterol (CHOL), high density lipoprotein (HDL) and low-density lipoprotein (LDL) and total cholesterol to HDL ratio (TC/HDL) in all the groups of the experimental design. There was significant ($p < 0.05$) increases in the levels of TAG, LDL and TC/HDL in group 2 and a significant ($p < 0.05$) decreased in the level of HDL in group 2 compared with group 1. There was significant ($p < 0.05$) decreases in the levels of TAG, LDL and TC/HDL in all the treated groups compared with group 2. No significant ($p < 0.05$) difference was seen in the level of cholesterol. There was a significant ($p < 0.05$) difference in the leaf protective and ameliorative groups in the levels of HDL, LDL and TC/HDL but no significant difference was seen in between the protective and ameliorative groups in the level of TAG. The hallmarks of dyslipidaemia in liver disease are elevated triacylglycerol and decreased HDL-C levels [31]. Changes in the lipid profile of the bio-membrane disrupt its fluidity, permeability, related enzyme activity, and transport system [32], which may have an impact on hepatic lipid transport. There is a wealth of data supporting the inverse relationship between HDL cholesterol and total body cholesterol. An abnormal blood lipid metabolism is a prerequisite for atherosclerosis to develop. In clinical work, TC, LDL-C, and TG are most frequently assessed. It has been demonstrated that a single lipid index is not a reliable indicator of cardiovascular disease. It has been demonstrated that a single lipid index is not a reliable indicator of cardiovascular disease [33]. There is ample evidence linking elevated serum total cholesterol (TC) and low levels of high-density lipoprotein cholesterol (HDL-C) to the risk of

cardiovascular disease [34]. Evidence suggests that the ratio of TC to HDL-C is a useful predictor of cardiovascular disease, and that it provides a better risk estimate than either TC or HDL-C on its own. It is unclear, how TC, HDL-C, and the TC/HDL-C ratio relate to the risks of stroke that is specific to a particular type of stroke [34]. Additionally, studies have shown that lowering plasma HDL cholesterol concentration can hasten the onset of atherosclerosis, which in turn can cause ischaemic heart disease by hindering the removal of cholesterol from the arterial wall [35].

Effect of ethanol leaf extract of *Annona squamosa* on haematological indices of acetaminophen induced hepatotoxicity.

Table 4. Result of Haematological Indices

GROUPS	WBC (10 ⁹ /L)	RBC (10 ¹² /L)	HGB (g/L)	PLT (10 ⁹ /L)	HCT (%)
1	58.17±3.24 ^a	0.20±0.01 ^b	89.00±2.12 ^c	1132.60±53.93 ^{bc}	1.36±0.22 ^b
2	80.24±5.59 ^b	0.12±0.03 ^a	62.80±5.93 ^a	824.80±38.91 ^a	0.58±0.37 ^a
3	55.8±6.54 ^a	0.19±0.07 ^b	79.20±4.44 ^b	1094.00±20.43 ^b	1.76±0.53 ^b
4	60.31±3.38 ^a	0.35±0.04 ^c	91.00±5.00 ^c	1170.00±38.08 ^c	2.04±0.66 ^b
5	59.02±3.64 ^a	0.32±0.04 ^c	89.40±5.18 ^c	1155.40±30.28 ^c	2.22±0.86 ^c

Results represent mean ± standard deviation of group results obtained (n=5). Values with different alphabet as superscripts in the same column are statistically significant (p < 0.05).

1=Normal control, 2= Hepatotoxic control, 3= Standard group, 4= Leaf Protective group, 5= Leaf Ameliorative group.

The results presented in table 4. above revealed the level of White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (HGB), Haematocrit (HCT) and Platelets (PLT). For WBC, no significant (< 0.05) difference was observed in all the treated groups compared with group 1 but there was a significant (< 0.05) increase in group 2 compared with group 1. RBC, HGB, HCT and PLT showed a significant (< 0.05) decrease in group 2 (hepatotoxic group) compared with group 1(normal control) and a significant (< 0.05) increase in all the treated groups compared with group 2). Studying blood parameters is important for risk assessment because alterations in the haematological system, when translated from animal research, have a higher predictive value for human toxicity [36]. In this study, there was a significant rise in the WBC count of the PCM-induced but untreated group, which can be explained by the attributed to the stimulation of immune system. It is clear from the notably significant rise in WBC values that an increase in WBC is a typical response to foreign chemicals that interfere with regular physiological functions. *A. squamosa* leaf was able to reduce the level of WBC in the treated groups bringing them close to the normal group. The body needs red blood cells (RBCs) for several functions. It helps move carbon IV oxide out of the body by moving it to the lung for exhalation and facilitating the movement of oxygen throughout the human system and anaemia is caused by a deficiency in red blood cells [37]. Defective haemopoiesis is the cause of the observed considerable drop in erythrocyte (RBC) count, haematocrit, and haemoglobin percent [38,39]. Blood platelet counts gradually decline in response to the loss of liver function, and thrombocytopenia develops as a key characteristic of both cirrhosis and chronic viral hepatitis. Numerous factors have been linked to the decrease in circulating platelets, including the production of anti-platelet antibodies, decreased bone marrow production, sequestration of platelets in the liver, and increased splenic clearance (often in conjunction with portal hypertension) [40]. A frequent side effect of long-term liver illness is thrombocytopenia, which is marked by decreased thrombopoietin (TPO) synthesis, decreased haematopoiesis, and increased splenic platelet destruction. It is often reported that patients with chronic liver disease frequently have a direct relationship between platelet count and liver functionality [41]. This study revealed an increase in the haemoglobin, red cell count, platelet count and haematocrits of groups treated with silymarin and *A. squamosa* leaf extract. The effect of the extracts on haematological indices of hepatoprotective and ameliorative groups were not significantly different from each other.

Conclusion

Ethanol leaf extracts of *Annona squamosa* showed to have a nephroprotective effect on paracetamol induced rats and a positive tendency in treatment and protection of cardiovascular disease with evidence as seen in the result above, it was also observed that the plant has haematopoiesis properties. But further analysis is needed to verify its safety dosage and should be used with caution in order to avoid overdose.

Compliance with Ethical Standards

Statement of Ethical Approval

All experiments on laboratory rats were performed according to protocols authorized by animal ethical committee of Department of Biochemistry, Federal University Wukari Taraba, Nigeria.

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest. The final manuscript was read and approved by all the writers.

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