

Renal Protective and Lipid Profile Modulatory Effects of Ethanol Extract of *Anthocleista vogelii* Stem Bark (EASB) in Renal Injury Rats Induced with Carbon-tetrachloride (CCl₄)

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ABSTRACT

The study evaluated the effects of ethanol extract of *Anthocleista vogelii* stem bark (EASB) on renal function indices and lipid profile in rats induced renal injury with CCl₄. Following a completely randomized experimental design, 30 rats were randomly distributed into 6 groups (n = 5). Group 1 was the normal control, group 2 was the negative control while group 3 was the silymarin control. Groups 4 – 6 were CCl₄ induced but treated with 100, 200, and 400 mg/kg EASB/day respectively. The study was conducted using appropriate methods. The results showed that the negative control had a significant (P<0.05) increase in the serum urea, creatinine, cholesterol, triacylglycerol (TAG), low-density lipoprotein (LDL) concentrations, and a significant reduction in the serum high-density lipoprotein (HDL) concentration relative to the normal control. Treatments with EASB lowered the serum creatinine, urea, and TAG concentrations when compared with the negative and silymarin controls respectively. The EASB at 100 mg/kg restored the serum HDL concentration to a normal level but 200 and 400 mg/kg EASB/day caused no significant (P>0.05) increase in the serum HDL concentrations relative to the negative control. The rats treated with EASB had elevated serum LDL and cholesterol concentrations in comparison with the negative and silymarin controls. Treatments with EASB prevented the tubular necrosis, degeneration, and infiltration of inflammatory leucocytes observed in the kidney histomorphology of the negative control. These findings suggest that EASB improves renal function and some lipid profile parameters but could cause dyslipidemia when taken in excess dose.

Keywords: *Anthocleista vogelii*; carbon tetrachloride; urea; creatinine; high-density lipoprotein; renal injury

INTRODUCTION

Carbon tetrachloride (CCl₄) is a very potent hepatotoxicant and is widely used in an experimental study to evaluate the therapeutic properties of medicinal plants and synthetic drugs in preventing damage to vital organs and tissues in the body or causing restoration of damaged organs or tissues to normalcy. Exposure to a high dose of CCl₄ has been shown to cause harm to various organs and tissues in the body including the kidneys, liver, lung, brain, testis, heart, etc. (Abraham *et al.*, 1999; Anand *et al.*, 2011). The kidney has been revealed to be one of the major targets of CCl₄ toxicity and several other environmental toxicants. The molecular mechanism of CCl₄-induced renal injury shows that when CCl₄ is metabolized in the liver via the cytochrome P₄₅₀ CYP2E1 it generates very lethal

trichloromethyl or trichloromethyl peroxy free radicals (Powell *et al.*, 2000). These radicals induce renal injury as a result of their binding to intracellular molecules, which leads to the death of kidney cells (Hermenean *et al.*, 2013).

Medicinal plants are generally recognized as plants whose parts contain chemical constituents with therapeutic potentials and can also serve as precursors for the synthesis of valuable drugs (Ogamba *et al.*, 2010). Different traditional therapeutic systems including Ayurvedic, Hindi, and Unani are used worldwide to abate ailments and this practice dates back to ancient times. Besides, plants serve as great sources of antioxidants and anti-ageing agents with curative potentials attributed to their phytochemical constituents including flavonoids, alkaloids, terpenoids, and phenolics to mention a few. Currently, ethnomedicinal plant sources are extensively utilized to treat or manage ailments,

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while many more are been evaluated for pharmacological activities such as antioxidant, antibiotic, anti-apoptotic, and anti-inflammatory effects (Abraham *et al.*, 1999). Several reports have indicated that plants with anti-oxidative properties could help in ameliorating hepatic and renal injury via free radical scavenging activities and the prevention of lipid peroxidation (Abraham *et al.*, 1999).

Anthocleista vogelii Planch well known as the “cabbage tree” is a member of the *Loganiaceae* family which is a common plant in tropical Africa, Cameroon, Sudan, and Sierra Leone (Igoli *et al.*, 2005). It is found across the Northern, Western and Eastern areas of Nigeria predominantly in marshy regions close to rivers and tropical rainforest (PROTA, 2018). Locally, it is known as “Kwari” in Hausa, ‘Apaoro’ in Yoruba, ‘Oriweni’ in Bini, and ‘Orimi’ in Benin (Keay *et al.*, 1964.). It has several species in the genus that are chiefly utilized for ethnomedicinal purposes and also harvested to serve as timber and raw materials for dyeing and making soap (Alaribe *et al.*, 2012). Several phytochemicals including saponins, flavonoids, terpenoids, carbohydrates, tannins, steroids, phenols, and fatty acids have been reported in the extracts from *A. vogelii* which were likely responsible for the medicinal properties and uses of this plant (Anyanwu *et al.*, 2013; Anyanwu *et al.*, 2018). The stem bark and leaf extracts of *A. vogelii* are used to treat malaria, diabetes, microbial infections, hypertension, rheumatism, fever, jaundice, and an ulcer (Alaribe *et al.*, 2012; Anyanwu *et al.*, 2013). The stem bark has also been reported to possess antidiabetic, antiulcerogenic, antiobesity, anti-infertility, antibiotic, and hepatoprotective properties (Oladimeji *et al.*, 2014; Osadebe *et al.*, 2014; Sunday *et al.*, 2016; Chukwu *et al.*, 2020). This study was therefore designed to investigate the renal protective and lipid profile modulatory effects of ethanol extract of *A. vogelii* stem bark (EASB) on rats induced renal injury with CCl₄-induction.

METHODOLOGY

Collection, identification, and extraction of the plant sample

The fresh stem barks of *Anthocleista vogelii* Planch were collected from the botanical garden at the Micheal Okpara University of Agriculture Umudike (MOUUAU), Umuahia, Abia State, Nigeria. The stem barks were identified and authenticated at the Herbarium unit of the Department of Forestry and Environmental Management, MOUUAU, and assigned specimen voucher number FHI 40448. The fresh stem barks were washed,

sliced into smaller pieces, and air-dried at room temperature till a constant dry weight was obtained. The dried sample was then pulverized into a coarse powder with a milling machine and weighed using an electric analytical weighing balance. A quantity, 500 g of the coarsely ground plant sample was weighed and macerated with 1.5 L of absolute ethanol in a clean sterile dry container and sealed with aluminium foil paper for 72 h with intermittent shaking to ensure even extraction of the constituents. Thereafter, it was sieved with a muslin cloth and filtered with a Whatman No. 1 filter paper. The resulting filtrate was concentrated with a rotary evaporator. The percentage yield of the extract was calculated accordingly and the extract was stored appropriately.

Collection and acclimatization of experimental animals

Thirty (30) Wistar albino rats weighing 140-150 g were purchased from the Animal House of the Department of Zoology and Environment Sciences, Faculty of Biological Sciences, University of Nigeria Nsukka. The rats were housed in steel cages at the Animal House of the Biochemistry Department, Natural Science College, MOUUAU for 7 days under twelve hours dark and light cycle with free access to standard animal feed and water *ad libitum*. The study adhered to the National Institute of Health’s guidelines for the care and use of laboratory animals (NIH, 1978, publ. no. 8023; NRC, 1985).

Ethical approval

The approval for the study was issued by the research ethical committee of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike with a reference number: MOUUAU/VPP/EC/18/003

Chemicals and reagents

All the chemicals and reagents used in this study were of standard analytical grade and were obtained from reputable manufacturers. The silymarin was obtained from Micro Labs Limited (India), carbon tetrachloride from Merck KGaA, Darmstadt, Germany, assay kits from Randox Co., UK and the absolute ethanol was obtained from BDH Chemicals, England. The rest of the chemicals and reagents used were obtained from the Department of Biochemistry Laboratory, MOUUAU.

Experimental design

The study adopted a completely randomized experimental design in which the 30 rats were randomly distributed into 6 groups

(n = 5). Group 1 was the normal control that received 2 mL/kg distilled water/day; group 2 was the negative control that was CCl₄ induced without any treatment while group 3 was the silymarin control which was CCl₄ induced but treated with 100 mg/kg silymarin/day. The groups 4 – 6 were CCl₄ induced rats treated with 100, 200, and 400 mg/kg EASB/day. The treatments of the CCl₄ induced with either silymarin or graded doses of EASB commenced 72 h after CCl₄ induction and the treatments lasted for 14 days. After treatment of the rats on the 14th day, the rats fasted overnight, and on the 15th day, blood samples were collected from the rats under mild anaesthesia and kidney tissues harvested for biochemical analyses and histological examination respectively.

Induction of renal injury

Renal injury was induced in the rats by the intraperitoneal administration of a single dose of 2000 mg/kg CCl₄ to the rats and allowing them to stay for 72 h without any treatment but with free access to standard feed and drinking water. The CCl₄ was mixed with olive oil in 2:1 (v/v) as a vehicle to temporarily mask the acute toxic effects of CCl₄ on the rats.

Biochemical analyses and histological examination

The serum creatinine concentrations in the rats were analysed with the method of Henry (1964) whereas the serum urea concentrations were analyzed with the Urease Berthelot method as described by Fawcett (1960) with the help of Randox commercial test kits. The serum total cholesterol and high-density lipoprotein concentrations (HDL) according to the methods outlined by Allain *et al.* (1974) and Albers *et al.* (1978) respectively. Also, the serum concentrations of triacylglycerol (TAG) were determined using the method of Albers *et al.* (1978) while the serum low-density lipoprotein cholesterol (LDL) was calculated using the Friedelwald equation (Friedelwald *et al.*, 1972). The kidney tissues were fixed in 10% phosphate-buffered formalin for 48 hours and the slides were prepared and examined as outlined in Uroko *et al.*, 2020.

Statistical analysis

The data obtained from the study were analyzed statistically one-way analysis of variance (ANOVA) and Duncan's multiple range comparison tests were performed on the means via Statistical Products and Service Solutions (SPSS) version 22.

The statistical significances of the analyzed data were established at a 95 % confidence level (P<0.05).

RESULT AND DISCUSSION

Results

Percentage yield of ethanol extract of *A. vogelii* stem bark (EASB)

The extraction of *A. vogelii* stem bark with ethanol gave a percentage yield of 12.2 % of the ethanol extract of *A. vogelii* stem bark (EASB) equivalent to 61 g of EASB.

Effects EASB on the serum creatinine concentrations of CCl₄-induced rats

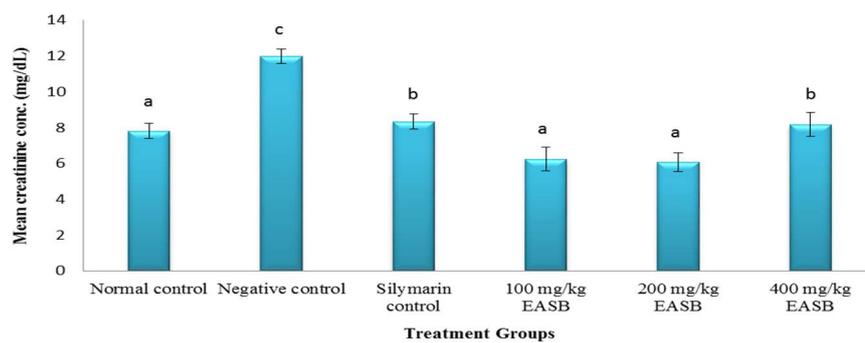
The result in Figure 1 showed CCl₄-induction caused a significant (P<0.05) increase in the serum creatinine concentrations in the rats but the treatment of the CCl₄-induced rats with 100, 200 and 400 mg/kg EASB/day respectively, resulted in significant (P<0.05) decline in the serum creatinine relative to the CCl₄-induced untreated rats. Treatment of CCl₄-induced rats with 100 and 200 mg/kg EASB/day respectively, effectively reversed the elevated serum creatinine concentrations to normal when compared with the normal control.

Effect EASB on the serum urea concentrations of CCl₄-induced rats

The serum urea concentration of the negative control rats that were CCl₄-induced but untreated was data significantly (P<0.05) elevated in comparison with the normal control unlike the CCl₄-induced rats treated with 100 and 200 mg/kg EASB/day respectively which exhibited no significant reduction in the serum urea concentrations (Figure 2). These showed that treated CCl₄-induced rats with 100 and 200 mg/kg EASB/day respectively were effective in reversing renal injury associated with CCl₄ toxicity in animals.

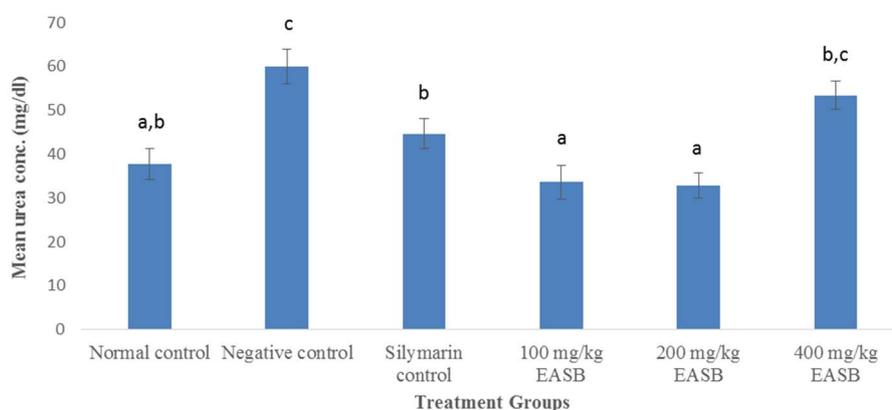
Effects of EASB on the serum total cholesterol (T. Chol) concentrations of CCl₄ induced rats

The CCl₄-induction elevated the serum total cholesterol concentrations in the negative control, silymarin control, and CCl₄-induced rats treated with 100, 200, and 400 mg/kg EASB/day respectively, when compared with the normal control (Figure 3). The CCl₄-induced treated with graded doses of EASB showed a further increase in the serum total cholesterol concentration relative to the CCl₄-induced untreated rats which suggest that EASB contributes to an increase in the serum cholesterol levels in rats.



Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 1. Serum creatinine concentrations of CCl₄-induced rats treated with EASB



Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 2. Serum urea concentrations of CCl₄-induced rats treated with EASB

Effects of EASB on the serum concentration of triacylglycerol (TAG) of CCl₄ induced rats

It was obvious in Figure 4 CCl₄ induction significantly ($p < 0.05$) elevated serum triacylglycerol (TAG) concentrations in the negative control, silymarin control, and CCl₄ induced rats treated with 100, 200, and 400 mg/kg EASB/day respectively, relative to the normal control. However, treatment of the CCl₄ induced rats with 100 and 400 mg/kg EASB/day respectively, significantly ($P < 0.05$) reversed the TAG concentrations in the treated rats when compared with the negative control.

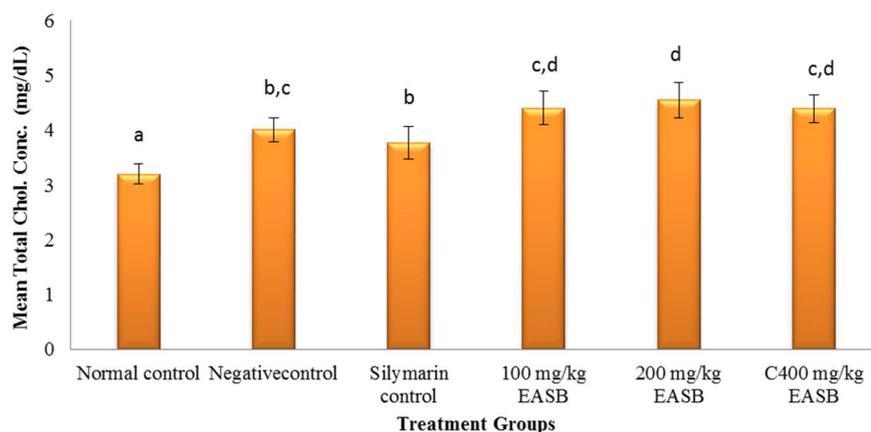
Effects of EASB on the high-density lipoprotein (HDL) concentrations of CCl₄ induced rats

The result in Figure 5 indicated a significant ($P < 0.05$) reduction in the serum high-density lipoprotein (HDL) concentrations of the negative control, silymarin control, and CCl₄ induced rats treated with 200 and 400 mg/k EASB/day respectively in comparison with the normal

control. Whereas, the CCl₄ induced rats treated with 100 mg/kg silymarin/day 100 mg/k EASB/day respectively, showed significantly ($P < 0.05$) elevated serum HDL concentrations when compared with the normal and negative controls.

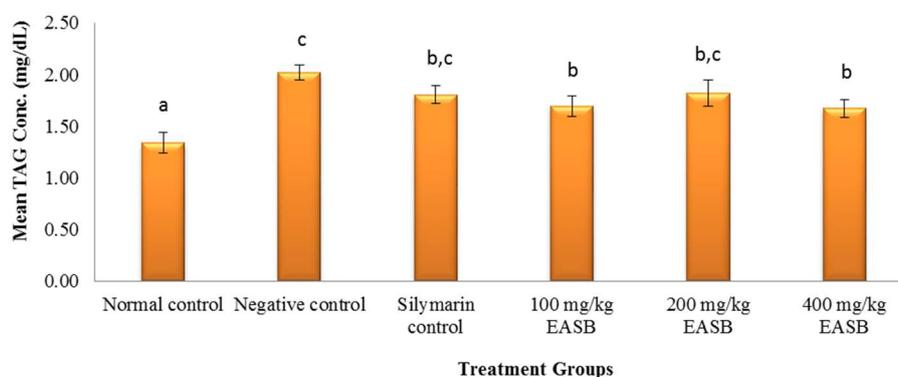
Effects of the EASB on the low-density lipoprotein (LDL) concentrations of CCl₄ induced rats

The result in Figure 6 indicated that the serum low-density lipoprotein (LDL) concentrations of the negative control, silymarin control, and CCl₄ induced rats treated with 100, 200 and 400 mg/kg EASB/day increased significantly ($P < 0.05$) relative to the normal control respectively. Treatments of the CCl₄ induced rats treated with 200 and 400 mg/kg EASB/day respectively significantly ($P < 0.05$) elevated the serum LDL concentrations when compared with the negative control unlike the silymarin treated CCl₄ induced rats.



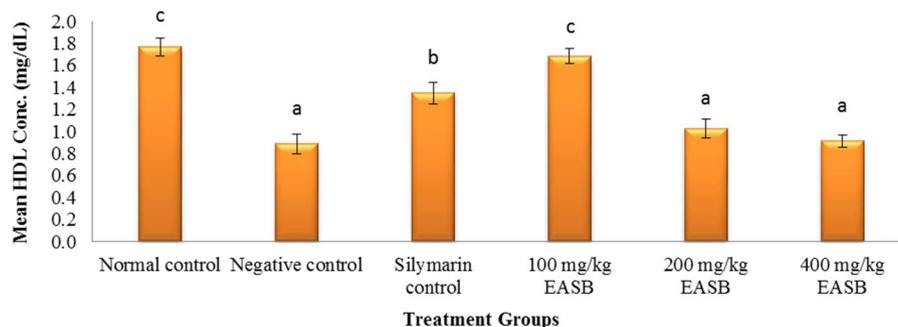
Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 3. Serum total cholesterol (T. Chol) concentrations of CCl_4 -induced rats treated with EASB



Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 4. Serum triacylglycerol (TAG) concentrations of CCl_4 -induced rats treated with EASB



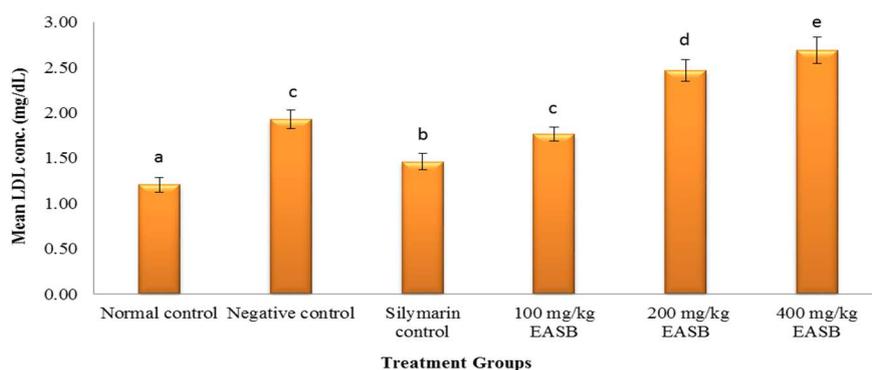
Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 5. Serum high-density lipoprotein (HDL) concentrations of CCl_4 -induced rats treated with EASB

Effects of EASB on kidney histomorphology of CCl_4 induced rats

Figures 7 – 12 show the effects of ethanol extract of *A. vogelii* on the kidney histomorphology in CCl_4 -induced Wistar rats. The kidneys collected from the rats in the normal control (Figure 7)

showed the normal renal histomorphology of normal laboratory rats, which comprises a normal Glomeruli (G) in normal Bowman's capsules (white arrow). In Figure 8, the kidney histomorphology of the untreated group (Group 2) indicated multifocal areas of renal tubular necrosis, tubular



Each bar represent mean \pm standard deviation (n = 5) and bars with the same superscripts are not significantly different ($P > 0.05$) from any paired mean.

Figure 6. Serum low-density lipoprotein (LDL) concentrations of CCl_4 -induced rats treated with EASB.

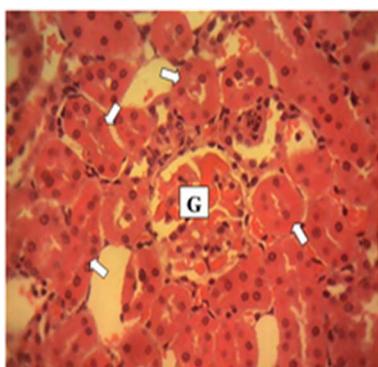


Figure 7. Kidney histomorphology of normal control

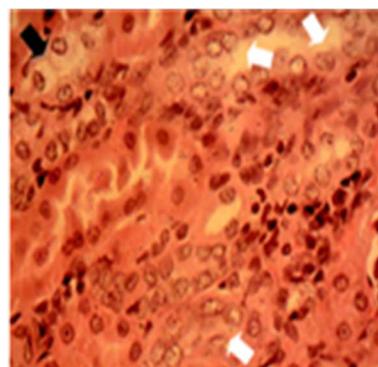


Figure 8. Kidney histomorphology of negative control

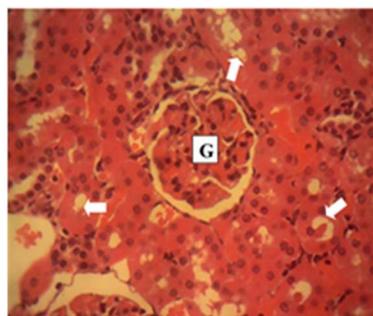


Figure 9. Kidney histomorphology of Silymarin control

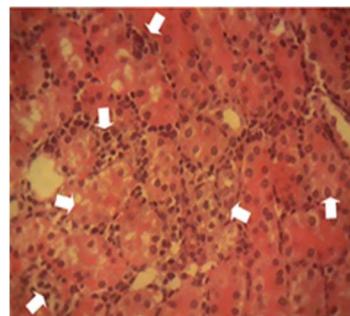


Figure 10. Kidney histomorphology of CCl_4 -induced rats treated with 100 mg/kg EASB/day

degeneration (black arrow), and regeneration (white arrow). The degenerating tubules indicated tubular lining epithelial cells with cloudy, vacuolated cytoplasm and relatively normal to hyperchromatic nuclear, whereas the regenerating cells indicated thin pale eosinophilic cytoplasm with large hyperchromatic nuclei. The Glomeruli were observed to be hemmed in by an avalanche of normal renal tubules in the cortex and the medulla. The kidney histomorphology of the positive control (Figure 9), and CCl_4 -induced rats treated with 100 and 200 mg/kg/day of EASB (Figures 10 and 11) respectively indicated the normal

structure of the renal histology of a normal laboratory rat. Multifocal areas of renal tubular necrosis with infiltration of inflammatory leucocytes were observed (arrow) in the CCl_4 -induced rats treated with 400 mg/kg/day of EASB (Figure 12).

Discussion

The study evaluated the renal protective and lipid profile modulatory effects of the ethanol extract of *A. vogelii* stem bark (EASB) in rats induced renal injury with carbon tetrachloride (CCl_4) administration. The kidney plays key

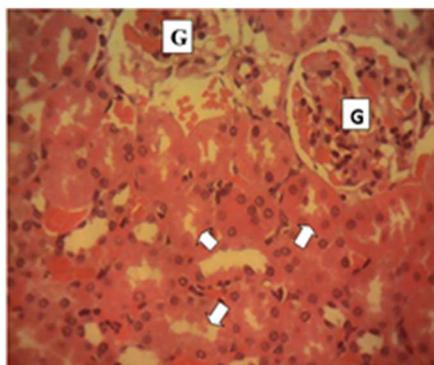


Figure 11. Kidney histomorphology of CCl₄-induced rats treated with 200 mg/kg EASB/day

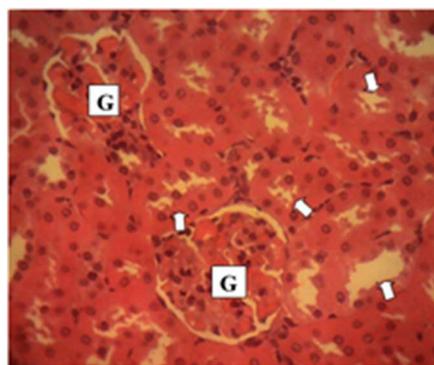


Figure 12. Kidney histomorphology of CCl₄-induced rats treated with 400 mg/kg EASB/day

functions in the body including maintaining a balanced constant internal environment, detoxification, and removal of some metabolites from the body via urine which predisposes it to the adverse effects of some toxic substances. Carbon tetrachloride is relatively nontoxic but when metabolized in the hepatocytes generates highly reactive metabolites including trichloromethyl and trichloromethyl peroxy radicals that can attack and elicit injury to various organs and tissues in the body including the kidney (Uroko *et al.*, 2020).

The high percentage yield of the extract obtained in this study suggests that the plant is rich in polar phytoconstituents extractable by polar solvent. Ethanol has been a polar solvent that can only extract polar compounds though some non-polar compounds may be present in trace amounts in the final extract obtained which is in agreement with the findings of Ncube *et al.* (2008).

The serum creatinine concentration is a very reliable glomerular filtration indicator and its rate of synthesis in the body approximately equals its rate of elimination and as such, the serum concentration of creatinine is considered inversely proportional to the prevailing glomerular filtration rate. The significantly elevated serum creatinine concentration of the negative control suggests a reduced glomerular filtration rate which could be associated with the renal injury caused by the CCl₄ in agreement with findings of Hsouna *et al.* (2011). The decreased glomerular rate caused a decline in the excretion of creatinine from urine and accumulation of creatinine in the blood. However, the significantly reduced serum creatinine concentrations in the silymarin control and CCl₄ induced rats treated with graded doses of EASB could be attributed to the renal protective and curative effects of silymarin and EASB respectively. The reductions in the serum creatinine concentrations of the silymarin and EASB treated groups showed the recovery of the rats from the

renal injury which resulted in improved glomerular filtration rate and improved renal function and agrees with findings of Hsouna *et al.* (2011) and Hermenean *et al.* (2013). The CCl₄ induced rats treated with 100 and 200 mg/kg EASB/day had the best recovery from renal injury and improved renal function depicted by very low serum creatinine concentrations slightly below the normal control and suggest that EASB has better therapeutic effects at lower concentrations.

The significantly elevated serum urea concentration in the negative control indicated the adverse health effects of CCl₄ on kidney function and its attendant renal impairment. The renal injury suffered by the negative control rats impaired the glomerular filtration rate and caused a decline in the ability of the kidney to excrete much of the urea via urine which is consistent with the findings of Hsouna *et al.* (2011). Although increased serum urea concentration could be due to its increased production, it may be applicable here as there is no evidence indicating that CCl₄ induces increased urea synthesis. The significant reductions of the serum urea concentration in the silymarin control, and all the CCl₄ induced rats treated with graded doses of EASB indicated significant recovery of the rats from the renal injury caused by the CCl₄ administration. The reduced serum urea concentration showed improvement in the glomerular filtration rats and possibly increased elimination of urea from the blood through urinary excretion in line with the findings of Hermenean *et al.* (2013). The EASB had better renal protective effects at lower doses (100 and 200 mg/kg EASB/day) than silymarin as it was able to restore serum urea concentration to a normal level not significantly below the normal control. This suggests that the EASB improved the glomerular filtration rate in the rats and these doses could be used to achieve much renal protection or curative instead of silymarin and

thus could be considered a close substitute to silymarin.

The serum total cholesterol is a component of the lipid profile found in the cell membranes and steroid hormones that are critical to maintaining normal biochemical functions in the body. The elevated serum total cholesterol concentrations in the negative control, silymarin control, and CCl₄ induced rats treated with graded doses of EASB could be attributed to the effects of CCl₄ toxicity that impaired the cholesterol metabolism. The CCl₄ could have damaged the hepatic cells there making them unable to metabolize the cholesterol and also partly due to insufficient serum albumin to transport them to the liver cells for catabolism. Treatments with either silymarin or graded doses of EASB were unable to improve cholesterol breakdown suggesting that there are serious adverse health effects experienced by the rats from the CCl₄ administration. The further elevation in the serum total cholesterol of CCl₄ induced rats treated with graded doses of EASB could be attributed to the effects of the phytochemical constituents of EASB on cholesterol such as saponins already shown to exhibit inhibitory effects on the cholesterol metabolism which is consistent with findings of Okwu (2001). The increased serum total cholesterol observed in the rats could predispose the rats to develop atherosclerosis and increase their risk for cardiovascular disorders. The uses of EASB for the management of renal impairment and other disorders should be monitored.

The significant elevation in the serum triacylglycerol (TAG) concentration observed in the negative control could be attributed to the toxic effects of CCl₄ metabolites on the lipase insulin-dependent enzyme which regulates the TAG concentrations. The reactive CCl₄ metabolites have the potentials of attacking and inactivating lipase through their free radical activities which inhibit lipase activity, prevent hydrolysis of TAG for catabolism, and its subsequent accumulation in the blood which is in agreement with the findings of Yost *et al.* (1995). The reductions in the serum TAG concentrations in the silymarin control and CCl₄ induced rats treated with graded doses of EASB relative to the negative control could be attributed to the therapeutic effects of the treatments that stimulated increased lipase synthesis or stabilized the activities of the circulating lipase enzymes. The increased lipase enzymes or stabilization of lipase could have increased TAG hydrolysis which resulted in the observed decrease in their serum TAG concentrations but neither silymarin nor EASB was

able to restore the serum TAG concentration to that of the normal control.

The decline in the serum HDL concentration in the negative control is associated with the adverse effects caused by CCl₄ toxicity in the rats. The low HDL concentration in the negative control may result in increased serum LDL concentration in the rats due to insufficient HDL concentration to transport LDL to the liver cells for metabolism thereby allowing LDL to accumulate in the blood. Impaired energy metabolism in the negative control rats could have led to the decreased serum HDL and subsequent increase in the serum TAG and LDL concentrations thereby making the rats prone to atherosclerosis and increased risk of cardiovascular disease (Singh and Rohatgi (2018). Besides, the significant increase in the serum HDL concentrations of the silymarin control and CCl₄ induced rats treated with 100 mg/kg EASB/day could be attributed to the effects of the treatments on the HDL metabolism possibly via up-regulation of glucose metabolism to ensure normal blood glucose level which promotes healthy amounts of HDL in the blood. The HDL concentration in CCl₄ induced rats treated with 100 mg/kg EASB/day would help to eliminate cholesterol and LDL from arterial walls and cells of non-hepatic tissues and return them to the liver for the metabolism which is in agreement with the findings of Duka *et al.* (2013). The high serum HDL concentrations in the silymarin control and CCl₄ induced rats treated with 100 mg/kg EASB/day will promote effective transport of LDL to the liver for catabolism and thus maintain normal serum LDL concentrations in the rats which would reduce their risk of cardiovascular diseases in line with findings of Singh and Rohatgi (2018). The no significant increase in the serum HDL concentrations of CCl₄ induced rats treated with 200 and 400 mg/kg EAS/day relative to negative control showed that EASB possesses insufficient therapeutic effects that could promote healthy levels of serum HDL concentrations in rats at higher doses. The renal injury caused by the CCl₄ toxicity could have contributed to the decline in the serum HDL concentration according to the findings of Shoji *et al.* (1997) that a decline in the renal function is associated with a decrease in the serum HDL levels. The inability of EASB to restore serum HDL concentration at higher doses suggests that the extract may contain trace amounts of phytochemicals that reduce serum HDL concentration when present in a relatively increased amount.

The elevated serum LDL concentration in the negative control suggests that CCl₄ causes accumulation of LDL in the blood and such rats

with increased serum LDL concentrations were exposed with increased risk of atherosclerosis due to the possibility of LDL clogging on the arterial walls thereby narrowing the walls and increasing the burden on the heart. This is consistent with the findings of Duka *et al.* (2013). Although there is a significant reduction in the serum LDL concentration in the silymarin control relative to the negative control, silymarin was unable to restore the serum LDL concentration to the normal control which suggests that the CCl₄ induction greatly caused an imbalance in the serum LDL concentration. The marked elevation in the serum LDL concentrations in the CCl₄ induced rats treated with graded doses of EASB could be attributed to the high serum TAG and low HDL concentrations observed in the rats. The EASB may have contributed to the increased serum LDL concentration in the rats by lowering the serum HDL concentrations which made HDL unable to transport LDL to the liver for catabolism. The increased serum LDL concentration and reduced serum HDL level caused an increased risk of cardiovascular diseases and its associated adverse health consequences in the rats as earlier demonstrated by Singh and Rohatgi (2018). The use of EASB for medicinal purposes should be monitored to prevent or minimize its dyslipidemic effects.

The kidney histomorphological changes together with the alterations in the kidney function parameters like serum urea, creatinine, and electrolyte concentrations are generally accepted as better indicators of renal function status. Carbon tetrachloride has been established to elicit significant alterations in the kidney histomorphology of animals and rodents like rats when administered appropriately and it's now used widely to induce renal injury in rats (Hermenean *et al.*, 2013). The multifocal areas of renal tubular necrosis, tubular degeneration, and regeneration observed in the kidney photomicrograph of the negative control rats suggest kidney injury due to the toxic effect of CCl₄ on the kidney histoarchitecture which further support the impaired renal functions in the rats. The adverse effects of CCl₄ on the kidney histomorphology of the negative control are consistent with the kidney histomorphological alterations observed by Hermenean *et al.* (2013) and Singh and Rohatgi (2018) respectively. However, the normal renal histomorphology consistent with a normal laboratory rat observed in the silymarin control, and CCl₄ induced rats treated with 100 and 200 mg/kg EASB/day showed that EASB possesses renal protective activities sufficient to ameliorate renal injury,

restore normal renal integrity and possibly improve renal functions. Also, the EASB possesses little or no renal protective and curative activity at a high dose as the kidney from the CCl₄ induced treated with 400 mg/kg EASB/day showed multifocal areas of renal tubular necrosis with infiltration of inflammatory leucocytes and suggests that EASB could not cure or ameliorate the renal injury at the administered dose.

CONCLUSION

The study revealed that the ethanol extract of *A. vogelii* stem bark (EASB) possesses renal protective properties most especially when administered at low doses as it could be counter-productive at higher doses. The EASB has positive effects on the lipid profile parameters concentrations but could raise the serum total cholesterol, triacylglycerol, and low-density lipoprotein concentrations when administered in higher doses. Thus, it is recommended that lower doses of EASB should be used for treating renal injury to maximize its therapeutic effects and prevents unhealthy lipid profile associated with its consumption in high dose.

CONFLICT OF INTERESTS

The authors have disclosed no conflict of interest.

REFERENCES

- Abraham, P.& Wilfred, G., 1999, 'Oxidative damage to the lipids and proteins of the lungs, testis, and kidney of rats during carbon tetrachloride intoxication', *Clin Chim Acta*. 289(1-2), 177-179.
- Alaribe, C.S., Coker, H.A., Shode, F.O., Ayoola, G., Adesegun, S.A., *et al.*, 2012, 'Antiplasmodial and phytochemical investigations of leaf extract of *Anthocleista vogelii* (Planch)' *J Nat Prod*. 5, 60-67.
- Albers, J.J., Warnick, G.R., Chenng, M.C., 1978, 'Quantitation of high-density lipoproteins', *Lipids*. 13(12), 926-932.
- Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W.F.P.C., Fu, P.C., 1974, 'Enzymatic determination of total serum cholesterol', *Clin Chem*. 20(4), 470-475.
- Anand, K.V., Anandhi, R., Pakkiyaraj, M., Geraldine, P., 2011, 'Protective effect of chrysin on carbon tetrachloride (CCl₄)-induced tissue injury in male Wistar rats. *Toxicol Ind Health*. 27(10), 923-933.
- Anyanwu, G.O., Onyeneke, E.C., Usunobun, U., Adegbeji, A.J., 2013. Impact of *Anthocleista vogelii* root bark ethanolic extract on weight reduction in high carbohydrate diet-induced

- obesity in male Wistar rats. *Afr J Biochem Res.* 7(11), 225-232.
- Anyanwu, P.I., Tarfa, F.D., Shamaki, D.E., Anyanwu, G.O., 2011, 'Chemical Composition, Antibacterial and Antifungal Activities of *Anthocleista vogelii* Planch Root Bark Extracts', In *IOP Conference Series: Earth and Environmental Science.* 210(1), 012006.
- Chukwu, C., Uroko, R., Egba, S., Adamude, F., Asadu, C., 2020, 'Effects of Combined Ethanol Extract of *Anthocleista Vogelii* and *Alstonia Boonei* Stem Barks on Liver Function Indices in Benign Prostatic Hyperplasia Induced Rats', *Niger J Pharm Sci.* 16(2), 191-201.
- Duka, A., Fotakis, P., Georgiadou, D., Katefides, A., Tzavlaki, K., *et al.*, 2013, 'ApoA-IV promotes the biogenesis of apoA-IV-containing HDL particles with the participation of ABCA1 and LCAT', *J Lipid Res.* 54(1), 107-115.
- Fawcett, J.K. & Scott, J., 1960, 'A rapid and precise method for the determination of urea', *J Clin Pathol.* 13(2), 156-159.
- Friedelwald, W.T., Levy, R.I., Fredrickson, D.S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge', *Clin Chem.* 18(6), 499-502.
- Henry, T.J., 1964, *Clinical chemistry principles and techniques* (2th ed.). New York: Harper and Row Publishers.
- Hermenean, A., Ardelean, A., Stan, M., Herman, H., Mihali, C.V., *et al.*, 2013, 'Protective effects of naringenin on carbon tetrachloride-induced acute nephrotoxicity in mouse kidney', *Chem Biol Interact.* 205(2), 138-147.
- Hsouana, A.B., Saoudi, M., Trigui, M., Jamoussi, K., Boudawara, T., Jaoua, S., El Feki, A., 2011, 'Characterization of bioactive compounds and ameliorative effects of *Ceratonia siliqua* leaf extract against CCl₄ induced hepatic oxidative damage and renal failure in rats' *Food Chem Toxicol.* 49(12), 3183-3191.
- Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A., Igoli N.P., 2005, 'Traditional medicine practice amongst the Igede People of Nigeria', Part II. *Afr J Tradit Complement. Altern Med.* 2(2), 134-152.
- Keay, R.W.J., Onochie, C.F.A., Stanfield, D.P., 1964, Nigerian tree. *Federal Department of Forestry Research*, Ibadan. Volume II. pp, 16-187.
- Ncube, N.S., Afolayan, A.J., Okoh, A.I., 2008, 'Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr J Biotechnol.* 7 (12), 1797-1806.
- NRC, 2010. *Guide for the Care and Use of Laboratory Animals.* 8th Edition, National Research Council (NRC), National Academies Press, Washington DC, USA.
- Ogamba, J.O., Eze, N.A., Ogamba, S.E., Chilaka, K.C.C., 2010, 'Phytochemical and biological activities of *Justicia secunda*', *Trop J Med Res.* 142 (1), 1-6.
- Okwu, D.E., 2001, 'Evaluation of the chemical composition of indigenous spices and flavouring agents', *J Sci.* 7, 455-459.
- Oladimeji, S.O., Igbalaye, J.O., Coleshowers, C.L., 2014, 'Immunological biomarkers determined in female rats administered with pro-fertility extract of *Anthocleista vogelii*', *J Nat Sci Res.* 4(8), 113-123.
- Osadebe, P.O., Uzor, P.F., Omeje, E.O., Agbo, M.O., Obonga, W.O., 2014, 'Hypoglycemic activity of the extract and fractions of *Anthocleista vogelii* (Planch) stem bark', *Trop J Pharm Res.* 13, 1437-1443.
- Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale (PROTA), Wageningen, Netherlands *Anthocleista vogelii*.
<<http://www.prota4u.org/search.asp>>. Accessed 11 December, 2020.
- Powell, S.R. 2000, 'The antioxidant properties of zinc', *J Nutr.* 130, 1447S-1454S.
- Shoji, T., Nishizawa, Y., Kawagishi, T., Tanaka, M., Kawasaki, K., *et al.*, 1997, 'Atherogenic lipoprotein changes in the absence of hyperlipidemia in patients with chronic renal failure treated by hemodialysis', *Atherosclerosis.* 131: 229-236.
- Singh, K., Rohatgi A., 2018, 'Examining the paradox of high high-density lipoprotein and elevated cardiovascular risk', *J Thorac Dis.* 10(1), 109-112.
- Sunday, R.M., Ilesanmi, O.R., Obuotor, E.M., 2016, 'Anti-diabetic effect of *Anthocleista vogelii* ethanolic root extract in alloxan-induced diabetic rats. *Res J Med Plant.* 10(1), 79-88.
- Uroko, R.I., Sangodare R.S.A., Onyeabo C., Agbafor A., Uchenna O.N., *et al.*, 2020, 'Investigation of antioxidant compositions and antioxidative activities of ethanol extract of *Alstonia boonei* stem bark', *Niger J Pharm Sci.* 16(1), 71-80.