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Evaluation of *in vitro* antioxidants activities, hepatoprotective and haematological effects of ethanol extract of *Anthocleista vogelii* stem bark (AVSB) on carbon tetrachloride (CCl_4) induced rats

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ABSTRACT: *Anthocleista vogelii* stem bark extract (AVSB) is used as a hepatoprotective protective and blood-boosting agent locally without any scientific evidence. This study evaluated the *in vitro* antioxidants activity, hepatoprotective and haematological effects of ethanol extract of AVSB on carbon tetrachloride (CCl_4) induced rats. Thirty rats distributed into 5 groups (n = 6) were used. Group 1 was the normal control rats without CCl_4 induction, group 2 was the CCl_4 control (CCl_4 induced untreated rats) while groups 3 – 6 were CCl_4 induced rats treated with 100 mg/kg/day of silymarin, 100, 200 and 500 mg/kg/day of AVSB respectively for 14 days. The results indicated high levels of total phenols, flavonoid, vitamin E, vitamin C, β-carotene and lycopene in the AVSB. The AVSB exhibited a dose-dependent increase in ferric reducing antioxidant power, DPPH and nitric oxide radicals scavenging activities similar to vitamin C, rutin and curcumin respectively. The CCl_4 induction significantly ($P<0.05$) elevated total bilirubin concentrations, serum ALT, AST and ALP activities and significantly ($P<0.05$) decreased PCV, RBC, WBC, platelet counts, and Hb, total protein and albumin concentrations and caused liver necrosis in the untreated rats relative to the normal control. Treatment with AVSB significantly reversed the altered serum ALT, AST and ALP activities, total bilirubin, total protein, albumin and Hb concentrations including haematological indices and liver histomorphology to normal compared with the CCl_4 control. The findings of this study indicated that the AVSB is rich in antioxidant components, *in vitro* antioxidant activity, and possesses hepatoprotective and improves haematological indices

KEYWORDS: *Anthocleista vogelii;* antioxidant activity; carbon tetrachloride; hepatoprotective; haematological indices.

1. INTRODUCTION

The liver is a very active organ responsible for the metabolism and detoxification of drugs and xenobiotics in the body, these predispose it to injury or oxidative attack from reactive metabolites generated from phagocytes, metabolism of prooxidants and incomplete transfer of electrons to oxygen in the electron transport chain. Insufficient amounts of antioxidants in the body to scavenge free radicals leads to oxidative stress, which could cause harmful effects on membranes, DNA, tissues, organs and other biomolecules [1]. It has been reported that oxidative stress could cause alcoholic liver disease and other liver disorders [2]. Plant extracts rich in antioxidants like phenols, flavonoids, β-carotene, vitamins C and E are capable of scavenging excess free radicals and reduce their levels to minimal concentrations and eventually ameliorate oxidative stress-induced tissues and cellular damage. They prevent oxidative stress-induced tissue damage because of their antioxidant properties that enable them, chelate metals, and reduce oxidants through the donation of hydrogen atoms and quenching of singlet oxygen [3]. Liver injury aside from damaging the integrity of the hepatocytes, impairs liver functions like protein synthesis, detoxification and metabolic functions carried out by the liver. To prevent liver damage and maintain normal liver functions, the health status of the liver could be indirectly monitored by measuring the serum activities of aspartate transaminase (AST), lactate dehydrogenase (LDH), alanine transaminase (ALT), and alkaline phosphatase (ALP) addition to total protein and bilirubin concentrations.

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Carbon tetrachloride is a well-known hepatotoxicant employed for the induction of liver injury in animals to enable investigations of hepatoprotective efficacy of synthetic drugs and traditional medicines against hepatic diseases. It induces hepatic injury and sometimes kidney injury by the oxidative attack of trichloromethyl (CCl_3•) and trichloromethyl peroxyl (Cl_3COO•) radicals produced from its metabolic break down in the liver by the cytochrome P450 enzymes [4, 5]. The endogenous antioxidant enzymes and non-enzymatic antioxidants present in the body fight to prevent oxidative stress and their associated adverse health effects but are overwhelmed under the condition of oxidative stress and leave the individual vulnerable to oxidative attack and damage. Treatment with antioxidant drugs like silymarin and plant extracts with high antioxidant activities reduce oxidative stress but the use of medicinal plants rich in antioxidant components and activities are on the increase because they have been found to possess a low risk of adverse health effects, cheap and readily available.

Anthocleista vogelii Planch commonly called the English cabbage tree belongs to the family of *Loganiaceae* and is used in traditional medicine because of its high therapeutic potentials against many diseases. *A. vogelii* extracts are useful in the treatment of various diseases including diabetes, fever, malaria, hypertension, stomach aches, indigestion, obesity, oxidative stress and bacterial infections such as typhoid and syphilis [6, 7]. The root and stem bark of *A. vogelii* possess pharmacological activities such as antiviral activities, anti-snake venom, anti-oedema and induce contraction for labour and abortion and reduce menstrual cycle pain [6]. The mixture of *A. vogelii* leaves and stem bark is a common contraceptive, anti- infertility and hepatoprotective herbal formulation in local traditional medicine, in Nigeria but there are little pieces of evidence to support these claims. The medicinal properties of *A. vogelii* extract are due partly to the pharmacological activities of the phytochemicals like glycosides, steroids, saponins, terpenoids, alkaloids and flavonoids found in the extract [8, 9]. This study evaluated the *in vitro* antioxidants activity, hepatoprotective and haematological effects of ethanol extract of *A. vogelii* stem bark (AVSB) on carbon tetrachloride (CCl_4) induced rats. The results of this study help in further understanding the medicinal properties of *A. vogelii* and validations of some of its therapeutic claims by local traditional medicine users.

2. RESULTS

2.1 Antioxidant contents in the AVSB

The data in table 1 indicated high levels of antioxidant constituents in the AVSB with the total phenolic and lycopene as the highest and least available antioxidant constituents in the AVSB respectively. Flavonoids were present in high concentration but lower than the total phenolic content. The trends of antioxidant levels in AVSB indicated that total phenolic > flavonoid > vitamin E > vitamin C > β-carotene > lycopene.

Table 1. Antioxidant constituents of AVSB.

Antioxidant constituents	Bioavailability (mg/100g)
Total phenolic	3120.76 ± 5.10
Flavonoid	1175.54 ± 7.53
β-carotene	1.58.52 ± 0.01
Lycopene	0.88.40 ± 0.01
Vitamin E	189.32 ± 0.05
Vitamin C	102.32 ± 0.87

Values are presented as mean ± standard deviation (n = 3)

2.2 Ferric reducing antioxidant power (FRAP) of AVSB

The AVSB indicated a dose-dependent increase in ferric reducing antioxidant power similar to vitamin C and rutin but much lower than either vitamin or rutin (Figure 1). The AVSB showed the highest ferric reducing antioxidant power at 1 mg/ml concentration like the rutin and vitamin C respectively.

2.3 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of AVSB

In Figure 2, the ethanol extract of AVSB exhibited dose-dependent 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities in comparison with the DPPH scavenging activities of vitamin C (standard antioxidant). The AVSB had the highest percentage DPPH inhibition of 54.36 % at 100 µg/ml concentration far below the 89.16 % recorded for vitamin C at the same concentration. The concentrations of AVSB and vitamin C required to achieve 50% inhibition of DPPH radicals (IC50) were 49 and 36 µg/ml respectively.

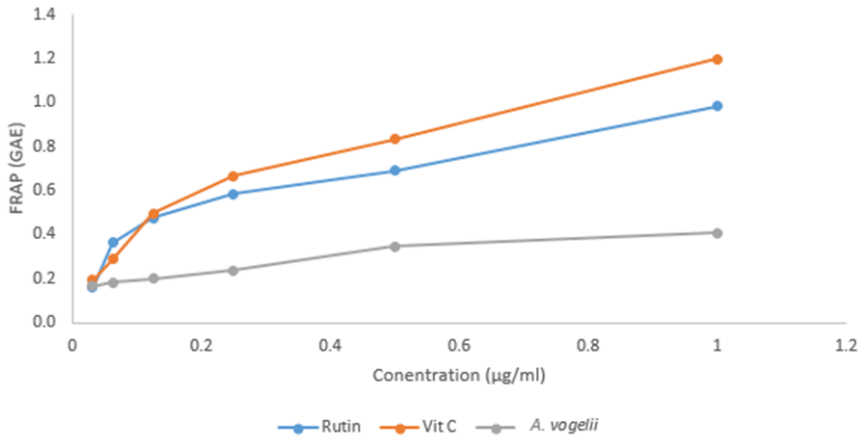

Figure 1. Ferric reducing antioxidant power of *A. vogelii*, vitamin C and rutin.

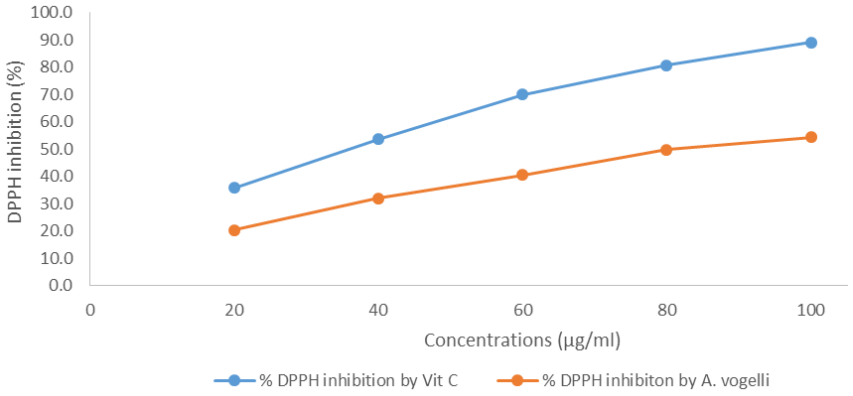

Figure 2. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of AVSB.

2.4 Nitric oxide scavenging activities of AVSB

The data in Figure 3 indicated a dose-dependent increase in nitric oxide scavenging activities of the AVSB relative to curcumin a standard antioxidant compound. The least (4.14 %) and highest (55.45 %) nitric oxide inhibition by the AVSB occurred at 20 and 100 µg/ml respectively, which were relatively lower than 17.44 and 82.64 % exhibited by curcumin at the corresponding concentrations. The AVSB had an IC50 (concentration required to achieve 50% inhibition of nitric oxide) of 62.80 µg/ml, which was higher than the IC50 value of 56 µg/ml recorded for curcumin.

2.5 Effects of AVSB on the alanine transaminase (ALT) activities of (CCl_4 induced rats

The carbon tetrachloride (CCl_4) induced rats showed significantly ($P < 0.05$) elevated alanine transaminase (ALT) activities when compared with the normal control rats, with the CCl_4 control rats having the highest ALT activities relative to the CCl_4 induced rats treated with silymarin and AVSB respectively (Figure 4). The CCl_4 induced rats treated with 100 mg/kg/d of silymarin, 200 and 500 mg/kg/d of AVSB respectively showed significant ($P < 0.05$) reductions in the ALT activities in comparison with the CCl_4 control. Besides, the CCl_4 induced rats treated with 100 mg/kg/d of AVSB showed no significant ($P > 0.05$) reduction in ALT activities when compared with the CCl_4 control.

2.6 Effects of AVSB on the aspartate transaminase (AST) activities of CCl_4 induced rats

The CCl_4 induction caused significant ($P < 0.05$) increases in the aspartate transaminase (AST) activities in the rats relative to the normal control (Figure 5). Treatment of the CCl_4 induced rats with 100 mg/kg/d of silymarin, 200 and 500 mg/kg/d of AVSB, respectively significantly ($P < 0.05$) decreased the AST activities of the respective rat groups relative to the CCl_4 control. The CCl_4 induced rats treated with 200 mg/kg/d of AVSB showed no significant ($P > 0.05$) increase in the AST activity when compared with the normal control while the CCl_4 induced rats treated with 500 mg/kg/d of AVSB showed significant ($P < 0.05$) reduction in the AST activity relative to the normal control. Besides, the CCl_4 induced rats treated with 100 mg/kg/day of AVSB showed no significant ($P > 0.05$) reduction in the AST activity when compared with CCl_4 control.

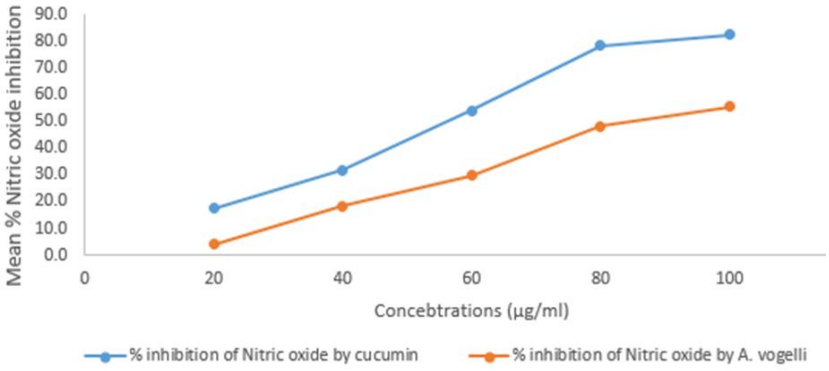

Figure 3. Percentage of nitric oxide inhibition by AVSB.

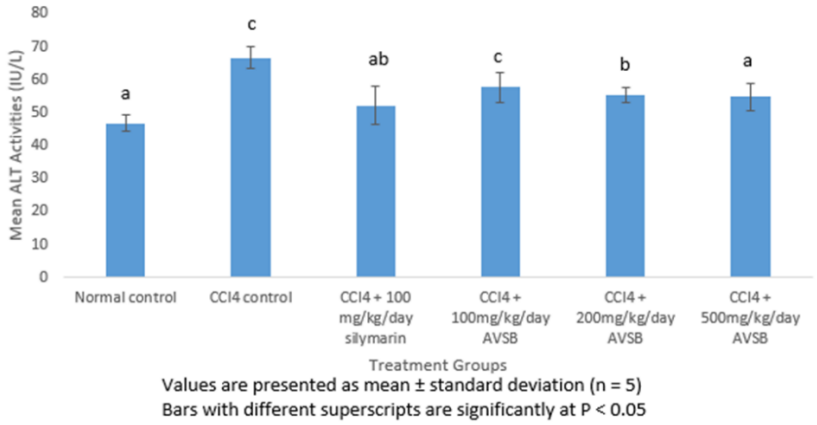

Figure 4. Alanine transaminase activities of CCl_4 induced rats treated with AVSB.

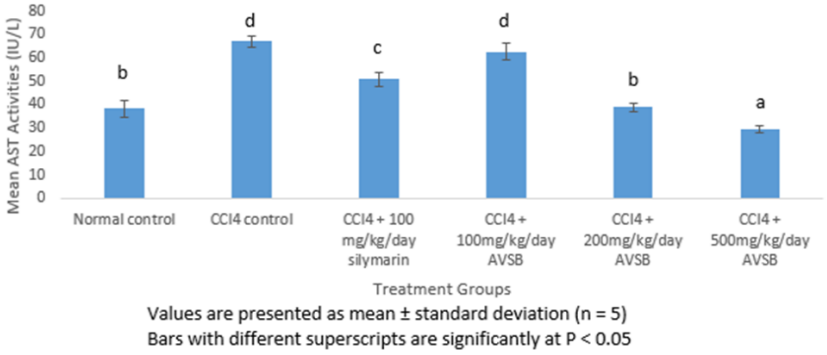

Figure 5. Aspartate transaminase activities of CCl_4 induced rats treated with AVSB.

2.7 Effects of AVSB on the alkaline phosphatase (ALP) activities of CCl_4 induced rats

The data in Figure 6 indicated significantly ($P < 0.05$) increases in the alkaline phosphatase (ALP) activities of the CCl_4 induced rats when compared with the normal control. Treatment of the CCl_4 induced rats with graded doses of AVSB caused dose-dependent significant ($P < 0.05$) decreases in the ALP activities relative to the CCl_4 control. The ALP activity of the CCl_4 induced rats treated with 100 mg/kg/d of silymarin decreased significantly ($P < 0.05$) when compared with the CCl_4 control but significantly ($P < 0.05$) higher than the normal control. The CCl_4 induced rats treated with 500 mg/kg/d of AVSB had the highest reduction in the ALP activity been non-significantly higher than the normal control.

2.8 Effects of AVSB on the total protein concentrations of CCl_4 induced rats

The CCl_4 induction caused significant ($P < 0.05$) reductions in the total protein concentrations of the rats as indicated in the CCl_4 control when compared with the normal control (Figure 7). The CCl_4 induced rats

treated with 100 mg/kg/d of silymarin and graded doses of AVSB respectively indicated a significant ($P < 0.05$) increase in the total protein concentrations when compared with the CCl_4 control with that of the CCl_4 induced rats treated with 500 mg/kg/d been significantly ($P < 0.05$) elevated relative to the normal control.

2.9 Effects of AVSB on the albumin concentrations of CCl_4 induced rats

The results in Figure 8 showed that CCl_4 induction caused significant ($P < 0.05$) reductions in the serum albumin concentrations of the CCl_4 induced rats as indicated in the CCl_4 control when compared with normal. The albumin concentrations of the CCl_4 induced rats treated with 100 mg/kg/d of silymarin, 100 and 200 mg/kg/d of AVSB respectively showed significant ($P < 0.05$) increases when compared with the CCl_4 control and no significant ($P > 0.05$) relative to the normal control. Also, the CCl_4 induced rats treated with 500 mg/kg/d of AVSB showed a significant ($P < 0.05$) increase in the albumin concentrations when compared to the normal control, CCl_4 control and silymarin treated groups respectively.

2.10 Effects of AVSB on the total bilirubin concentrations of CCl_4 induced rats

The total bilirubin concentrations in Figure 9 indicated that CCl_4 induction significantly ($P > 0.05$) elevated total bilirubin concentrations in the CCl_4 control when compared with normal control. The CCl_4 induced rats treated with 100 mg/kg/d of silymarin, and graded doses of the AVSB respectively showed a significant ($P < 0.05$) decrease in the total bilirubin concentration when compared with CCl_4 control but significantly ($P < 0.05$) higher than the normal control.

2.11 Effects of AVSB on the haematological indices of CCl_4 induced rats

The percentage packed cell volume (PCV) in Table 2 indicated significant ($P < 0.05$) reductions in the PCV of the CCl_4 induced rats relative to the normal control. However, the CCl_4 induced rats treated with graded doses of AVSB showed no significant ($P > 0.05$) decreases in the PCV when compared with the normal control and CCl_4 control respectively while the CCl_4 induced rats treated with 100 mg/kg/d of silymarin indicated no significant ($P > 0.05$) increase in PCV relative to the normal control.

The white blood cell (WBC) counts of the CCl_4 control, CCl_4 induced rats treated with 100 mg/kg/d of silymarin, and 500 mg/kg/d of the AVSB respectively significantly ($P < 0.05$) decreased when compared with the normal control (Table 2). However, the CCl_4 induced rats treated with 100 and 200 mg/kg/d of AVSB respectively showed no significant ($P > 0.05$) increase in the WBC counts relative to the normal control. The rats induced CCl_4 and treated with 100 and 200 mg/kg/d of AVSB respectively showed a significant ($P < 0.05$) increase in the WBC counts when compared with the CCl_4 control. Besides, the CCl_4 induced rats treated with 100 mg/kg/d of silymarin and 500 mg/kg/d of AVSB, respectively showed significant ($P < 0.05$) decreases in the WBC counts relative to the CCl_4 control.

The red blood cell (RBC) counts in Table 2 indicated a significant ($P < 0.05$) decrease in the RBC counts of the CCl_4 induced rats (CCl_4 control) relative to the normal control rats. The CCl_4 induced rats treated with 100 mg/kg/d of silymarin and 500 mg/kg/d of AVSB respectively showed a significant ($P < 0.05$) increase in RBC counts when compared with the CCl_4 control. However, the CCl_4 induced rats treated with 100 and 200 mg/kg/d of AVSB respectively showed no significant ($P > 0.05$) increase in RBC counts relative to the CCl_4 control.

In Table 2, the haemoglobin (Hb) concentrations of CCl_4 induced untreated rats (CCl_4 control) showed a significant ($P < 0.05$) decreased when compared with the normal control. The CCl_4 induced rats treated with 100 mg/kg/d of silymarin, 200 and 500 mg/kg/d of AVSB respectively showed significant ($P < 0.05$) increase in Hb concentrations when compared with the CCl_4 control and no significant ($P > 0.05$) increase in Hb concentration relative to the normal control. Besides the CCl_4 induced rats treated with 100 mg/kg/d of AVSB showed no significant ($P > 0.05$) increase in Hb concentration when compared with CCl_4 control.

The CCl_4 induced untreated rats in Table 2 indicated a significant ($P < 0.05$) decrease in the platelet count when compared with the normal control. The platelet counts of CCl_4 induced rats treated with silymarin and graded doses of AVSB respectively showed a significant ($P < 0.05$) increase when compared with the CCl_4 control. The CCl_4 induced rats treated with 100 mg/kg/d of silymarin, 100, 200 and 500 mg/kg/d of AVSB respectively showed no significant ($P > 0.05$) decreases in platelet counts relative to the normal control.

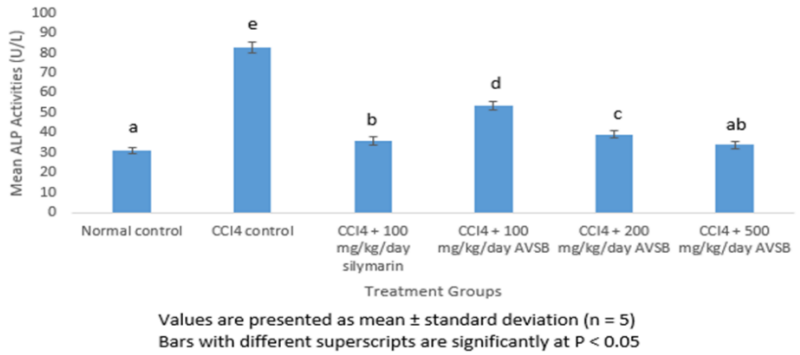

Figure 6. Alkaline phosphatase activities of CCl_4 induced rats treated with AVSB.

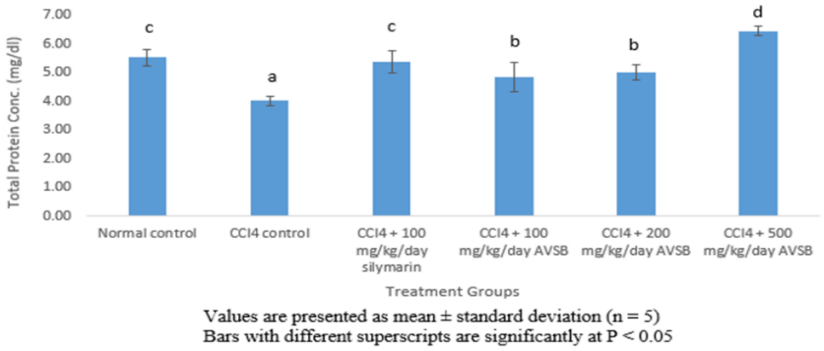

Figure 7. Total protein concentrations of CCl_4 induced rats treated with AVSB.

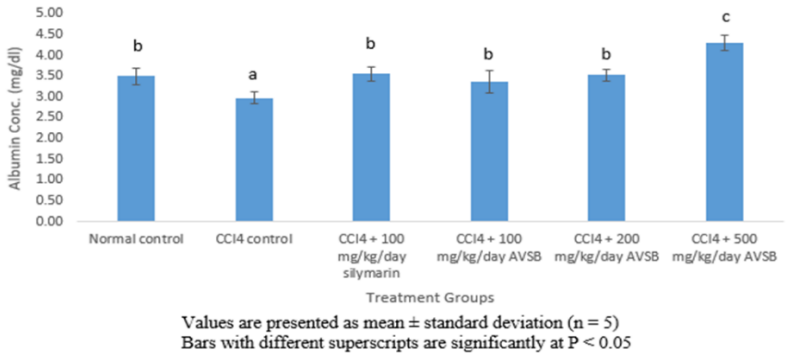

Figure 8. Albumin concentrations of CCl_4 induced rats treated with AVSB.

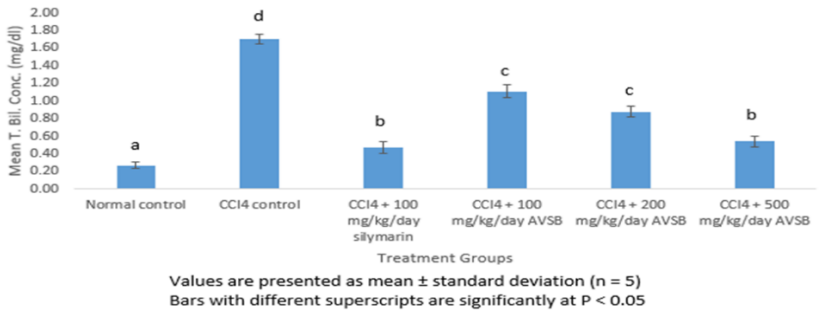

Figure 9. Total bilirubin concentrations of CCl_4 induced rats treated with AVSB.

Table 2. Haematological indices of carbon tetrachloride-induced rats treated with AVSB.

Treatment Groups	PCV %	WBC $X10^9/L$	RBC $X10^{12}/L$	HB g/dL	Platelets $X10^{12}/L$
Normal control	45.00±4.36^b	6.73±0.31^c	4.92±0.16bc	16.80±0.20^{bc}	5.61±0.40^b
CCl_4 control	36.67±3.06^a	5.73±0.23^{ab}	4.83±0.17a	16.20±0.21^a	3.07±0.12^a
CCl_4 + 100 mg/kg/day silymarin	49.00±1.00^b	5.13±0.31^a	4.95±0.06c	17.00±0.53^c	4.87±0.31^b
CCl_4 + 100 mg/kg/day AVSB	43.67±8.96^b	6.81±0.20^c	4.89±0.24^{ab}	16.33±0.23^{ab}	4.74±0.10^b
CCl_4 + 200 mg/kg/day AVSB	44.32±3.46^b	6.13±0.31^b	4.87±0.09ab	17.03±0.25^c	5.03±0.25^b
CCl_4 + 500 mg/kg/day AVSB	43.56±1.75^b	5.40±0.53^a	4.95±0.08^c	17.04±0.22^c	5.33±0.21^b

Values are presented as mean ± standard deviation (n = 5); Values with different superscripts are significantly different at $P < 0.05$.

2.12 Effects of AVSB on the liver histomorphology of CCl_4 induced rats

The photomicrograph showing the liver histomorphology of normal control rats in Figure 10a indicated the normal hepatic histology for laboratory rodents. The liver section showed numerous partially demarcated hepatic lobules consisting of hepatocytes arranged in interconnecting cords around the central veins (V). The cords radiate towards the periphery of the hepatic lobule where it meets with the components of the portal areas (P) (Hepatic artery; hepatic vein and bile ductile). The hepatic lobules also showed clear hepatic sinusoids. The liver photomicrograph of the liver from the carbon tetrachloride-induced untreated rats manifested a widespread random area of necrosis with infiltration of mononuclear cells (white arrow) and moderate periportal infiltration of inflammatory leucocytes (black arrow) as shown in Figure 10b. The liver photomicrograph of carbon tetrachloride-induced rats treated with 100 mg/kg/d of silymarin in Figure 10c indicated normal hepatic histology of a laboratory rodent with a central vein (V) and portal area (P). However, the liver photomicrographs in Figures 10d and 10e showed the liver histomorphology of carbon tetrachloride-induced rats treated with 100 and 200 mg/kg/d of AVSB respectively. The liver photomicrograph of CCl_4 induced rats treated with 100 mg/kg/d of AVSB extract showed a mild infiltration of mononuclear leucocytes around the portal areas (arrow) indicated with a letter (P) (Figure 10d). Similarly, the rat liver from CCl_4 induced rats treated with 200 mg/kg/d of AVSB extract showed multifocal random aggregations of inflammatory leucocytes (white arrow) in the hepatic parenchyma. The letters (V) and (P) represent the central vein and portal area respectively. The liver photomicrograph of a liver section from CCl_4 induced rats treated with 500 mg/kg/d of AVSB extract showed the normal hepatic histology of a normal laboratory rodent (Figure 10f).

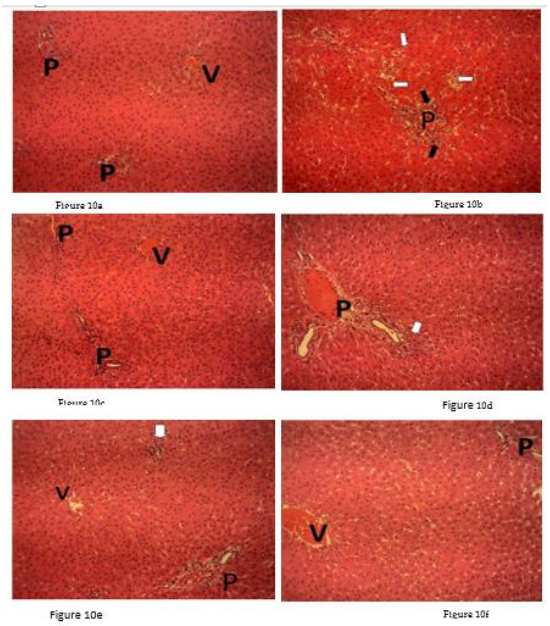

Figure 10. Figures 10a – d are the photomicrographs of the liver sections from the normal control, CCl_4 control, rats induced CCl_4 treated with 100 mg/kg/d of silymarin, and CCl4 induced rats treated with 100, 200 and 500 mg/kg/d of AVSB respectively.

3. DISCUSSION

This study evaluated the antioxidant composition, *in vitro* antioxidant activities of AVSB and its hepatoprotective and haematological effects on the CCl_4 induced rats. CCl_4 is a potent hepatotoxicant that could severely damage the liver and impair its functions because of the toxic effects of highly reactive trichloromethyl (CCl_3.) and trichloromethyl peroxyl radicals generated from CCl_4 breakdown in the body [23]. These reactive free radicals are capable of eliciting lipid peroxidation and oxidative damage to various biomolecules and organs especially the liver that adversely affects the stability, integrity and functions. Medicinal plant extracts rich in antioxidant components are effective in the management of oxidative stress and protecting the liver cells from hepatotoxicants and oxidative damage.

The antioxidant phytochemicals inhibit free radical attack on biomolecules and oxidative damage and could help to prevent the toxic effects of CCl_4 on hepatocytes and promote good health. The high levels of antioxidant components including total phenols and flavonoids detected in the AVSB suggest that the extract could be rich in antioxidant properties that would prevent lipid peroxidation and oxidative damage associated with the excess free radical attack on biomolecules. The antioxidant components present in the AVSB could supplement the endogenous antioxidants in preventing oxidative stress and inducing repair of cells and organs damaged by free radicals in line with findings of Jaouad and Torsten [24]. The high flavonoid contents in AVSB could contribute much to its antioxidant activity possibly by chelating reactive metals or by scavenging excess reactive radicals, which prevents oxidative damage and are in line with the mechanism of antioxidant activity earlier suggested [25]. Plant extracts rich in β-carotene, and lycopene like AVSB possess antioxidant activities to scavenge free radical and reduce oxidative stress-related diseases like various cancer, diabetes, ageing and cardiovascular diseases is in line with the previous findings [26]. Vitamin C is one of the most commonly consumed vitamins that have antioxidant activity due to its ability to donate an electron to reactive free radicals thereby making them stable and help regeneration of vitamin E (α-tocopherol) in the body. Thus, the high content of vitamin C in AVSB could be beneficial in the management of oxidative stress and promote a healthy life. The high levels of antioxidant components in the AVSB could effectively scavenge free radicals and inhibit oxidative damage to biomolecules and injuries to various tissues and organs in the body [25].

The dose-dependent increases observed in the absorbance with the increasing concentrations of the AVSB indicated a reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) possibly via hydrogen donation from the antioxidant components in the extract. The ferric reducing antioxidant power of AVSB could be attributed to antioxidant components like flavonoids, ascorbic acid, β-carotene and vitamin E present in large amounts in the extract. The ferric reducing power of the extract was markedly low relative to rutin and vitamin C respectively suggesting that there may be other phytochemicals present in the extract that inhibit its antioxidant activity. Plant extracts with antioxidant activity like the AVSB have been shown to reduce the risk of oxidative stress associated diseases like diabetes, cardiovascular and neurodegenerative diseases.

The DPPH is a stable radical commonly employed in the investigation of *in vitro* antioxidant activity of bioactive compounds like flavonoids, phenols, vitamins C and E because they can reduce DPPH radical to their respective hydrazine by donating an electron to the unpaired nitrogen atom. It is one of the most reliable means of evaluating antioxidant activities of plant extracts, drugs and foods based on their ability to scavenge free radicals by serving as hydrogen donors. The dose-dependent DPPH radical scavenging activity of the AVSB indicated that the extract possesses significant free radical scavenging activity, especially at higher doses, which are in line with the previous findings [27]. The extract could serve as a potent antioxidant by donating a hydrogen atom to unpaired free radicals like vitamin C and other antioxidant compounds. The lower DPPH scavenging activity demonstrated by the extract at each of the concentrations tested when compared with vitamin C indicated interference by other phytoconstituents present in the extract. The high IC50 value obtained for the extract relative to vitamin C further indicated that increased doses of the extract are required to achieve effective DPPH scavenging activity comparable to vitamin C.

Nitric oxide is a multifunctional second messenger generated from the conversion of arginine to citrulline by the action of arginine synthase in endothelial cells, neurons and phagocytes. Due to the presence of unpaired electron in nitric oxide, it functions as a free radical and reacts with superoxide radicals to give a very reactive peroxynitrite anion that is highly toxic to the biological system [28]. The nitric oxide plays important physiological roles in the regulation of blood pressure, erection in men and inflammatory reactions but when present in high concentration could induce oxidative stress that damage DNA, membrane integrity, tissues, and organs in line with the report of Dzoyem and Eloff [29]. The dose-dependent increase in the nitric oxide scavenging activity demonstrated by the AVSB could be attributed to its rich antioxidant components

like flavonoids, phenols, vitamins C and E which are in agreement with the previous report that plant extracts rich in flavonoids and phenols are potent scavengers of nitric oxide [30]. The high level of flavonoids, phenols, β-carotene, lycopene, vitamins C and E present in the AVSB possess redox properties that enable them to function as reducing agents via electron donation and metal chelation. These could be responsible for nitric oxide scavenging and other antioxidant activities exhibited by the plant extract in this study in line with the findings of Pietta [31]. The nitric oxide scavenging activity of the AVSB was lower than curcumin, which suggests that the extract contained other non-antioxidant components that interfered with its antioxidant activity.

The marked increases in the activities of serum hepatic marker enzymes including alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) indicated hepatotoxic effects of carbon tetrachloride (CCl_4) in the rats induced without any treatment and agrees with earlier findings [32, 33]. The serum ALT, AST and ALP activities are usually insignificant due to their very low concentrations in the extra-hepatic environment under normal health conditions and their increased activities occur mainly when there are hepatic injuries. This is because they can easily leak outside hepatocytes due to damaged hepatic membrane and increased permeability. The toxic effects of CCl_4 induction caused could have damaged liver injury, compromised liver integrity and architecture and elevated their concentrations and activities in the serum. However, kidney and heart tissues could have contributed to increased AST and ALP activities observed in the rats as CCl_4 has shown to have adverse effects on these organs as such their increased activities in the serum are a relatively less specific indicator of hepatic injury, unlike ALT activity.

The dose-dependent reductions in the serum ALT, AST and ALP activities in the CCl_4 induced rats treated with silymarin and AVSB respectively showed that AVSB has hepatoprotective effects similar to that of silymarin, which improved hepatic integrity and prevented leakage of these enzymes to the extrahepatic tissues. The reductions of ALT, AST and ALP activities are consistent with the findings that reductions in the activities of the hepatic enzyme of CCl_4 induced rats are an indication of recovery from hepatic injury [34]. The treatment with AVSB could have improved hepatic integrity by scavenging reactive free radicals like trichloromethyl and trichloromethyl peroxyl radicals generated from biotransformation of CCl_4 in the rats that are responsible for the oxidative stress that damage hepatocytes and impair their functions. The high antioxidant components like phenols, flavonoids, β-carotene and lycopene could scavenge free radicals, dilute the adverse effects of many oxidants, and not limited to trichloromethyl and trichloromethyl peroxyl radicals. The AVSB possesses hepatoprotective activity comparable to silymarin preferably at higher doses and may as an alternative to silymarin in the management of hepatic disorders. These hepatoprotective effects of AVSB against CCl_4 toxicity in rats give credence to its use in traditional medicine for the management of hepatic disorders. However, the major hepatoprotective bioactive principle in AVSB needed to be isolated to enable its full utilization in the management of hepatic disorders and diseases with minimal adverse effects. The elevated hepatic enzymes activities in the CCl_4 induced rats treated with a lower dose of AVSB suggest that the extract possesses insignificant hepatoprotective activity at a lower dose and requires administration of higher doses to achieve better hepatoprotective effects and are consistent with the earlier findings [32].

The significant reductions of total protein and albumin concentrations in the CCl_4 induced untreated rats could be attributed to the adverse effects of CCl_4 toxicity on the hepatocytes and probably the endoplasmic reticulum that impaired their ability to synthesize sufficient amounts of total protein including albumin. Liver cells synthesize proteins including albumin needed to maintain normal biochemical functions in the body and its measurement could be used to monitor liver functions as earlier suggested [35]. The marked elevation in total protein and albumin concentrations of CCl_4 induced rats treated with graded doses of the AVSB indicated recovery of the rats from hepatic injury and restoration of hepatic functions, which are in agreement with the previous findings [36]. The extract probably prevented CCl_4 metabolites from causing severe oxidative damage to the hepatocytes by antioxidant activity and stimulation of endogenous antioxidants that could have maintained the integrity and functions of the hepatocytes. Albumin plays a vital role in the transport and maintenance of adequate levels of bilirubin, minerals, fatty acid, drugs and some xenobiotic and its increased concentrations in the AVSB treated CCl_4 induced rats could be responsible for the low levels of serum total bilirubin recorded in these animals.

The elevated serum total bilirubin levels in the CCl_4 induced untreated rats could be attributed to the hepatic injury suffered by the animals that impaired their liver functions and low concentrations of albumin available in these animals to transport bilirubin to the liver for biotransformation and excretion. However, the lower levels of total bilirubin in the CCl_4 induced rats treated with graded doses of AVSB indicated hepatic

recovery from CCl_4 toxicity and improved protein synthesis including albumin required to transport bilirubin to the liver for detoxification which in line with findings of Uroko *et al*., [34].

The significant reductions in the percentage packed cell volume (PCV), haemoglobin (Hb), platelets, red blood cell (RBC) and white blood cell (WBC) counts of rats induced carbon tetrachloride untreated indicated toxic effects of CCl_4 on the blood cell which might have caused haemolysis of the blood cells or hindered the ability of bone marrow to produce sufficient blood cells. The reductions in PCV, RBC, Hb, WBC and platelets counts are consistent with previous findings on the haematotoxic effects of CCl_4 on the rats [37]. However, the significant dose-dependent increases in the PCV, Hb, RBC, and WBC counts in the CCl_4 induced rats treated with the AVSB can be attributed to haematoprotective effects of the effects of the extract. The extract prevented the blood cells from haemolysis and maintained normal bone marrow function and reversed the possible anaemia and insufficient haemoglobin concentration caused by the CCl_4 induction in the untreated rats. The improved haematological indices in the CCl_4 induced rats treated with AVSB could be attributed to its high levels of antioxidant components like flavonoids, phenols, vitamins C and E that ameliorated the adverse effects of reactive CCl_4 metabolites on the haematological indices and other tissues in the body as previously reported [38]. However, treated with AVSB reversed the significant decreases in platelet counts of the CCl_4 induced rats to normal levels and suggest that the AVSB could prevent blood loss from bleeding.

The wide spread necrosis observed in the liver of the CCl_4 induced untreated rats could be attributed to the hepatotoxic effects of CCl_4 which are in agreement with the previous findings [4, 5]. These findings suggest that the toxic reactive metabolites of CCl_4 such as trichloromethy and trichloromethyl peroxyl radicals attacked hepatic cells and caused various degrees of hepatic injury possibly via oxidative mechanism. However, the mild infiltration of mononuclear leucocytes around portal areas and multifocal random aggregations of inflammatory leucocytes in the CCl_4 induced rats treated with 100 and 200 mg/kg/d of AVSB extract respectively are indicative of the hepatoprotective effects of AVSB extract. The extract was able to prevent CCl_4 induced necrosis in the rats but not attain complete protection of the hepatocytes from the toxic effects of CCl_4 as the rats suffered mild hepatic injury contrary to the CCl_4 induced rats treated with silymarin.

4. CONCLUSION

The findings of this study revealed that the AVSB contains high levels of antioxidants including flavonoids, phenols, β-carotene, vitamins C and E and exhibits dose-dependent *in vitro* antioxidant activities as indicated by its ferric reducing antioxidant power, DPPH, and nitric oxide scavenging activities. The AVSB demonstrated hepatoprotective and haematoprotective effects against carbon tetrachloride-induced toxicity and effectively restored normal hepatic integrity and functions and maintained normal haematological indices in the CCl_4 induced rats treated with it.

5. MATERIALS AND METHODS

5.1.1 Collection and identification of plant material

The fresh *Anthocleista vogelii* stem barks were collected from the botanical garden at the Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The stem barks were properly identified and authenticated at the Department of Forestry and Environmental Management, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, with a voucher number FHI 40448.

5.1.2 Chemicals and reagents

The study used high qualities laboratory chemicals and reagents sourced from well-known manufacturers and distributors of high-grade analytical chemicals and reagents. The ethanol was sourced from Sigma-Aldrich (USA), silymarin from Micro Labs Limited (India), carbon tetrachloride from Merck KGaA, Darmstadt, Germany, while other chemicals and reagents were obtained from chemical stores at Onitsha, Anambra State, Nigeria.

5.2.1 Preparation and extraction of plant material

The fresh *Anthocleista vogelii* stem barks were handpicked, washed in running water, sliced into small pieces and dried under shade until they were properly dried. The dried *A. vogelii* stem bark sample was pulverized into a coarse powder using a milling machine and stored inside a sterile clean dry container. Five hundred grams (500 g) of the coarsely ground *Anthocleista vogelii* stem barks were soaked in 1.5 L of absolute

ethanol for 72 h, filtered with mesh cloth followed with Whatman No. 1 filter paper and the filtrate concentrated in a Water bath at 50℃ until all the ethanol has evaporated, weighed and percentage yield calculated [10].

5.2.2 Experimental animals

Thirty male Wistar albino rats weighing 140 – 150 g were purchased from the University of Nigeria, Nsukka, Enugu State, Nigeria and acclimatized to the environmental condition at the Animal House of the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. The rats were acclimatized under 12 h dark/light cycles with unhindered access to standard feed and drinking water *ad libitum* for 14 days.

2.2.3 Experimental design for animal study

The study utilized six groups of rats containing five rats per group. Group 1 was the normal control rats without CCl_4 induction but received distilled water 2 ml/kg/d for 14 days and group 2 was the CCl_4 control that was CCl_4 induced but received no treatment. Groups 3 – 6 were treatment groups that were CCl_4 induced but treated with 100 mg/kg/d silymarin, 100, 200 and 500 mg/kg/d AVSB respectively for 14 days. On completing the treatment on the 14th day, the rats fasted overnight, blood samples were collected, and livers harvested on the 15th day for biochemical and haematological analyses, and histological examinations respectively.

5.2.4 Induction of liver injury

Liver injury was induced in the rats by subjecting the rats to overnight fasting followed by the intraperitoneal administration of 2 ml/kg (i.e. 2000 mg/kg) carbon tetrachloride (CCl_4) mixed with olive oil in the ratio of 2:1 (v/v) on day 1 and allowed to stay for 72 h without any treatment to enable CCl_4 to initiate sufficient oxidative damage on the liver cells.

5.2.5 Determination of the antioxidant contents

The flavonoids and total phenols contents in the ethanol extract of *A. vogelii* stem bark (AVSB) were determined using the methods described by Harborne, and Trease and Evans respectively [11, 12]. The β-carotene and lycopene content in the AVSB were determined according to the methods of Nagata and Yamashita while the vitamins C (ascorbic acid) and E (α-tocopherol) contents were quantified using the methods of Omaye *et al.,* and Desai respectively [13, 14, 15].

5.2.6 Determination of hepatoprotective and haematological indices

The alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were assayed with the methods of Reitman and Frankel using Randox commercial kits [16]. The total bilirubin was determined using the method described by Jendrassik and Grof [17]. The serum total protein concentration was quantified with the Biuret method as modified by Lubran while the albumin concentration was quantified with the method of Doumas *et al.,* [18, 19]. The haematological indices including percentage packed cell volume (PCV), haemoglobin concentrations, red blood cell, white blood cell and platelet counts were quantified with an automated haematology Analyzer–MC-2800 (Mindray Company, China).

5.2.7 Determination of the *in vitro* antioxidant activities

The *in vitro* antioxidant activities including ferric reducing antioxidant power (FRAP), DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitric oxide (NO·) scavenging free radicals activities of the AVSB were quantified with the methods of Oyaizu; Hatano *et al*., and Marcocci *et al*., respectively [20, 21, 22].

5.2.8 Ethical issues

The study was conducted in line with the regulations of the Research Ethics Committee of Iranian Ethical Guidelines for the use of animals in research and the guidelines of the Research Ethics Committee of Michael Okpara University of Agriculture Umudike (MOUAU) for experiments with animals. The ethical clearance was duly approved by the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with the Ethical Number: MOUAU/VPP/EC/18/003"

5.2.9 Statistical analysis

The data obtained from the animal study were statistically analyzed with a Statistical Products and Service Solutions (SPSS) version 22 using one-way analysis of variance and Duncan's multiple range comparison post hoc test (LSD) and the level of statistical significance obtained at ($P < 0.05$). The results were presented as mean ± standard deviation (n = 5).

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Conflict of interest statement: The authors have declared no conflict of interest.

Ethics committee approval: All experiments conducted in this study were approved by Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with the approval number of MOUAU/VPP/EC/18/003.

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