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Hepatoprotective effects of *Allanblackia gabonensis* aqueous trunk bark extract on carbon tetrachloride-induced chronic liver damage in Wistar rats

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ABSTRACT

Objectives: Natural bioactive compounds protect against oxidative stress-induced diseases. Studies have demonstrated antioxidant properties of *Allanblackia gabonensis* (member of *Clusiaceae* family), which is used for liver diseases. This work was designed to investigate the hepatoprotective effects of *A. gabonensis* aqueous trunk bark extract against carbon tetrachloride (CCl_4)-induced chronic liver injury.

Materials and Methods: Rats were divided into six groups of five rats each. Rats of control and CCl_4 groups received distilled water orally from week 1 to week 12. *A. gabonensis* aqueous extract was given orally to preventive (PREV) test group (200 mg/kg) from week 1 to week 12. SIM group and two curative groups received silymarin 25 mg/kg and extract (100 or 200 mg/kg) from week 8 to week 12. CCl_4 was injected hypodermically to induce chronic liver injury to all groups except control, 2 h after treatment, from week 1 to week 12. All rats were often weighed and were sacrificed 12 weeks later under anesthesia and blood was collected in ethylene diamine tetra-acetic acid tubes and plain tubes for hematological profiling and serum preparation, respectively. Liver and kidney functions were assessed by measuring alanine transaminase (ALT), aspartate aminotransferase (AST) serum activities, serum creatinine, total bilirubin, and total protein levels. Superoxide dismutase (SOD), catalase, glutathione (GSH), and malondialdehyde (MDA) were assessed. Histology of the liver and kidney was done.

Results: Administration of CCl_4 to rats resulted in significant ($P < 0.05$) impairment of the animals' weight growth. ALT activity, creatinine, total bilirubin, and MDA levels were significantly increased. Total proteins, GSH levels, SOD, and catalase activities were decreased in the CCl_4 group compared to control. PREV or curative administration of *A. gabonensis* extract (100 or 200 mg/kg) significantly reduced liver injury by preventing significant elevation of ALT activity, creatinine, and total bilirubin levels and exhibited significant reduction in the levels of MDA, compared to the CCl_4-group. These effects of *A. gabonensis* extract were evident by a marked improvement of the liver and kidney histological architectures.

Conclusion: The results revealed antioxidant and anti-inflammatory hepatoprotective effects of the aqueous extract of *A. gabonensis* and constituted a scientific basis for further research on this plant.

Keywords: *Allanblackia gabonensis*, hepatoprotective, rats, carbon tetrachloride, antioxidant

INTRODUCTION

Many xenobiotics enter the liver through portal vein after absorption.[1] Carbon tetrachloride (CCl_4) was found to be one of the best characterized animal models of xenobiotic-induced oxidative stress-mediated liver toxicity.[2] CCl_4 is the commonly used method to screen hepatoprotective activity of drugs.[1] Fouad *et al*.,[3] reported that the conversion of CCl_4 by cytochrome P450 yields two free radicals trichloromethyl radical ($\cdot CCl_3$) and peroxyltrichloromethyl radical ($\cdot OOCCl_3$). The hepatotoxic effect of most chemicals is characterized by increased free radical production, increased tissue lipid peroxidation, transaminases, bilirubin, total cholesterol and triglycerides, and increased alkaline phosphatase activity.[4] It was reported that oxidative stress caused by the accumulation of reactive oxygen species in the liver is the primary pathogenic mechanism of chemical-induced liver injury.[5] The liver, whether in a human or a rodent, performs several functions that are crucial for life, such as drug metabolism, protein synthesis, detoxification, micronutrients storage, and cholesterol production.[6] It also contains diverse liver enzymes involved in the metabolism of drugs and chemicals. Oxidative stress is known to cause liver diseases and there are many liver enzymes with antioxidant potential to prevent or to stop the oxidative stress process and therefore improve the liver function.

Medicinal plants contain antioxidant compounds with an important hepatoprotective effect.[7] Among numerous medicinal plants, *Allanblackia gabonensis* which belongs to the *Clusiaceae* family, has been found to contain antioxidant compounds.[8,9] *A. gabonensis* is a sub-montane species, that is found above 500 m altitude above sea level.[10] This plant is largely used in traditional medicine to improve virility in men and to treat infection such as dysenteries, cold, and tooth ache.[11,12] Cameroonian population also uses it to relieve pain, rheumatism dysentery, toothache, and inflammations.[13] The previous reports showed its antimicrobial, antileishmanial and antibacterial,[13-15] analgesic and anti-inflammatory properties,[16] hepato-nephroprotective and antioxidant effects against acute acetaminophen-induced liver and kidney disorders in rats,[8] and anticancer activities.[17,18]

Phytochemical studies showed that the stem bark of *A. gabonensis* contains xanthone, benzophenone, flavonoid, and phytosterol.[14] Nganou *et al*.,[15] following a successive chromatography of the methanol stem bark extract of the *A. gabonensis*, have isolated a new polyprenylated benzophenone along with the known kaempferol, morelloflavone, morelloflavone 7-O-β-d-glucopyranoside, β-stosterol 3-O-β-d-glucopyranoside, and β-sitosterol. High-performance liquid chromatography confirmed the presence of 1,3,6,7-tetrahydroxy-2-(3-methylbut-2-enyl) xanthone, Allan xanthones A and D, some flavonoids, and phytosterols.[14,15] Based on the above information, and given that no studies have been done on chronic liver damage to our knowledge, we aimed to study the hepatoprotective effects of *A. gabonensis* aqueous trunk bark extract against CCl_4-induced chronic liver damage in rats.

MATERIALS AND METHODS

Plant material

A. gabonensis trunk bark was collected in June 2007, from Kola mountain at Nkolbisson, Center region of Cameroon and identified at the National Herbarium of Cameroon, where a voucher specimen N° 23255/SRF/Cam was deposited.

Preparation of extract

The air-dried trunk bark was powdered, and 500 g of powder was extracted by decoction in distilled water (1.5 L) for 15 min. After filtration, the filtrate was concentrated in oven at 55°C to obtain brown residue of 41.5 g (8.3% yield), which was stored in the refrigerator (4°C) in a sealed labeled bottle for further study.

Animals

Three-month-old albino rats (30) of both sexes (15 males and 15 females), weighing 130–160 g, were used in this study to comply with the requirement of the experimental model of hepatotoxicity due to CCl_4 that is suitable for rats. All rats were bred in animal house of the Department of Animal Biology and Physiology, University of Yaoundé I, Cameroon. Rats were housed in cages under standard laboratory conditions (12:12 light/dark cycle at 25 ± 8°C), with free access to the water and standard commercial diet. The experiment was conducted in accordance with the institutional guidelines approved by the National Ethics Committee of Cameroon (No. FWAIRD 0001954).

Experimental design

Assessment of the hepatoprotective activity of *A. gabonensis* aqueous extract was carried out following procedure described by Mu *et al*.[19] which was modified by including a preventive (PREV) group of rats to appreciate both PREV and curative effects.

Rats were divided into six groups of five animals each and they were fasted 12 h before the administration of different substances. The control group (group 1) received orally distilled water without any additional treatment from week 1 to week 12. The CCl_4 group (group 2) and PREV group (group 3) were, respectively, given orally distilled water and a daily dose of *A. gabonensis* aqueous trunk bark extract (200 mg/kg) from week 1 to week 12. The Silymarin (SIM)

group (group 4) and curative groups (group 5 and 6), respectively, received orally SIM (25 mg/kg) and plant extract (100 and 200 mg/kg) once daily, from week 8 to week 12. SIM is a hepatoprotective agent widely used to treat liver damage of various origins. The CCl_4 dissolved in olive oil (50% solution) was injected hypodermically twice weekly to all groups except control to induce chronic liver injury, from the 1st week to the last week (12th week). The first administration of CCl_4 was at a dose of 3 mL/kg and the following doses were at 2 mL/kg. All rats were weighed regularly during the experiment after 2 h of fasting and had free access to water and a standard commercial diet the rest of the time.

At the end of the treatment, 12 weeks later, all rats were sacrificed under anesthesia and blood was collected in ethylene diamine tetra-acetic acid tubes for hematological profiling, and in plain tubes for serum preparation by centrifugation (6000 rpm) for 20 min. The liver, kidney, and heart were dissected from the rats, rinsed in 0.9% sodium chloride and weighed. A sample of these organs was cold ground and homogenized in Tris-hydrochloride buffer (50 mM pH: 7.4) (liver and kidney) or in Mc Even (heart). The 20% homogenate obtained each time was centrifuged at 6000 rpm for 20 min at 4°C and the supernatant recovered. The serum and supernatant were stored at −20°C and used for biochemical analysis. Liver and kidney samples were processed for histological analysis.[20]

Measurement biochemical parameters

Alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin levels were estimated using specific kits (Fortress diagnostic, Antrim, UK), while total protein and creatinine levels were determined according to the procedure described by Bilanda *et al*.[21] Total cholesterol, superoxide dismutase (SOD), catalase, malondialdehyde (MDA), and nitric oxide were quantified as previously described,[22-25] while the determination of reduced glutathione (GSH) was performed using a method described by Kouemou *et al*.[26]

Statistics analysis

Data were provided as microphotography or mean ± standard error of the mean and difference between groups were assessed by one-way analysis of variance followed by Dunnett's test using Graph Pad Instat Software (version 5.03). $P < 0.05$ was regarded as statistically significant.

RESULTS

Effect of *A. gabonensis* extract on body weight gain

Results showed the normal weight growth of the rats of control group [Figure 1a]. Rats treated with CCl_4 significantly lost weight every week in comparison with control group. A reduction of 87.50% was recorded in week 9th. *A. gabonensis*

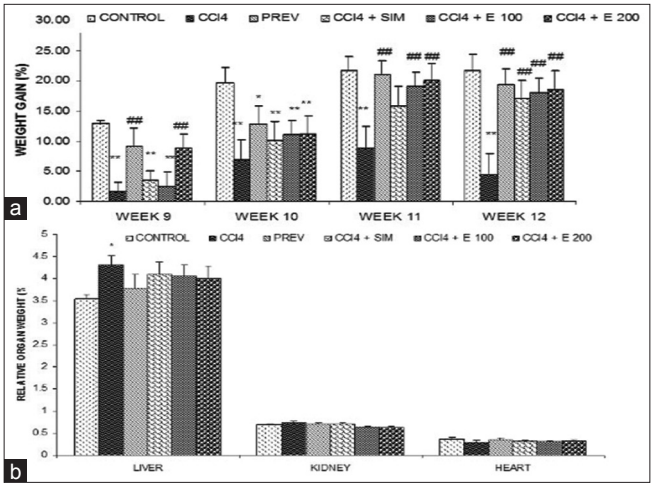

Figure 1: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on body weight gain and relative organ weight of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. *$P < 0.05$, **$P < 0.01$ compared to the control group, ##p<0.01 compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

at the dose of 200 mg/kg administered in PREV manner significantly prevent the weight loss of rats compared to CCl_4 group and when given in the curative way, *A. gabonensis* (100 or 200 mg/kg) and SIM (25 mg/kg) significantly improved the weight growth from week 11th when compared to CCl_4 group.

Effect of *A. gabonensis* extract on relative organ weight

The relative liver weight of CCl_4 group was significantly higher (21.81%) compared to the control group. SIM (25 m/kg) and *A. gabonensis* (100 or 200 mg/kg) administered preventively or curatively prevented significant increase in liver weight compared to CCl_4 group. No significant changes were found in the kidney and heart compared to the control rats [Figure 1b].

Effect of *A. gabonensis* on hematological parameters

The number of platelets was significantly decreased in CCl_4 group compared to control. *A. gabonensis* (100 or 200 mg/kg) administered preventively or curatively improved the number of platelets [Figure 2a]. No significant modification of white blood cells, red blood cells, and hematocrits was noted compared to the control.

Effect of *A. gabonensis* on total protein levels

Total protein levels were significantly decreased (29.80%) in the serum of CCl_4 group compared to control group. PREV treatment of rats with *A. gabonensis* extract (200 mg/kg) or curative administration of *A. gabonensis* extract (100 or

200 mg/kg) or SIM (25 mg/kg) caused significant increase ($P < 0.05$) in the serum total proteins level in comparison with CCl_4 group. No significant difference was observed in liver and kidney as compared to the control and CCl_4 groups. PREV treatment of rats with *A. gabonensis* extract (200 mg/kg) induced a significant increase in the total protein level in the rat's heart compared to CCl_4 group [Figure 2b].

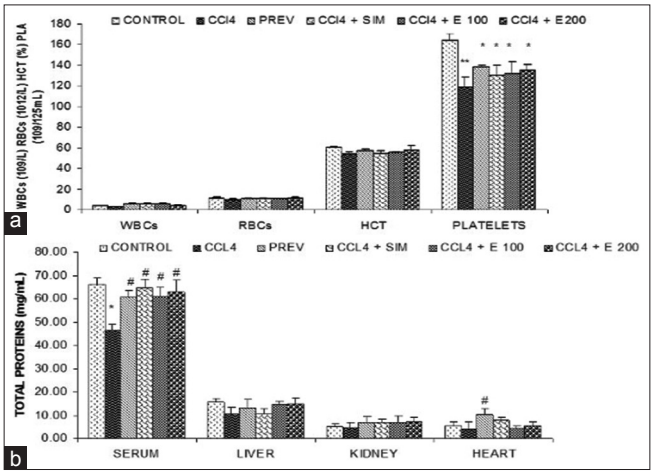

Figure 2: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on hematological parameters and total proteins of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. $^*P < 0.05$, $^{**}P < 0.01$ compared to the control group. # $p < 0.05$ compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

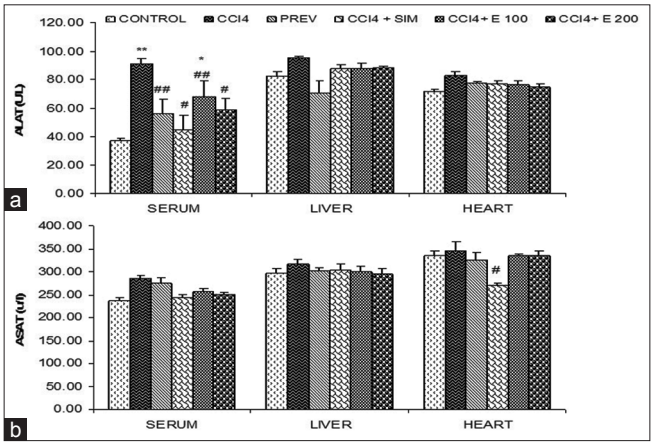

Figure 3: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on transaminase levels of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. $^*P < 0.05$, $^{**}P < 0.01$ compared to the control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

Effect of *A. gabonensis* on transaminase activity

A significant increase in ALT activity (125.98%) in the serum of the CCl_4 group was recorded compared to control. *A. gabonensis* extract (200 mg/kg) prevented a significant increase in ALT activity and when given in the curative way, *A. gabonensis* extract (100 or 200 mg/kg) and SIM significantly reduced serum ALT activity when compared to CCl_4 group. No significant modification of ALT activity was observed in the liver and heart as compared to the control group [Figure 3a].

No significant modifications were found in AST activity in serum, liver and in heart compared to control group. However, rats treated with SIM presented a significant decrease (28.13%) of AST activity in the heart as compared to CCl_4 group [Figure 3b].

Effect of *A. gabonensis* aqueous extract on bilirubin level

Results showed a significant increase of bilirubin level in the liver of CCl_4 group compared to the control group. Treatment with SIM and *A. gabonensis* extract (100 or 200 mg/kg) hindered the significant elevation of bilirubin in the liver [Figure 4a].

Effect of *A. gabonensis* extract on creatinine level

In the kidney, creatinine level was found to be significantly increased in the CCl_4 group compared to the control. PREV

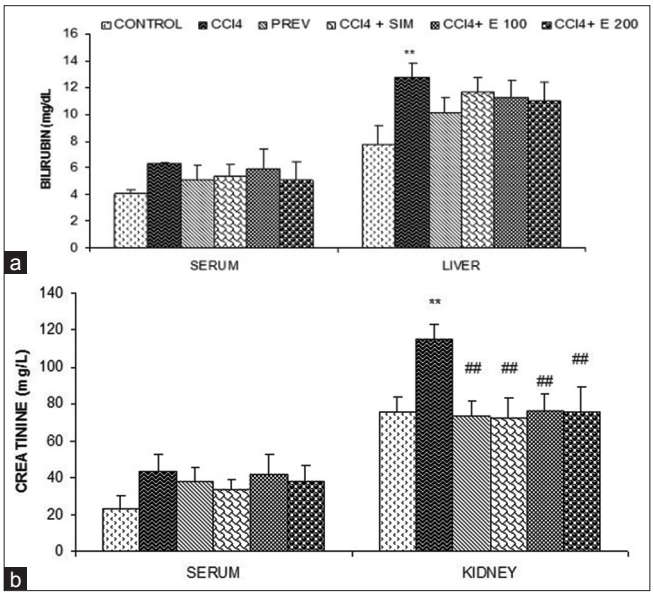

Figure 4: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on bilirubin and creatinine levels of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. $^{**}P < 0.01$ compared to the control group. $^{\#\#}P < 0.01$ compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

and curative administration of *A. gabonensis* extract or SIM to rats significantly prevented increase in the renal creatinine level as compared to CCl_4 group [Figure 4b]. No significant variations were found in the serum following treatment.

Effect of *A. gabonensis* extract on SOD activities

Injection of CCl_4 caused a significant decrease of SOD activities in the liver (59.77%), kidney (71.63%), and heart (57.67%) as compared to control [Figure 5a]. PREV administration of *A. gabonensis* extract (200 mg/kg) to rats significantly prevented the decrease of the SOD activity in the liver, kidney, and heart as compared to CCl_4 group. Administration of SIM or *A. gabonensis* extracts (100 or 200 mg/kg) after induction of liver injury triggered significant elevation of SOD activity in all tissues as compared to CCl_4 group.

Effect of *A. gabonensis* extract on GSH level

Injection of CCl_4 caused a significant decrease in GSH level in the liver (62.54%) and kidney (54.33%) compared to control. Results showed significant increases in GSH level in the liver of the rats treated both preventively and curatively with the plant aqueous extract and SIM as compared to CCl_4 group [Figure 5b]. A significant increase in the heart GSH level was observed in the PREV group compared to CCl_4 group.

Effect of *A. gabonensis* on catalase activity

CCl_4 administration caused a significant decrease (26.67%) of catalase activity in CCl_4 group compared to control group. Treatment with *A. gabonensis* or SIM significantly improved the catalase activity as compared to CCl_4 [Figure 6a].

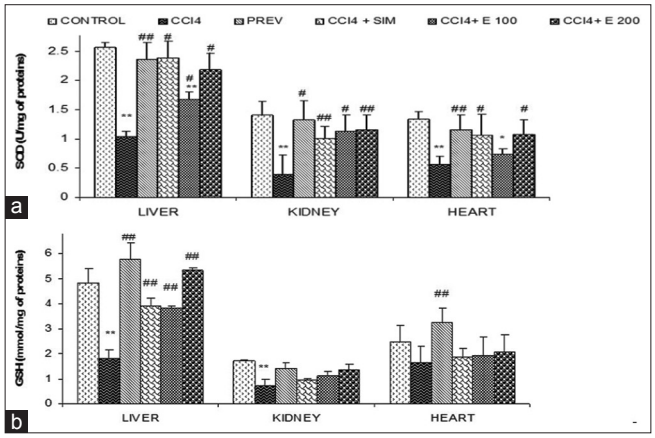

Figure 5: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on superoxydismutase and glutathione activities of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

Effect of *A. gabonensis* extract on MDA level

MDA levels were significantly increased in the liver (59.43%), kidney (28.75%), and heart (33.65%) after administration of CCl_4, compared to control. Treatment with *A. gabonensis* at 200 mg/kg in PREV study, and *A. gabonensis* (100 or 200 mg/kg) and SIM (25 mg/kg) in curative study significantly decreased MDA levels in the liver when compared to CCl_4 group [Figure 6b].

Effect of *A. gabonensis* extract on nitrite oxide level

The administration of CCl_4 caused a non-significant increase of nitrite oxide in the liver (27.32%), kidney (20.36%), and heart (3.51%) in comparison to the control. *A. gabonensis* or

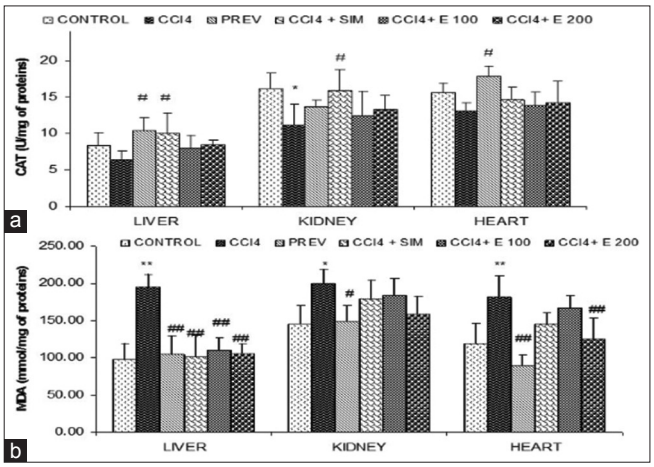

Figure 6: (a and b) Effect of *Allanblackia gabonensis* aqueous extract on catalase activity of rat treated with carbon tetrachloride (CCl_4) Each bar represents mean ± SEM, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to CCl_4 group. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

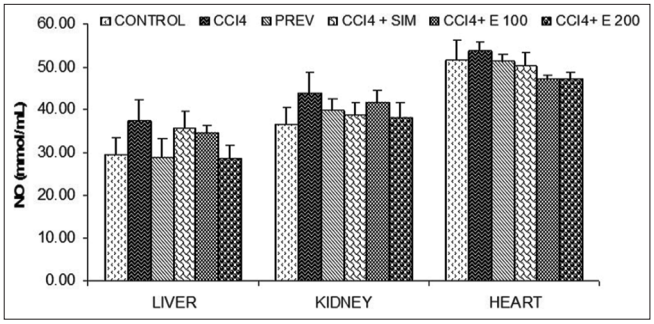

Figure 7: Effect of *Allanblackia gabonensis* aqueous extract on nitrite oxide levels of rat treated with carbon tetrachloride (CCl_4). Each bar represents mean ± SEM, $n = 5$. PREV: Preventive group (E 200 mg/kg + CCl_4). SIM: Silymarin at the dose of 25 mg/kg. E 100 or 200: Extract of trunk bark of *A. gabonensis* at the dose of 100 or 200 mg/kg.

SIM did not induce any significant changes in nitrite oxide levels in the liver, kidney, and heart when compared to control and CCl_4 groups [Figure 7].

Effect of *A. gabonensis* aqueous extract on kidney histological structure

The kidney shows a normal appearance of structures such as the glomerulus, Bowman's capsule, urinary space, and renal tubules in the control group [Figure 8a]. The kidney of the CCl_4 rats shows tubular clarification, the urinary space is somewhat large, but most importantly, there is extensive necrosis of the renal tubules [Figure 8b]. In the PREV group, tubular clarification was observed, and the other structures are normal [Figure 8c]. Rats treated with SIM show slight tubular clearing and a very large urine space [Figure 8d]. The renal section of rats treated curatively with the plant extract shows normal architecture except for the tubular clarification observed especially at the lowest dose [Figure 8e and f].

Effect of *A. gabonensis* aqueous extract on liver histological structure

The livers of the control rats showed a normal appearance with hepatocytes cells well-organized around the portal space [Figure 9a]. However, microphotography of the livers of the CCl_4 group of rats showed steatosis and necrosis of the hepatocytes [Figure 9b]. A PREV treatment with aqueous extract of *A. gabonensis* prevented the installation of the deteriorations observed in the CCl_4 treated group [Figure 9c]. The livers of rats treated with SIM showed a significant reduction in lesions as compared to the control [Figure 9d]. The livers of rats treated with plant extract at 100 or 200 mg/kg showed mild steatosis and necrosis of hepatocytes [Figure 9e and f].

DISCUSSION

Hepatoprotective activity of *A. gabonensis* was done on CCl_4-induced rat chronic liver damage since it has been demonstrated to possess antioxidant, anti-inflammatory properties, and protective effects against acute acetaminophen-induced liver and kidney disorders in rats.[8] This work has shown that the aqueous extract of *A. gabonensis* trunk bark may protect the liver against chronic damage caused by CCl_4 by improving the weight growth of rats, the biomarkers of the liver and kidney functions and by reducing oxidative stress induced by prolonged treatment with CCl_4.

Our results showed a significant decrease in the body mass of the CCl_4-treated rats compared to control group. Change in body weight is an adequate index to evaluate the seriousness of pathologies and to appreciate the normal functioning of the body. Thus, a loss of body mass is indicative of a state of dysfunction within an organism.[20] Rats treated with *A. gabonensis* extract showed a markedly improved weight growth of rats compared to CCl_4 group, suggesting beneficial effect of the extract against CCl_4-induced chronic liver injury. It has been reported that many plants have the ability to protect against liver injury.[27] The aptitude of plant component to inhibit the aromatized activity of cytochrome P-450 by favoring liver regeneration is another and interesting factor in the hepatoprotective effect.[28]

It is important to find compounds that can prevent liver damage from free radicals generated by toxic chemicals.[29] Many compounds known to be beneficial against CCl_4-mediated liver injury exert their protective action through toxin-mediated lipid peroxidation either through a decreased production of CCl_4-derived free radicals or by the antioxidant activity of the protective agents themselves.[30,31] Recently,

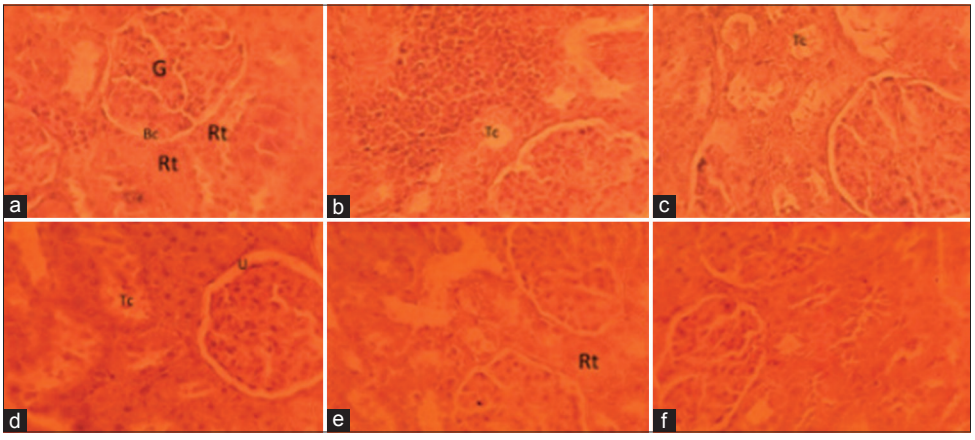

Figure 8: Microphotography of kidney of rats treated with carbon tetrachloride (CCl_4) and *Allanblackia gabonensis* renal histological sections stained with hematoxylin eosin (×400), (a) kidney section of control rat, (b) kidney section of CCl_4 rat, (c) kidney section of preventive rat (E 200 mg/kg + CCl_4), (d) kidney section of CCl_4+Silymarin 25mg/kg, (e) kidney section of CCl_4 + E 100 mg/kg, and (f) kidney section of CCl_4 + E 200 mg/kg. G: Glomerulus, Bc: Bowman's capsule, Rt: Renal tubule, U: Urinary space, Tn: Tubular necrosis, Tc: Tubular clarification.

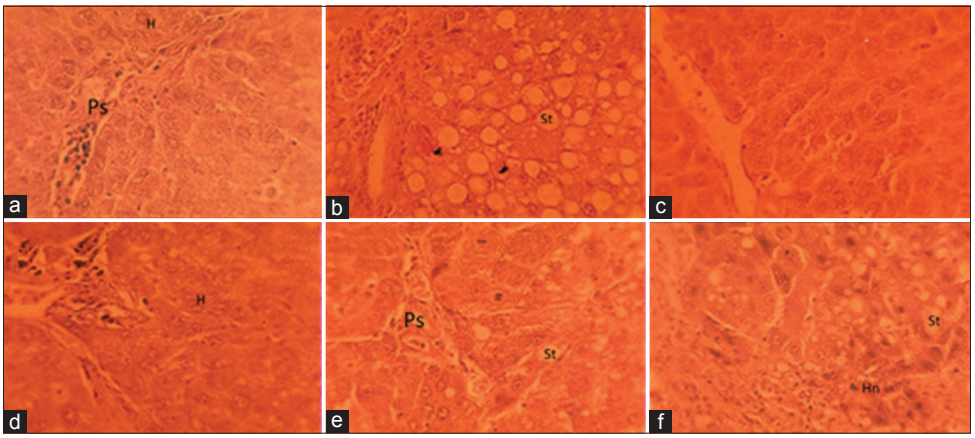

Figure 9: Microphotography of liver of rat treated with carbon tetrachloride (CCl_4) and *Allanblackia gabonensis* histological sections stained with hematoxylin eosin (×400). (a) Liver section of control rat, (b) liver section of CCl_4 rat, (c) liver section of preventive rat (E 200 mg/kg + CCl_4), and (d) liver section of CCl_4 +Sily25. (e) Liver section of CCl_4 + E 100 mg/kg and (f) liver section of CCl_4 + E 200 mg/kg. H: Hepatocyte, St: Steatosis, Ps: Portal space, Hn: Hepatocyte necrosis.

interest has increased considerably in finding naturally occurring antioxidants for use in food due to their potential in health promotion and disease prevention, and their high safety and consumer acceptability.[32] In search of novel sources of antioxidants in the past years, medicinal plants have been extensively studied for their antioxidant activity.[33]

Liver function tests such as ALT, AST, and total proteins can be used to assess liver status. Transaminase is a cytosolic enzyme that contributes to metabolic activity of the liver. The elevated plasma activity of this enzyme indicates hepatocyte damage.[34] Our results displayed a significantly increased level of ALT in CCl_4 group when compared to the control group and this corroborate studies that demonstrated an increase in plasma levels of ALT due to CCl_4 injection to rat.[35] Administration of *A. gabonensis* aqueous extract has prevented significant elevation of ALT activity in PREV study and decrease considerably the level of ALT in curative study compared to CCl_4 control. An adequate serum protein level indicates normal physiological activity of hepatocytes.[34] Rats treated with *A. gabonensis* aqueous extract showed a total serum protein level comparable to that of the control, regardless of treatment. These results showed that extract may have a hepatoprotective activity against liver damage due to chronic administration of CCl_4.

Chronic administration of CCl_4 has caused a significant decrease of platelets. Blood coagulation requires that platelets be in sufficient size, number, and function in the absence of which a satisfactory plug may not occur, and bleeding may continue as a result of a breach in the vascular endothelium.[36] *A. gabonensis* extract has elevated the level of platelets compared to control group. Similar results were obtained by Keon *et al*.[37]

The metabolism of toxic chemicals generates free radicals that cause liver damage.[29] Many compounds that have a beneficial effect against CCl_4-induced liver damage exert their protective action on CCl_4-induced lipid peroxidation, either by causing a decrease in the production of CCl_4-derived free radicals, or by exerting direct antioxidant activity (scavenging activity) or indirectly through activation of the endogenous antioxidant system.[30,31] The results of this study showed that CCl_4 intoxication of study rats, resulted in a significant reduction of reduced GSH level, and decreased in SOD and catalase activity compared to the control. Treatment with *A. gabonensis* extract has prevented a significant decrease in reduced GSH level and in SOD and catalase activity compared to control group in both PREV and curative studies. These results suggest that *A. gabonensis* extract would provide the tissue with better protection against oxidative stress by contributing to the neutralization of free radicals. The PREV and curative administration of *A. gabonensis* extract to the rats maintained MDA levels in the liver tissue similar to that of the control rats and would confirm the protective properties of the plant extract against CCl_4-induced liver damage in rat.

Histopathological analysis of liver sections from CCl_4 rats provides support for the previous biochemical analyses. Observation of the liver sections showed hepatic lesions, such as hepatocyte steatosis and necrosis. PREV or curative administration of the aqueous extract of *A. gabonensis* remarkably reduced these hepatic lesions, thus preserving the appearance of the liver parenchyma. In the kidneys, the extract also reduced necrosis of the renal tubules. This hepato- and nephroprotective activity of the aqueous extract may be due to the antioxidant properties of this plant. The previous phytochemical study of the stem bark of *A. gabonensis* resulted in the identification, isolation and characterization of xanthone derivatives, one polyisoprenylated benzophenone, guttiferone F, one flavanol, epicathechin, two phytosterols,

β-sitosterol, and campesterol.[14,38] The antioxidant effectiveness in natural substances was reported to be mostly due to the presence of phenol compounds.[39-42] Moreover, strong relationship between the phenols and antioxidant activity was shown by Velioglu *et al.*,[43] Kahkonen *et al*.,[44] and Javanmardi *et al.*[45] *A. gabonensis* showed its ability to preserve and restore the normal functional status of liver under the toxic effects of CCl_4.

CONCLUSION

The results of our study show that the aqueous extract of *A. gabonensis* trunk bark has PREV and curative effects against CCl_4-induced chronic liver injury in rats. This extract reduced the hepatoxicity induced by CCl_4 administration as shown by the rats' body weight increase and the liver markers. These protective effects may be related to the antioxidant properties of the aqueous extract of the plant. These results show evidence for a better exploitation of this plant, whose antioxidant properties have been demonstrated and which could be useful for a drug formulation against liver diseases.

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Disclaimer

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Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest as far as this work is concerned.

REFERENCES

1. Mahmoodzadeh Y, Mazani M, Rezagholizadeh L. Hepatoprotective effect of methanolic *Tanacetum parthenium* extract on CCl_4-induced liver damage in rats. *Toxicol Rep.* 2017;4:455-462. doi: 10.1016/j.toxrep.2017.08.003
2. Ouassou H, Bouhrim M, Daoudi NE, *et al*. Evaluation of hepatoprotective activity of *Caralluma europaea* stem extract against CCl_4-induced hepatic damage in wistar rats. *Adv Pharmacol Pharm Sci*. 2021;2021:8883040. doi.org/10.1155/2021/8883040
3. Fouad D, Badr A, Attia HA. Hepatoprotective activity of raspberry ketone is mediated via inhibition of the NF-κB/TNF-α/caspase axis and mitochondrial apoptosis in chemically induced acute liver injury. *Toxicol Res* (*Camb*). 2019;8:663-676.
4. Shanmugam G, Ayyavu M, Rao DM, *et al*. Hepatoprotective effect of *Caralluma umbellate* against acetaminophen induced oxidative stress and liver damage in rat. *J Pharm Res*. 2013;6:342-345.
5. Souza CF, Baldissera MD, Descovi SN, *et al*. Serum and hepatic oxidative damage induced by a diet contaminated with fungal mycotoxin in freshwater silver catfish *Rhamdia quelen*: Involvement on disease pathogenesis. *Microb Pathog*. 2018;124:82-88.
6. Wahid A, Hamed AN, Eltahir HM, *et al*. Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl_4-induced chronic hepatotoxicity in rats. *BMC Complement Altern Med*. 2016;16:263.
7. Al-Snai A, Mousa H, Majid WJ. Medicinal plants possessed hepatoprotective activity. *IOSR J Pharm*. 2019;9:26-56.
8. Vouffo YE, Metchi DM, Temdie GJ, *et al*. Hepatho-nephroprotective and antioxidant effect of stem bark of *Allanblackia gabonensis* aqueous extract against acetaminophen-induced liver and kidney disorders in rats. *J Exp Integr Med*. 2012;4:337-344.
9. Fankam AG, Atsafack SS, Njateng GS, *et al*. Antioxidant and antifungal activities of *Myrianthus arboreus* P. Beauv. (*Moraceae*), *Allanblackia gabonensis* Pellegr. (*Clusiaceae*) and three other Cameroonian medicinal plants. *Investig Med Chem Pharmacol*. 2021;4:52. doi.org/10.31183/imcp.2021.00052
10. Kyereh D, Maňourová A, Hendre PS, *et al*. Diversity, chemical composition, and domestication potential of *Allanblackia parviflora* A. Chev. West Africa. *Forests*. 2021;12:1758. doi.org/10.3390/f12121758
11. Raponda-Walker A, Sillans R. Les Plantes Utiles du Gabon. 1st ed. London: Lechevalier; 1961.
12. Vivien J, Faure JJ. Fruitiers Sauvages d'Afrique: Especes du Cameroun, Carnoe-France. Nguila-Kerou. France: Ministère Français de la Coopération et CTA; 1996.
13. Fankam AG, Kuiate JR, Kuete V. Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis*, *Combretum molle* and *Gladiolus quartinianus* against Gram-negative bacteria including multi-drug resistant phenotypes. *BMC Complement Altern Med*. 2015;15:206-218.
14. Azebaze AG, Ouahouo BM, Vardamides JC, *et al*. Antimicrobial and antileishmanial xanthones from the stem bark of *Allanblackia gabonensis* (Guttiferacae). *Nat Prod Res*. 2008;22:333-341.
15. Nganou BK, Simo KI, Fankam AG, *et al*. Guttiferone BL with antibacterial activity from the fruits of *Allanblackia gabonensis*. *Nat Prod Res*. 2019;33:2638-2646.
16. Ymele VE, Dongmo AB, Dimo T. Analgesic and anti-inflammatory effect of the aqueous extract of the stem bark of *Allanblackia gabonensis* (Guttiferacae). *Inflammopharmacol*.

2011;21:21-30.
17. Kuete V, Fankam AG, Wiench B, *et al*. Cytotoxicity and modes of action of the methanol extracts of six Cameroonian medicinal plants against multidrug-resistant tumor cells. *Evid Based Complement Alternat Med*. 2013;2013:285903. doi.org/10.1155/2013/285903
18. Fankam AG, Das R, Mallick A, *et al*. Cytotoxicity of the extracts and fractions from *Allanblackia gabonensis* (*Clusiaceae*) towards a panel of cancer cell lines. *S Afr J Bot*. 2017;111:29-36.
19. Mu Y, Liu P, Du G, *et al*. Action mechanism of Yi Guang Jiang decoction on CCl_4 induced cirrhosis in rats. *J Ethnopharmacol*. 2009;121:35-42.
20. Temdie GR, Kamdem GB, Minoue MG, *et al*. Safety assessment of *Markhamia tomentosa* (Benth.) K. Schum. (*Bignoniaceae*) leaves extracts, highlight the psychostimulant effect of the methanol extract. *J Exp Appl Trop Biol*. 2021;1:37-47.
21. Bilanda DC, Andomo MY, Dzeufiet DP, *et al*. Methanol extract of *Allanblackia gabonensis* (Guttiferacae) prevents hypertension and oxidative stress induced by chronic sucrose consumption in rat. *Pharmacologyonline*. 2013;1:194-200.
22. Dzeufiet DP, Mogueo A, Bilanda DC, *et al*. Antihypertensive potential of the aqueous extract which combine leaf of *Persea americana* Mill. (*Lauraceae*), stems and leaf of *Cymbopogon citratus* (D.C) Stapf. (*Poaceae*), fruits of *Citrus medical* L. (*Rutaceae*) as well as honey in ethanol and sucrose experimental model. *BMC Complement Alternat Med*. 2014;14:507. doi:10.1186/1472-6882-14-507
23. Donfack MM, Atsamo AD, Temdie GR, *et al*. Antihypertensive effects of the *Vitex cienkowskii* (*Verbenaceae*) stem-bark extract on L-NAME-induced hypertensive rats. *Evid Based Complement Alternat Med*. 2021;2021:6668919. doi.org/10.1155/2021/6668919
24. Fotio AL, Nguepi MS, Tonfack LB, *et al*. Acetaminophen induces liver injury and depletes glutathione in mice brain: Prevention by *Moringa oleifera* extract. *S Afr J Bot*. 2019;129:317-323. doi:10.1016/j.sajb.2019.08.037
25. Fotio AL, Olleros ML, Vesin D, *et al*. *In vitro* inhibition of LPS and Mycobacterium bovis BCG-induced inflammatory cytokines and *in vivo* protection from GaIN/LPS-mediated liver injury by the medicinal plant *Sclerocarya birrea*. *Int J Immunopathol Pharmacol*. 2010;23:61-72.
26. Kouemou EN, Fotio LA, Dongmo NM, *et al*. Antioxidant activities of *Dichrocephala integrifolia* (Linn.f.) O. Kuntze (*Asteraceae*) in a mice model of D-Galactose-induced oxidative stress and accelerated aging. *Int J Pharmacol Phytochem Ethnomed*. 2017;8:41-53.
27. Murugesan GS, Sathishkumar M, Jayabalan R, *et al*. Hepatoprotective and curative properties of Kombucha tea against carbon tetrachloride-induced toxicity. *J Microbiol Biotechnol*. 2009;19:397-402. doi: 10.4014/jmb.0806.374
28. Aly AA, Hassan MM, Badr EH, *et al*. Protective effect of extracts from dates (*Phonix dactylifera* L.) on carbon tetrachloride-induced hepatotoxicity in rats. *Int J Appl Res Vet Med*. 2004;2:176-180.
29. Sohair RF, Ahh S. Antioxidant effect of Egyptian freshwater *Procambarus clarkii* extract in rats' liver and erythrocytes. *Afr J Pharm Pharmacol*. 2011;5:776-785.
30. Jayatilaka KA, Thabrew MI, Perera DJ. Effect of *Melothria maderaspatana* on carbon tetrachloride-induced changes in rat hepatic microsomal drug-metabolizing enzyme activity. *J Ethnopharmacol*. 1990;30:97-105.
31. Thabrew MI, Joice PD, Rajatissa W. A comparative study of the efficacy of *Pavetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Med*. 1987;53:239-241. doi: 10.1055/s-2006-962691
32. Gorinstein S, Yamamoto K, Katrich E, *et al*. Antioxidative properties of Jaffa sweeties and grapefruit and their influence on lipid metabolism and plasma antioxidative potential in rats. *Biosci Biotechnol Biochem*. 2003;67:907-910.
33. Maria K, Petko D, Milan C, *et al*. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. *Acta Biochim Pol*. 2010;57:229-234.
34. Temdie GR, Boumzina DE, Minoue KM, *et al*. Hepatoprotective activity of *Markhamia tomentosa* (Benth) K. Schum (*Bignoniaceae*) aqueous leaves extract against CCl_4-induced subacute liver injury in rat. *EC Pharmacol Toxicol*. 2022;10:17-31.
35. Ragab A, Nasr NE, Kahilo K. Protective effect of glycyrrhizc acid against carbon tetrachloride-induced liver fibrosis in rats: Role of integrin subunit β like 1 (itg β L1). *Slovenian Vet Res*. 2019;56:673-679.
36. Salawi OA, Aliyu M, Tijani AY. Haematological studies on the ethanolic stem bark extract of *Pterocarpus erinaceus* poie (*Fabaceae*). *Afr J Biotechnol*. 2008;7:1212-1215.
37. Keon WK, Yoon GK, Min KC, *et al*. Oltipraz regenerates cirrhotic liver through CCAAT/enhancer binding protein-mediated stellate cell inactivation. *FASEB J*. 2002;16:1988-1990. doi: 10.1096/fj.02-0406fje
38. Ymele VE, Dongmo AB, Dimo T. Analgesic and anti-inflammatory effect of the aqueous extract of the stem bark of *Allanblackia gabonensis* (Guttiferacae). *Inflammopharmacol*. 2013;21:21-30.
39. Gutfinger T. Polyphones in olive oils. *J Am Oil Chem Soc*. 1981;58:966-968.
40. Hayes F, Kato H. Antioxidative components of sweet potatoes. *J Nutr Sci Vitaminol* (*Tokyo*). 1984;30:37-46.
41. Yen GC, Duh PD. Anti-oxidative properties of metabolic extracts from peanuts hulls. *J Am Oil Chem*. 1993;70:383-386.
42. Udomchotphruet S, Phuwapraisirisan P, Sichaem J, *et al*. Xanthones from the stems of *Cratoxylum cochinchinense*. *Phytochemistry*. 2012;73:148-151.
43. Velioglu YS, Mazza G, Gao L, *et al*. Antioxidant activity and total phenolics in selected fruits, vegetables and grain product. *J Agric Food Chem*. 1998;46:4113-4117.
44. Kahkonen MP, Hopia AT, Vuorela HJ, *et al*. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem*. 1999;47:3954-3962.
45. Javanmardi J, Stushnff C, Locke E, *et al*. Antioxidant activity and total phenolic content of Iranian *Ocimum accessions*. *Food Chem*. 2003;83:547-550.

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