Hepatho-nephroprotective and antioxidant effect of stem bark of *Allanblackia gabonensis* aqueous extract against acetaminophen-induced liver and kidney disorders in rats

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Abstract

**Objective:** *Allanblackia gabonensis* (Guttiferae) is a plant used in traditional medicine to treat some inflammatory diseases. As oxidative stress promotes the development of acetaminophen (APAP)-induced hepatotoxicity, the aim of the present study was to evaluate the hepato-nephroprotective and antioxidant effect of aqueous extract of *A. gabonensis* on APAP-induced liver and kidney damage.

**Methods:** *A. gabonensis* was given daily *per os* during 7 days, followed by APAP which was given 2 h after the 6th dose for preventive effect, whereas for curative testing *A. gabonensis* was administrated 30 min after APAP (2 g/kg). Preventive and curative effects were observed by following biochemical parameters analysis: transaminases, bilirubin, creatinine, nitric oxide, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).

**Results:** The aqueous extract of *A. gabonensis* at the dose of 100 and 200 mg/kg produced significant hepato-nephroprotective activity by reducing the serum effect of MDA while it significantly produced an increase in enzymatic antioxidant activities (SOD and CAT) and non enzymatic antioxidant (GSH) levels. *A. gabonensis* also showed a significant decrease in transaminase, bilirubin and creatinine in APAP intoxicated rats at the doses of 100 and 200 mg/kg.

**Conclusion:** From this study it can be concluded that aqueous extract of *A. gabonensis* may possess hepato-nephroprotective activities which can be partly attributed to its antioxidant properties.

Key words:

Acetaminophen; *Allanblackia gabonensis*; Antioxidants; Hepato-nephroprotective

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Introduction

The liver is the largest vital organ which has evolved to regulate many important metabolic functions [1]. It is also responsible for maintaining the body's internal milieu and protects itself from challenges it faces during its functioning [2] and faces the immense task of detoxification of xenobiotic agents [3]. Any changes in liver anatomy, or injury or impairment of its functions, may lead to many types of complications in one’s health [4]. The management of liver disease continues to be a challenge to modern medicine [5]. About 20,000 deaths are known every year to be due to liver disorders [6]. Oxidative stress plays an important role in the pathogenesis of toxic liver diseases and other hepatic alterations [7]. Moreover hepatitis is considered to be one of the most serious health problems [8] because liver is a powerful organ involved in a wide number of metabolic pathways. Advanced pharmacological search needs to be conducted quickly to save more people with liver diseases. Most of the hepatoprotective drugs belong in the group of free-radical scavengers; their mechanisms of action involve membrane stabilization, neutralization of free radicals and immunomodulation [7]. Reactive oxygen species (ROS) and free radicals generally play an important role in the pathogenesis of a large number of diseases including hepatitis, AIDS, and cardiovascular disorders [9]. Moreover, chronic illnesses are often connected to the production of toxic chemicals that inflame and cause oxidative stress [10].
N-acetyl-para-aminophenol (paracetamol or acetaminophen; APAP) was discovered in Germany at the end of the 19th century [5]. It is frequently used for the relief of pains, fever, headaches, and minor musculoskeletal pain. Although it is widely used as an analgesic, antipyretic drug, it is generally known as a hepatotoxic agent for the screening of the antihepatotoxic effects of a large variety of traditional medicinal plants [11]. APAP in over doses results in hepatotoxicity and nephrotoxicity in men and experimental animals [12]. APAP toxicity is caused by the formation of toxic metabolites by cytochrome P-450 [6]. It is known to be implicated in analgesic-abuse nephropathy [13]. An overdose of the widely used analgesic and antipyretic APAP is known to be one of the most common pharmaceutical product poisonings in the United States [14]. Medicines available in the pharmacies do not totally counteract some hepatitis forms, hepatotoxicity or nephrotoxicity. In addition, steroids, vaccines, and antiviral drugs that are employed as therapy for liver diseases have potential adverse effects especially when administered for long periods [15]. Therefore, researchers are looking for alternative remedies in hepatic problems. Natural products are believed to be an important source of new chemical substances with potential therapeutic application [16].

Silymarin is an extract from the seed of Silybum marianum, that consists of at least seven flavonolignans and the flavonoid taxifolin [17]. Silymarin has been used to treat a range of liver disorders including hepatitis, cirrhosis, and poisoning from wild mushrooms [18]. Allanblackia gabonensis, belonging to the family of Guttiferae, is commonly grown in tropical Africa (Cameroon, Democratic Republic of Congo, etc) between around 500 and 1750 m above sea level [19]. A. gabonensis is generally used in traditional medicine to improve virility in men and to treat infection such as dysenteries, colds, and tooth aches [20, 21]. People also use it to relieve pain, inflammation and rheumatism. Previous reports showed its antimicrobial, antileishmanial [22], analgesic and anti-inflammatory properties [23]. Phytochemical studies showed that the stem bark of A. gabonensis possess xanthones, benzophenone, flavonoid, and phytosterol [22]. Many studies have been conducted on traditional medicines in an attempt to develop new drugs for liver diseases [24-26]. Hence, the current experiment was designed to evaluate the preventive and curative effects of aqueous extract of A. gabonensis against APAP-induced liver and kidney damage in rats.

Materials and methods

Plant material
The stem bark of A. gabonensis were collected in June 2007 from Kola Mountain at Nkolbisson, Centre Region, Cameroon and identified by an authorized botanist at the National Herbarium where a voucher specimen N°23255/SRF/Cam is deposited for future reference.

Preparation of extract
The air dried stem bark was powdered (1000 g) and extracted by decoction in distilled water (3000 ml) for 15 min and kept cool for 1 h. After filtration, the filtrate was concentrated in an oven at 55ºC producing brown residue yielding 83 g (8.3%). High-performance liquid chromatography (HPLC) confirmed the presence of phytosterols, xanthone (derivatives), epicatechin, and phenol (derivatives) [23].

Animals
Wistar rats (120-150 g) of both sexes were used for this study. Animals were bred in the animal house of the Faculty of Science, University of Yaounde I. They were fed with standard food and water ad libitum. The “principle of laboratory animal care” was followed in this study [27].

For the first set of experimentation (preventive test), previously described methods were used [28, 29]. Animals were randomly divided into 5 mixed groups (n = 5). Animals in Group 1 (negative control) were administered only distilled water throughout the duration of the experiment. Those in Group 2 (APAP treated) received distilled water for 7 days followed by oral administration of a single dose of 2 g/kg of APAP on the 6th day. Animals in Group 3 (positive control) received orally silymarin (25 mg/kg), a reference substance purchased from the Sigma-Aldrich (Munich, Germany), for 7 days followed by 2 g/kg of APAP on the 6th day. Animals in Groups 4 and 5 were administered 100 and 200 mg/kg of the aqueous plant extract once daily for 7 consecutive days followed by 2 g/kg of APAP on the 6th day of extract administration. After 48 h of APAP feeding, rats were sacrificed by decapitation and their blood was collected for the estimation of biochemical parameters.

For the second set of experimentation (curative test), the method of Shih-Chang et al was used [30]. Rats were divided into five groups of five animals each. All animals except controls received APAP (2 g/kg, p.o.). Group 1 received distilled water (10 ml/kg, p.o.) and was used as a normal control. Group 2 received APAP only. Group 3 received APAP, followed 1/2 h later by silymarin (25 mg/kg), a reference substance dissolved in distilled...
Group 4 and 5 received APAP followed 30 min later by stem bark extract (100 and 200 mg/kg, p.o.). After 24 h, rats were sacrificed by decapitation and their blood was collected for the estimation of biochemical parameters.

Two hours after collection, the blood was centrifuged for biochemical analysis; i.e. transaminases and bilirubin were estimated by using specific kits (Fortress Diagnostics, Antrim, Northern Ireland, UK), total protein by the method of Gornall et al [31], creatinine by Bartels and Bohner [32], cholesterol by Schettle and Nussel [33], superoxide dismutase (SOD) by Misra and Fridovich [34], catalase (CAT) by Sinha [35], reduced glutathione (GSH) by Ellmann [36], malondialdehyde (MDA) by Wilbur et al [37], and nitric oxide (NO) assay was performed using the Griess method [38]. All these parameters were determined by measurement of the optical density of the reaction products at the corresponding wavelengths with spectrophotometer (Genesys 20, Thermo Fisher Scientific, Waltham, MA, USA).

Liver, kidney, and heart were quickly removed washed with 0.9% NaCl, weighed, and placed on ice. Liver and kidney were then homogenized in a Tris-HCl (50 mM, pH 7.4) buffer (20% homogenate), centrifuged and the supernatant recovered for biochemical analysis as described above. The heart was homogenised in McEven solution.

**Statistical analysis**

The results were presented as means ± SEM and analysed with Graph Pad Instat Software. The comparisons within the experimental groups were made using one way analysis of variance (ANOVA) followed by Dunnett’s test; p values less than 0.05 were considered significant.

**Results**

**Effect of A. gabonensis on APAP-induced hepatocellular toxicity**

Administration of APAP resulted in a marked increase in blood transaminase activities and bilirubin levels compared with the control (Figs.1-3). Administration of the aqueous extract induces significant decreases in the activities of serum SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase) (Fig.1), and serum bilirubin levels (Fig.3) compared with the APAP-treated group. The variation of serum creatinine level was not statistically significant (Fig.2) compared with the APAP-treated group. These results were observed as well in preventive as in curative treatments.
Effect of A. gabonensis on proteins, relative weight, and nitric oxide levels in APAP-induced hepato-nephrotoxicity

A significant decrease of liver weight was observed during preventive and curative studies in the APAP group compared to the normal group (p < 0.01). The liver weight in the silymarin and APAP + 200 mg/kg extract groups were significantly increased in comparison with the APAP group (p < 0.05) (Fig.4). No significant modifications were found in the kidney weight. APAP induced a decrease in serum protein levels compared to the control. This decrease was only corrected by aqueous extract of A. gabonensis at a dose of 200 mg/kg (curative; p < 0.05) (Fig.5). No significant changes were found on liver and kidney proteins levels during preventive and curative studies.

Nitric oxide levels were increased in liver, kidney, and serum of the APAP group (*p<0.05) compared to the control rats in both studies. Whereas, in the case of A. gabonensis treated rats, liver and kidney NO was significantly lower than the APAP-treated group (Fig.6).

Effect of A. gabonensis in APAP-induced oxidative stress in rats

A significant decrease of liver and kidney CAT (p < 0.05), SOD (p < 0.01), and GSH (p < 0.01) level was observed in APAP group during preventive studies (p < 0.05) as compared to the control animals (Fig.7).

No significant variation was found for CAT during curative studies in both liver and kidney. However, a significant increase of CAT was observed in the APAP + 100 mg/kg extract group compared to the APAP-toxicity group (p < 0.05). A significant increase was observed for SOD in the APAP + 200 mg/kg extract group in the liver during the preventive test while this increase was observed in both the APAP + 100 and 200 mg/kg extract groups during the curative test. Silymarin used as reference substance significantly corrected the decrease of SOD level induced by APAP. A significant increase of GSH level was observed in the serum of the APAP + 200 mg/kg extract group as compared to the APAP group during preventive and curative studies (p < 0.01).

MDA levels during preventive and curative studies were increased significantly in the livers of the APAP group as compared to the control group (p < 0.01). In the liver, a significant decrease was observed in the APAP + 100 mg/kg extract group (preventive) and also in the APAP + 100 and 200 mg/kg extract groups (curative treatment) as compared to the APAP group (p < 0.05).

Discussion

The metabolism of APAP is 90-95% hepatic and its excretion by the kidney. APAP (also called paracetamol) is an effective, well-tolerated, household, over-the-counter analgesic and antipyretic alternative to aspirin. Its ingestion in large doses or chronic use is commonly associated with hepatotoxicity and nephrotoxicity in humans.
and animals [39, 40]. It has been reported that many plants have the ability to protect against liver injury [41]. The aptitude of plant components to inhibit the aromatase activity of cytochrome 450 by favouring liver regeneration is another and interesting factor in the hepatoprotective effect [42].

High doses of paracetamol have been demonstrated to elevate serum levels of SGPT and SGOT [43, 44]. Paracetamol intoxication may lead to fulminant hepatotoxicity, which includes significant increases in transaminases and creatinine. The ability of *A. gabonensis* to prevent increase in the activities of these enzymes is primary evidence indicative of their hepatoprotective activity [44].

The drug-induced nephrotoxicities are often associated with marked elevations in blood urea nitrogen, serum creatinine, and acute tubular necrosis [45]. Thus, biochemical parameters such as serum creatinine for example have been used to investigate drug-induced nephrotoxicity in animals and man [46]. In the present study, the administration of APAP was characterized by light elevation in the circulating level serum creatinine and light decrease in the kidney. These light changes were not significatively modified by pre-treatment or treatment with aqueous extract of *A. gabonensis*.

Bilirubin is a yellow pigment produced when heme is catabolized. Hepatocytes render bilirubin water-soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the liver can process; damage or obstruction of excretory ducts of the liver impairs its ability to excrete normal amounts of bilirubin. Serum bilirubin is considered to be one of the true tests of liver functions since it reflects the ability of the liver to take-up and process bilirubin into bile. Elevated levels may indicate severe illness [47]. High levels of total bilirubin in the APAP-induced treated rats may be due to APAP toxicity. This may have resulted in hyperbilirubinemia [48]. The significant reduction in the level of total bilirubin in the serum of *A. gabonensis* pre-treated rats associated to the reduction of the level of transaminases suggested the hepatoprotective potential of the plant extracts against APAP intoxication.

The reduction in the serum and liver levels of proteins in the APAP intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthetic function of the liver. In particular, it reduces proteins production by the liver, and by extension, its serum quantity [49]. Pre-treatment with the aqueous extracts of *A. gabonensis* ameliorated the imbalance in protein level (curative) significantly in serum at a dose of 200 mg/kg, whereas the variation of this protein level was not significant in the liver and kidney. It is evident that the *A. gabonensis* extract was able to reduce all the elevated levels of SGPT, SGOT, and bilirubin as an indication of the stabilisation of plasma membrane as well as repair of hepatic tissue damages caused by hepatotoxins [50].

The treatment of rats with APAP induced depletion in levels of GSH, CAT, and SOD in the kidney and liver, justifying the damage of these.
The treatment with aqueous extract of *A. gabonensis* afforded protection from such APAP-induced liver damage by enhancing the levels of these parameters. Possible mechanisms that may be responsible for the protection of APAP-induced damage by aqueous extract of *A. gabonensis* extract include the following: (1) *A. gabonensis* extract by itself could act as a free radical scavenger intercepting those radicals involved in APAP metabolism by microsomal enzymes. This may suggest that a decrease of oxidative stress in this experiment plays a role in the mechanism of hepatoprotective properties. Thus, by trapping oxygen-related free radicals, *A. gabonensis* extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidative processes leading to MDA formation. (2) *A. gabonensis* extract significantly increases the content of GSH in the liver and kidney. Our results also showed that the increased levels of GSH and SOD were mostly observed during curative studies. It has been established that reactive oxygen easily inactivated SOD and CAT, which consequently decreased their activities in APAP-intoxicated animals [52]. Our results suggest that a higher content of glutathione in the blood and liver would afford the tissue a better protection against oxidative stress, thus contributing to the abolishment of APAP-induced hepatotoxicity.

Furthermore, the level of MDA was increased in the group receiving APAP administration. This modification may be due to an increase in the production of free radicals which are involved in the initiation of lipid peroxidation and consequently cell damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals [5]. The pre-treatment and post-treatment with the *A. gabonensis* extract reduced the amount of MDA in the liver and kidney. This result indicated that decreasing the formation of lipid peroxidation is also one of the events in preventing oxidative toxicity by APAP.

The previous phytochemical study of the stem bark of *A. gabonensis* has resulted in the identification, isolation, and characterization of xanthone derivatives, named allanxanthone D, allanxanthone A, 1.5-dihydroxyxanthone, 1,7-dihydroxyxanthone and 1,3,6,7-tetrahydroxy-2-(3-methylbut-2-enyl)xanthon, forbexanthone, 6-deoxyisojacareubin, one polyisoprenylated benzo-phenone, guttiferone F, one flavanol, epicathechin, two phytosterols, β-sitosterol and campesterol [22, 23]. The antioxidant effectiveness in natural substances was reported to be mostly due to the presence of phenol compounds [53-56]. Moreover, the strong relationship between the phenols and antioxidant activity has been shown [57-59]. Thus, the presence of phenolic compounds in our plant extract might be responsible for its antioxidant activity.

Previous studies have demonstrated that APAP administration leads to upregulation of iNOS protein and nitric oxide production in hepatocytes. It has been reported that NO is implicated in the progression of APAP-induced liver injury [60]. Furthermore, aminoguanidine, an inhibitor of iNOS, abrogated this response as well as APAP-induced hepatotoxicity [61]. In our study, in a similar manner there was upregulation in NO production in the APAP-treated group, probably via stimulation through NFκB; *A. gabonensis* intake in rats inhibited NO production, thereby alleviating liver or renal inflammation and damage.

In conclusion, the present study has demonstrated that the *A. gabonensis* extract has hepatoprotective effect against APAP-induced hepatotoxicity in rats. From the results, it is evident that post-treatment with aqueous extract of *A. gabonensis* lightly reduced the APAP-toxicity compared to the pretreatment. Since this plant is rich in phenol compounds, the activities of the extract could be attributed to this class of compounds. The enhanced levels of antioxidant enzymes, transaminases reduction, and reduced amount of lipid peroxides are suggested to be the major mechanisms of plant extract in preventing the development of liver and renal damage induced by APAP.

References


