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Possible *In-Vitro* Antioxidant Potential of *Gossypium Barbadense* Leaf Aqueous Extract and It's Effect on Lipid Profile and Liver Enzymes of Albino Rats.

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ABSTRACT

Gossypium barbadense belongs to the family Malvaceae and is used in folk medicine in treatment of malaria, hypertension, delay/irregular menstruation, convulsion, dysentery, antipyretics and ulcers. This study aimed at evaluating possible *in vitro* antioxidant potential of aqueous extract of *Gossypium Barbadense* Leaf (AGB) and its effect on lipid profile and liver enzymes of Albino rats *in vivo*. *In vitro* anti-lipoperoxidative property was investigated in the liver, kidney and heart tissues. Thirty male albino rats divided into (6) different groups were administered for 14 days 250, 350, 500, 750 and 1000 mg/kg body weight of AGB (groups B-F) while group A serve as control. AST, ALT serum protein lipids were determined. Phytochemical screening detects cardiac glycosides, flavonoids, saponin, steroids and terpenoids. AGB (100 mg/ml) showed 30.42% DPPH inhibition and the inhibition is dose dependent. A concentration dependent inhibition of Malondialdehyde (MDA) in the liver and heart homogenate was observed, while AGB did not offer such protection in the kidney tissue. A significantly ($p<0.05$) increase in triglycerides and cholesterol was observed with 350 mg/kg to 1000 mg/kg AGB compared with the control with corresponding decrease in serum protein. An increase in AST activity (87 and 91%) and ALT (94 and 97%) at 750 gm/kg and 1000 mg/kg AGB, respectively was observed. AGB though possess *in vitro* antioxidant properties, but it exhibits toxic potential *in vivo.*

Keywords: ALT (alanine aminotransferase), AST (Aspartate aminotransferase), cholesterol, *Gossypium barbadense*, *in vivo* toxicity, Lipid peroxidation, plasma triglycerides, protein

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INTRODUCTION

Gossypium barbadense is a plant well known forthe cotton it produces. It also has some medicinal applications in emetics, veneral diseases, tumors, paralysis, epilepsy, convulsions, spasm and cutaneous and subcutaneous parasitic infection [1]. It has antifungal properties and contains the chemical gossypol, making it less susceptible to insect damage [2, 3]. Sometimes it is used as a male anti-fertility drug [4]. In Suriname's traditional medicine, the leaves of G. barbadense are used to treat hypertension and delayed or irregular menstruation and malaria [5]. In a preliminary report gossypol which is an active constituent of *G. barbadense*, was reported to have an in vitro antimalarial activity against the human pathogen *Plasmodium falciparum* [6]. Recent research evaluates the antimalarial activity of the AGB in mice and concluded that AGB is a potent antimalarial if used in combination [7]. Study has shown that AGB decreases the tension of phenylephrine by stimulating isolated guinea pig aorta rings by 15 to 35% [5]. Methanolic extract of the leaf of G. Barbadense has antibacterial and wound healing properties [8]. This leaf extract is also used by traditional practitioners to cure gonorrhea. Beneficial use of medicinal plants has been reported over years [9]. The therapeutic uses of AGB has also been reported by various researchers [10], but its effect on the liver enzymes, lipid profile and its antioxidant potential, has not been looked into. Hence, this study is designed to determine the in vivo effect of the AGB on the liver enzymes and lipid profiles as well as in vitro antioxidant properties.

MATERIALS AND METHODS

Chemicals

Randox kits (Randox Laboratories LTD. 55 Diamond Road. Crumlin. Co. Antrim. United Kingdom BT 29 4QY) were used for the determination of ALT, AST, Triglycerides, Total protein and Teco diagnostics kits for cholesterol. Other reagents are of analytical grade and are products of BDN, Poole,England.

Collection of Plant materials

Cotton leaves were obtained from a farm in Sango Ota, Ogun State, Nigeria. The leaves were air dried and blended in analytically clean blender. Powdered cotton leaves (1 kg) were soaked in 10 L of distilled water for 24 h. The extract using a Buckner funnel and Whatman No.1 filter paper. The filtrate was freeze dried using freeze drier (LABFREEZ FD-12-MR) and then stored at 4^{o}C until required for use.

Experimental animals

30 Adult male albino rats (*Rattus norvegicus*) weighing between 200g-300 g were purchased from Akure Ondo State. The Rats were kept in stainless-steel cages in a room maintained at 26- 29°C with a 12 h light-dark cycle. They were acclimatized for two weeks given normal laboratory chow produced by Top Feed Nigeria Limited. The Rats had access to food and water *ad libitum.* The rats were divided into six different groups of five animals each. Group A (control group) was administered 0.5ml distilled water. Group B-F was given 250 gm, 350gm, 500 gm, 750 gm and 1000 gm/kg body weight of the AGB, respectively for 14 days.

Anaesthetization of animals and isolation of tissue

At the end of 14th day, the rats were fasted for 24 h and then sacrificed by cervical dislocation and then dissected to take blood from the heart. The blood was collected into clean dry beakers for serum preparation. Tissues (liver, kidney heart) were harvested into 0.25M ice cold sucrose solution, homogenized and centrifuge at 3500 rpm at for 10 min in an MSC bench centrifuge (Beckman and Hirsch, Burlington, IO, USA) and used for the assays.

Phytochemical screening

Simple standard chemical tests were carried out for phytochemical screening as described by (9) and such tests were used to detect the presence of bioactive agents such as the alkaloids, tannins, saponins, flavonoids, phlobatannins, anthraquinones and cardiac glycosides in AGB.

Assay for protein and enzyme activities

The protein concentrations in the tissue of experimental rats were determined following the method reported by Gornal *et al*. (1949). Cupric ions in alkaline solution form a purple colored complex with any compound containing repeated-CONH-links such as proteins. The activities of AST and ALT level in the serum and tissue of experimental animals was assayed using commercial kits (Randox Laboratories).

***Invitro* lipid peroxidation**

Rats were sacrificed by cervical dislocation and dissected. Liver, kidney, heart were harvested and weighed on analytical weighing balance and washed with ice-cold phosphate buffered saline. Each tissue were homogenized in ice-cold phosphate buffer (1:10wlv) and centrifuged at 6000rpm for 10 min. The clear supernatant obtained was used for lipid peroxidation assay.

Statistical analysis

The experimental results were expressed as mean ± Standard Error of Mean (SEM) of three replicates and were subjected to one way analysis of variance followed by Duncan's multiple range tests. Significant levels were tested at $p<0.05$. The SPSS 11.0 (SPSS Inc., Chicago, USA), was used for this analysis.

RESULTS

The phytochemical constituent of AGB revealed the presence of cardiac glycosides, steroids, saponin, flavonoids and terpenoids and is presented in Table1.

The aqueous extract of AGB leaf was evaluated for the flavonoids content. In the present study, the total flavonoid content was1.57±0.64 µg rutin equivalent/g of dried weight as shown in Table 2

The % DPPH inhibitory effects of AGB 100 gm/ml, 75 gm/ml, 50gm/ml and 25 gm/ml AGB inhibits 30.42±1.68%, 27.37±0.23%, 20.39±1.73% and 18.55±0.56% of the DPPH-induced free radical, respectively (Table 3). These values correspond to 10 fold increase inhibition when 75 gm/ml extract preparation is compared to 100 gm/ml and 25 gm/ml preparation compared with 50gm/ml, respectively.

The ability of AGB to inhibit Fe^{2+} induced lipid Peroxidation in liver heart and kidney homogenates are presented in Table4. A dose dependent inhibition of lipid peroxides was observed in all the tissues studied. An indication of a potent lipid peroxide inhibition by AGB.

Table 1: The phytochemical constituents of AGB

PHYTOCHEMICALS	RESULTS
Alkaloids	Absent
Saponin	present
Tannin	Absent
Phlobatanin	Absent
Anthraquinone	Absent
Cardiac glycosides	Present
Flavonoids	Present
Steroid	Present
Terpenoids	Present

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Table 2: Flavonoids in AGB

Flavonoid content (Rutin equivalent (µg/ml)
1.57 ± 0.64

Flavonoid content is expressed in µg/ml Rutin equivalent of Gossypium barbadense leaves aqueous extract.

Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table 3: The DPPH radical scavenging activity of AGB

Extract concentration mg/ml	%DPPH
100	30.42 ± 1.68^{C}
75	27.37 ± 0.23^{b}
50	20.39 ± 1.73^{a}
25	18.55 ± 0.56^{a}

Values are the average of three separate experiments and represented as mean ± standard deviation. Values having the same letter are not significantly different (P≥0.05).

Table 4: the effect of AGB on Fe^{2+} induced lipid peroxidation in the heart, liver and kidney homogenates

Test (mg/ml)	Liver homogenate	Kidney homogenate	Heart homogenate
Basal	0.047 ± 0.01^{a}	0.043 ± 0.002^{b}	0.079 ± 0.002^{c}
Control	0.112 ± 0.025^{d}	0.098 ± 0.002^{e}	0.120 ± 0.002^{f}
20	0.068 ± 0.001^{c}	0.072 ± 0.002^{d}	0.101 ± 0.001^{e}
40	0.058 ± 0.002^{b}	0.069 ± 0.001^{d}	0.089 ± 0.001^{d}
80	0.045 ± 0.001^{a}	0.055 ± 0.002^{c}	0.065 ± 0.001^{b}
100	0.0403 ± 0.001^{a}	0.036 ± 0.002^{a}	0.059 ± 0.001^{a}

Values are the average of three experiments and represented as mean ± standard deviation. Values having the same letter are not significantly different (P≥0.05).

Table 5: The effect of AGB on serum lipid profile of rats

Extract conc (mg/kg bw)	triglyceride mg/dl	cholesterol mg/ml
Control	2.54 ± 0.22^{a}	1.32 ± 0.21^{a}
250	7.06 ± 0.77^{b}	2.107 ± 0.41^{a}
350	13.83 ± 2.05^{c}	2.16 ± 0.39^{a}
500	16.29 ± 2.87^{d}	2.33 ± 0.168^{a}
750	16.57 ± 1.64^{d}	3.94 ± 0.73^{b}
1000	16.04 ± 0.78^{d}	5.59 ± 2.05^{c}

Data are presented as mean ± standard deviation n=5. Values having the same letter are not significantly different (P≥0.05).

Table 6: The effect of AGB on serum protein and liver enzyme activities of rats

Extract conc. mg/kg bw	Protein conc g/l	ALT activity IU/L	AST activity IU/L
Control	74.92±8.56^{c}	4.08±2.02^{a}	7.42±2.50^{a}
250	57.70±12.97^{b}	10.01±0.96^{b}	13.77±2.47^{a}
350	49.21±14.84^{ab}	24.72±4.59^{c}	16.99±2.23^{a}
500	42.17±5.44^{a}	41.53±1.07^{d}	29.02±4.17^{b}
750	49.05±7.04^{ab}	82.85±5.71^{e}	67.12±7.97^{c}
1000	37.71±5.54^{a}	89.52±1.90^{f}	71.90±3.74^{c}

Data are presented as mean ± standard deviation =5. Values having the same letter are not significantly different (P≥0.05).

The effects of AGB on serum lipid profile of rats are shown in Table 5. An increase in triglyceride and cholesterol was observed.

The serum protein, Aspartatate aminotransferase (AST) and Alanine aminotransferase (ALT) activity in following administration of AGB are shown in Table 6. Exposure to 750 gm/kg and 1000 gm/kg extract resulted in 67.12 and 71.90% increases in the AST activity compared with the control.

DISCUSSION

The phytochemical footprint of a plant is the sole determinant of the biological activities of a plant [12-14]. The presence of cardiac glycosides in any plant is an indication of its potential cardiotoxicity. [15] reported cardiotoxic responses in plant exposed to high dosages of plant containing cardiac glycosides. Cardiac glycosides are potentially toxic at high dosage of administration due to their inhibitory action on the ATP-based pumps conspicuously present in the heart especially the Na+ /K+ ATPase [16]. [14] however reported therapeutic use of cardiac glycosides at low dosages in the treatment of congestive heart failure. The isolation of enzymes of the phenyl propanoid pathway and chalcone synthase [17] is an indication that Gossypium barbadense synthesizes flavonoids. Flavonoids which contain hydroxyl functional groups are responsible for antioxidant effects in some medicinal plants [18]. It also decreases capillary fragility and exert a cortisone-like effect on tissues [19]. The mechanisms of action of flavonoids are Said to be either through scavenging or chelating process [20, 21]. Since most flavonoids are stored in plant tissues as glycosides as does the cardiac glycosides, high activity of glycosyl-transferaseare present in some tropic plants [22] including Gossypium barbadense further establishes the presence of flavonoids and cardiac glycosides thus, consistent with the phytochemical screening result. The antioxidant and anti-lipoperoxidative properties of the Gossypium barbadense have been traced to the presence of flavonoids and its derivatives [20]. The presence of Steroids in Gossypium barbadense is consistent with the initial discovery of cardiac glycoside which also has a steroid nucleus; this is also consistent with the presence of the enzymes of the mevalonate pathway, squalene synthase in tissues of Gossypium barbadense [16]. Terpenoids also called Isoprenoids are present in all living organisms, but with an unusual diversity in plants [22]. These isoprenoids have many different functions. In primary metabolism, they function as membrane constituents, photosynthetic pigments, electron transport carriers, growth substances and plant hormones [23]. They act as glucosyl carriers in glucosylation reactions and are involved in the regulation of cell growth. They make plants toxic or indigestible therefore, they act as a defense measure against herbivores [24]. They act as antibiotics to protect the plant from pathogenic microorganisms [23]. This explains why the extract shown antibacterial and wound healing properties (8) Therefore, most isoprenoids are formed only in response to infection by bacteria or fungi [24]. Plant isoprenoids are important commercially, for example, as aroma substances for food, beverages and cosmetics, vitamins (A, D and E), natural insecticides (e.g., pyrethrin), solvents (e.g., turpentine) and as rubber and gutta-percha [25]. The plant *et al*., isoprenoids also comprise important natural substances, which are utilized as pharmaceuticals or their precursors (26). Terpenoids also have antioxidant property but are known for their anti-microbial property [27]. At higher concentration, they exhibit fungalstatic property and recently they are being studied for their antiviral properties [28]. This explains the antifungi and antibacterial properties that the AGB exhibits (8). The use of Gossypium barbadense in folk medicine would be justified on the basis of the occurrence and distribution of the phytochemicals present in the plant [7]. Flavonoids are large compounds

occurring ubiquitously in food plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure [13, 18]. Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups [24]. DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. The assay is based on the measurements of the' ability of plant extract to scavenge the stable radical DPPH [13, 29]. 100 gm/ml solution of the extract yielded 31.42% DPPH inhibition and 25 gm/ml solution of the extract gave 18.55% DPPH inhibition. The dose dependent increase in the percentage inhibition of AGB is due to the presence of flavonoids, phenols and saponins. Flavonoids and saponins possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, anticarcinogenic, free radical-scavenging activities and metal chelators and also decrease cardiovascular complications [30, 31]. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as Advanced Lipoxidation End-products (ALE), in analogy to Advanced Glycation End products (AGE) [32,33]. Free radical species are highly reactive and capable of attacking the unsaturated bonds of the phospholipid membrane [34]. The product of this attack is the low molecular weight aldehydes and dialdehyde capable of reacting with thiobarbiturate to form MDA-TBA adduct [35]. Therefore, the higher the MDA, the higher the level of lipid peroxidation in such sample [35]. Table 4 shows the MDA level in the heart, liver and kidney homogenate exposed AGB; there was concentration dependent reduction in the level of MDA formed. This might be due to the presence of phenols, flavonoids and saponins in the extracts. To explain this finding, it could be explained that liver tissue has better extract assimilating property compared to the cardiac tissue of the heart. This may be particularly true because the liver has various transportation mechanisms for different compounds not found in other tissues of the body [36]. Therefore, the liver would be able to maximize the antioxidant phytochemicals in the extract at low concentration than the heart but at higher extract concentration, this advantage is cancelled out as a result of saturating effect. The MDA in the kidney homogenate shows that only 100 mg/ml AGB shows slight MDA reducing effect while 20 gm/ml to 80 gm/ml does not show promising anti-lipoperoxidative effect. The metabolic function of the kidney may explain this finding. The excretory function of the kidney may not allow the kidney tissue to accumulate the bioactive phytochemical therefore disallowing the AGB from performing its antioxidant role [37, 38]. Triglycerides do not exist in the blood stream as free TAGs rather, in complex form such as chylomicra, Very Low Density Lipoprotein (VLDL) and low density lipoprotein (LDL). While VLDL and LDL are derived from the liver upon endogenous synthesis of TAG and cholestryl esters, chylomicra is formed from the intestinal cells after digestion and absorption of exogenous or dietary lipids [39]. Table 5 shows the lipid profile (triglyceride and cholesterol) concentration of rats administered different dosages of AGB. The control group has the least recorded concentration of triglycerides. Since the extract administered was the aqueous, the increased concentration of triglycerides cannot be linked to the lipid composition of the extract rather, the lipogenic property of one or more of the phytochemical composition of the extract [39]. The best candidate for this activity would be the steroids, capable of activating HMG-coAreductase or at saturating concentration, prevents feed-back inhibitory actions on the enzyme [40]. Terpenoids also could demonstrate activating effect on acetyl coA carboxylase by selectively inhibiting the binding of free fatty acids to the regulatory subunit of acetyl CoA carboxylase [41]. A dose-dependent decrease in the protein content of the plasma with an increasing dosage of administration coupled with increased triglycerides in the plasma indicates that the plant extract may be able to induce fatty liver resulting from low mobility of VLDL due to the inability of the liver to synthesis protein [42]. Cardiac glycosides have been reported to possess anti-translation and protein misfolding properties [43]. Alternatively, if the liver synthesizes protein in adequate quantity but reduced plasma protein is observed, it may also indicate that the extract is causing renal excretion of protein this observation is consistent with steroidal functions [43]. The dose-dependent increase in plasma cholesterol Table 5 is also consistent with the increased triglycerides observed. Since the packaging of VLDL and LDL requires the presence of triglycerides as the core component [30]. Aspartate aminotransferase is a pyridoxal phosphate (PLP)-dependent enzyme of the muscles. Its metabolic function is reversible transfer of amino nitrogen from an amino acid to oxaloacetate to form aspartate and an alpha-keto acid which can further undergo oxidative reactions to form carbon-IV-oxide and water [42]. The enzyme is well distributed in both skeletal muscle and cardiac muscle and thus, changes in the tissue activity of this enzyme is associated with severe health complications [7]. A decreased activity of AST in the heart tissue is a primary diagnosis for myocardial infarction and other related cardiovascular complications while the decreased activity of the enzyme in the skeletal muscle points the onset of muscular dystrophy [44,45]. To authenticate this claim, serum/plasma activity of the enzyme indicates higher AST activities in the entire experimental group compared to the control. Further analysis shows that administration of 250 gm/kg and 350 gm/kg doses resulted in fairly similar activity if AST must be closely monitored to

establish necrosis in these tissues. Table 6 shows the serum protein, AST and ALT activity in rats responding to varied doses of AGB. Exposure to 750 gm/kg and 1000 gm/kg extract resulted in 67.12 and 71.90% increases in the AST activity compared with the control. For toxicity to be established, the recorded value must be about five times the normal value which means at 750 gm/kg and 1000 gm/kg dosages, AGB is toxic to rat muscles [7,30]. This finding could have resulted from the cardiotoxic activities of cardiac glycosides which were detected in the plant sample during the phytochemical screening procedure. Similar trend was also reported for ALT. However, the tissue distribution of ALT differs from AST [45, 46]. ALT is more of liver enzyme and it reversibly transfers amino nitrogen between an amino acid and pyruvate in a PLP dependent reaction. The data in Table 6 shows that AGB is toxic to the liver at dosages 350 gm/kg, 750 gm/kg and 1000 gm/kg body weight preparations. The hepatotoxicity of the extract may not be due to cardiac glycosides but steroids and terpenoids which have been characterized as disruptors of cell membrane and inhibitors of phospholipid synthesis respectively [47]. Evidence from the present study shows that, the phytochemical screening of Gossy*pium barbadense* indicates the presence of flavonoids, its concentration in the aqueous extract is relatively low and the reduced % DPPH inhibition at higher concentration of extract confirms that finding. Dose-dependent decrease in the *in vitro* tissue MDA may be due to multiplicity of effects; interaction between steroid nuclei and thiobarbiturate, free radicals scavenging of terpenoids and flavonoids.However, the triglyceride, cholesterol and protein data imply inherent toxicity which was subsequently confirmed by the serum pattern of ALT and AST enzymes.

CONCLUSION

The data presented in this study clearly indicate the hyperlipidemic and hepatoxic potential of aqueous extract of *Gossypium barbadense* in albino rats.

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