RESEARCH ARTICLE

Spathodea companulata (African tulip tree) stem and root barks extracts ameliorated N-nitroso diethylamine induced hepatic impairment in male rats

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ABSTRACT

Spathodea campanulata Beauv. is an important plant widely used in traditional medicine for the treatment of hepatic disease. In the present study, we evaluate the phytochemical composition and hepatoprotective potential effect of the stem and root bark extracts of *Spathodea companulata* on dimethylnitrosamine (DEN) induced hepatic impairment in albino rats. Phytochemical compositions were analyzed using standard protocols. Forty-five male rats were grouped into 9 (A-I) of 5 rats each. Groups A-F were treated with stem bark extract, root bark extract, quercetin, DEN + stem bark extract, DEN + root bark extract, DEN + quercetin respectively while groups G-1 serve as control groups and were treated with corn oil only (Vehicle control), DEN only (Toxicant control), and distilled water (normal control) respectively. DEN was given at 25 mg/kg b.w., i.p on a weekly basis while all treatments were administered at 100 mg/kg BW orally for 60 days. Serum transaminase activities, body weight, and liver body weight ratio were evaluated. Results revealed the presence of alkaloids, cardiac glycoside, saponins, anthraquinone, steroids, and flavonoids in stem bark extract of *Spathodea companulata***,** while the root bark contains cardiac glycoside, saponins, anthraquinonoid, steroids, and flavonoid. The DEN non-treated rat exhibited significant ($p<0.05$) weight loss and increase serum alanine transaminase (ALT) and aspartate transaminase (AST) activities when compared with the normal control. Treatment with stem and root bark extracts of *Spathodea companulata* significantly ameliorated the toxicant induce alterations in the transaminase activity and body weight loss when compared with the DEN non-treated rats. In conclusion, this study provides scientific validation of the use of this plant in traditional medicine for the management of hepatic disorders.

Keywords: *Spathodea companulata; diethylnitrosamine; hepatic injury; hepatoprotective*

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1.0 INTRODUCTION

Diethyl nitrosamine (DEN) is a semi-volatile, tasteless organic chemical, highly toxic especially to the liver, and a suspected human carcinogen produced as a by-product of several industrial processes e.g. the manufacturing of unsymmetrical dimethylhydrazine, which is a component of rocket fuel [1]. During water treatment via chlorination or chloramination of organic nitrogen-containing wastewater can lead to the production of DEN at potentially harmful levels [2]. DEN can also be formed or be leached during treatment of water by anion exchange resins and also present at very low levels in certain foodstuffs, especially those cooked,

smoked, or cured such as meat or fish, in addition to being present in beer and tobacco smoke [3]. The parenteral or oral administration of the smallest quantities of diethylnitrosamine (DEN) or dimethylnitrosamine (DMN) results in severe liver damage[4-6]. Most prominent are intense neutrophilic infiltration, extensive centrilobular haemorrhagic necrosis, bile duct proliferation, fibrosis, and bridging necrosis that ends in hepatocarcinogenesis [4,6]

The medicinal plant is defined as any plant with one or more of its organs containing a substance that can be used for the therapeutic purpose or which can be used as precursors for the synthesis of antimicrobial, anti-infectious, anti-tumor activities and anti- antioxidant drug [7-11] Plants are presently the source of medicines for many people of different ages in many countries of the world, where diseases are treated primarily with traditional medicine obtained from plants [10,12].

Spathodea campanulata P. Beauv. (family; Bignoniaceae), commonly referred to as the African tulip it's one of the world's most spectacular flowering trees which is commonly uses in traditional medicine for treatment of several diseases[13]. It is extensively distributed throughout Africa and is cultivated as an ornamental tree in tropical and sub-tropical countries, including American countries[13-15]. The flowers are employed as a diuretic and anti-inflammatory agent, while the leaves are used against kidney diseases, urethral inflammations, and as an antidote against animal poisons [13]. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, Stomach aches, diarrhea and sedative purpose [13,15,16]. It is, therefore, become relevant to evaluate the hepatoprotective and hepatoprotective potential effect of the stem and root bark extracts of *Spathodea companulata* at a single concentration on dimethylnitrosamine (DEN) induced toxicity in albino rats.

2.0 Materials and Methods

2.1 Plant Sample Collection and extraction

The stem bark and root bark of *Spathodea companulata* were collected at Ibadan. The plant was identified and authenticated at National Research Institute for Chemical Technology, Zaria, where voucher number 1613 was deposited. The plant was rinsed under clean running water, air dried and pulverized. The powdered sample was stored in an air tight container until it is ready for use. Five hundred gram (500 g) of powdered plant sample was extracted with methanol (2 liters) using reflux extractor. The concentrated extract and fractions were stored in refrigerator at 4 $^{\circ}$C.

2.2 Phytochemical analysis

The phytochemical screening of the powdered aqueous extract obtained from the plant was carried out using standard qualitative procedures of Trease and Evans [17], and Sofowora, [18]. This is with the view to access the secondary metabolites present in stem bark and root bark of *Spathodea companulata* extracts.

2.3 Experimental Animal

Forty-five healthy male Wistar rats weighing 120-150g were purchased from Central Animal House, College of Medicine, University of Ibadan, Nigeria for the study. The rats were acclimatized for a period of one week after purchase. They were housed five (5) rats per cage in plastic cages placed in a well-ventilated animal house, provided with rat pellets purchased from Top feeds distributors, and provided water *ad libitum.* They were subjected to natural

photoperiod of 12-hour light: dark cycle, for the period of acclimatization and observation until they were sacrificed.

2.4 Experimental Design

Forty-five (45) male rats were grouped into 9 (A-I) of 5 rats each. Groups A-F were treated with stem bark extract, root bark extract, quercetin, DEN + stem bark extract, DEN + root bark extract, DEN + quercetin respectively while groups G-1 serve as control groups and were treated with corn oil only (Vehicle control), DEN only (Toxicant control), and distilled water (normal control) respectively. DEN were given at 25 mg/kg b.w., i.p on a weekly basis while all treatments were administered at 100 mg/kg BW orally for 60 days.

2.5 Collection of blood sample

Twenty-four hours after the last treatment, the animals were sacrificed by cervical dislocation. Blood was collected in non-heparinized tubes by ocular puncture and allowed to clot. Serum was then separated by centrifugation of the clotted blood at 3000 rpm for ten (10) minutes with a table centrifuge [19]. The liver was harvested with dissecting scissors and forceps and were quickly rinsed in ice-cold 1.15% potassium chloride (KCl) solution, blotted dry and weighed. The liver body weight ratio were computed as described previously [20]

2.6 Determination of transaminase activities

All biochemical analyses were conducted using Randox Diagonistic kit (Randox Laboratories Ltd, Crumlin, UK). Alanine transminase (ALT) was analysed on the principle of catalytic action of ALT on alanine and α – oxoglutarate to form pyruvate and glutamate [21]. Aspartate transaminase (AST) was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine [22].

2.7 Statistical Analysis

All quantitative data were expressed as means ± standard deviation. Treated and control groups were compared using one-way ANOVA. A 95% confidence level was used to determine statistically significant differences between treated ad control groups. P values less than 0.05 ($p < 0.05$) will be considered as indicative of significance.

3.0 Results and Discussion

Medicinal plants have serve as source of therapeutic agents for the treatments of various ailments, some of which are without scientific credence to substantiate or refute such claims. The preliminary pharmacological screening of crude extracts of medicinal plants, which is a step towards the successful isolation of bioactive principles, will serve as lead for drug development and promote conservation and sustainability of ancient knowledge [23].

Our phytochemical studies revealed the presence of alkaloids, cardiac glycoside, saponins, anthraquinone, steroids, and flavonoids in stem bark extract of *Spathodea campanulata* (Table 1). The bark is reported to contain sterols, triterpenoids, tranorin, vannillic acid, ferulic acid, verminoside, pelargonidin diglycoside, maldivin, and tannins *[24]* . Leaves contain polyhydroxy sterol spathodol, quercetin, and chlorogenic acid [15]. *Spathodea campanulata* has been used in folk medicine for the treatment of several diseases. The therapeutic properties of this plant therefore could be attributed to the presence of the

secondary metabolite identified in the stem bark and root bark extracts of *Spathodea companulata*

Table 1: Phytochemical composition on stem bark and root bark extracts of *Spathodea companulata*

TEST	STEM BARK	ROOT BARK
alkaloid	**+ +**	**-**
Cardaic Glycoside	**+**	**+**
Saponin	**+ +**	**+ +**
Tannis	**-**	**-**
Anthraquinone	**-**	**+**
Steriod	**+ +**	**+ +**
Flavonoid	**++**	**+ +**

+: Fairly present, + +: Moderately present, + + +: Excessively present

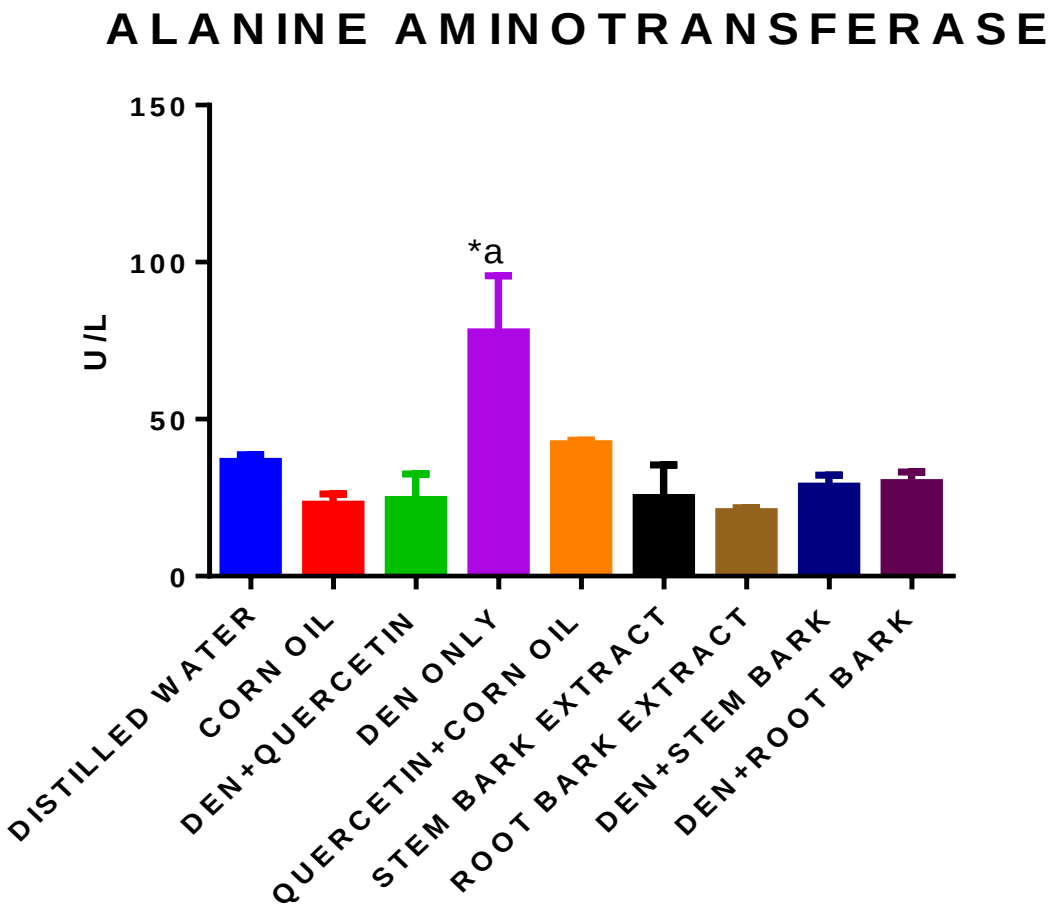

Figure 1: Effect of stem bark and root bark extracts of *Spathodea companulata* on alanine transaminase activities in DEN intoxicated rats. Data are MEAN±SEM of 5 replicates. The superscript "a" and "*" indicate values with significant differences from other groups at $p < 0.05$.

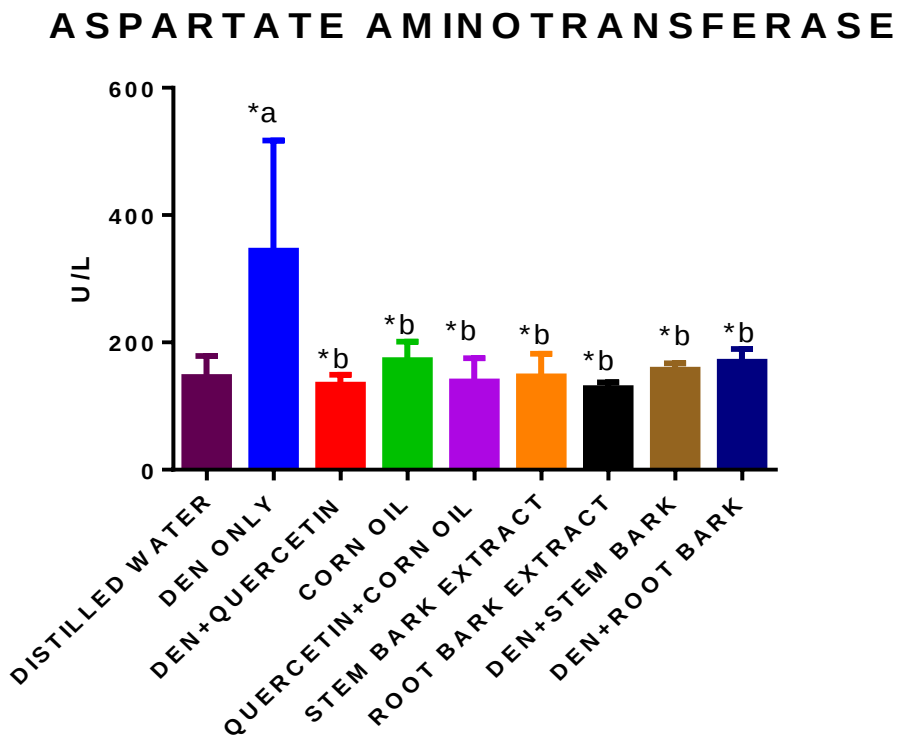

Figure 2: Effect of stem bark and root bark extracts of *Spathodea companulata* on aspartate transaminase activities in DEN intoxicated rats**.** Data are MEAN±SEM of 5 replicates. The superscript "a,b" and "*" indicate the significant differences between the groups at p <0.05.

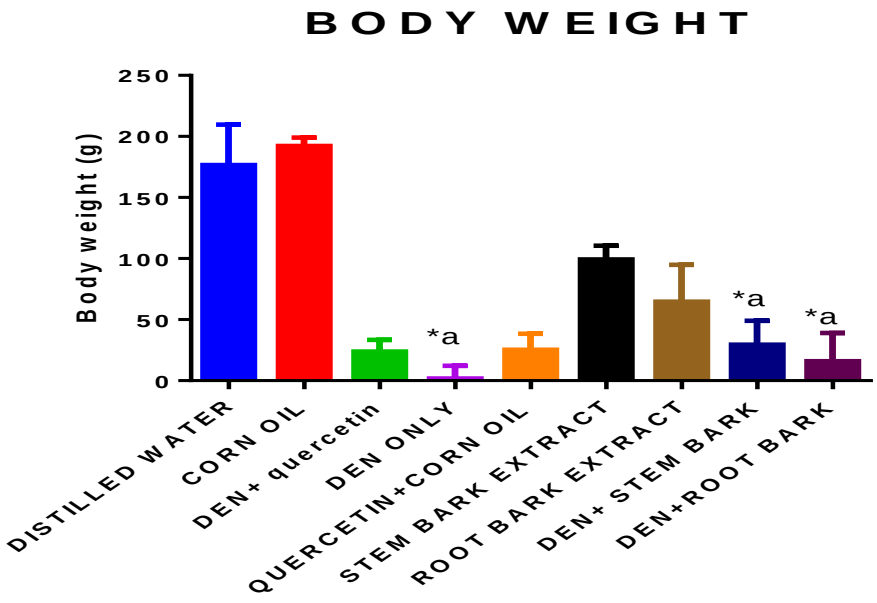

Figure 3: Effect of stem bark and root bark extracts of *Spathodea companulata* on body weight gain of DEN intoxicated rats. Data are MEAN±SEM of 5 replicates. The superscript "a" and "*" indicate values with significant differences from other groups at p <0.05.

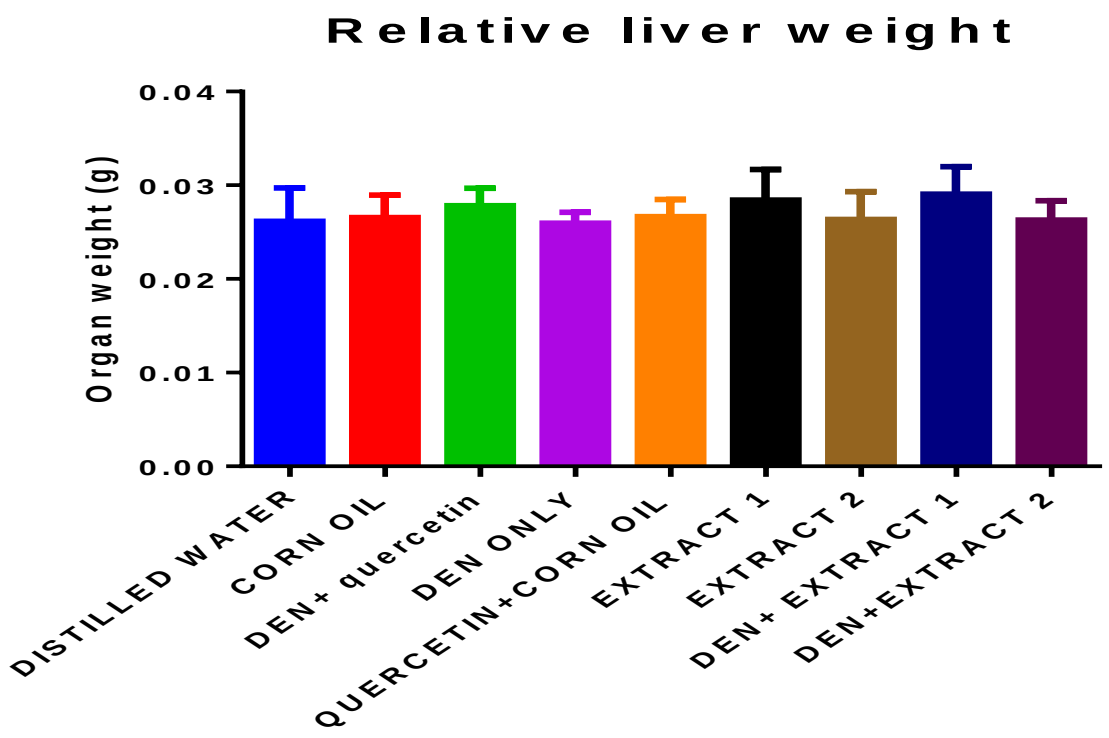

Figure 4: Effect of stem bark and root bark extracts of *Spathodea companulata* on relative liver body weight gain of DEN intoxicated rats. Data are MEAN±SEM of 5 replicates.

Several studies have established the toxicity of Diethylnitrosoamine (DEN), also known as *N*-nitrosodiethylamine, which is widely used as a carcinogen in experimental animal models which affect mostly the liver and gives rise to hepatic tumors [4]. Consequently, in this study we evaluated the protective effect of the stem and root barks extract of *Spathodea companulata* on DEN-induced liver impairment

Hepatic enzymes which include Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are markers for early acute damage [25,26] due to their localization in periportal hepatocytes as a reflection in oxidative phosphorylation and gluconeogenesis. There was a significant increase in the level of aspartate aminotransferase (figure 1) in the DEN administered group indicative of liver damage. The enzymes may be released into blood plasma and serum, and their levels may continue to increase due to cellular damage in the liver [27]. The increase in the activities of liver enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood while there was a significant decrease in the groups[20]. There is a significant increase in the AST levels in the DEN group as compared with the control (Figure 1).

There was a significant decrease in the extracts and quercetin groups as compared with the control. However, the group treated with DEN + root bark group had a non-significant difference as compared with the DEN control while those treated with DEN + stem bark extracts showed a significant decrease in AST level which might indicate that the extracts were able to reduce cellular damage in d liver and prevent the enzymes from leaking into the blood plasma [28]. There was a significant decrease in the level of alanine transaminase (figure 2) in groups DEN+ stem bark and DEN+ root bark when compared to the positive

control which indicates that the extracts were able to mitigate the effect of DEN in destroying the hepatic enzymes.

The percentage body weight change in the DEN + Extract groups significantly decreased as compared to the control (figure 3) whereas, no significant difference was seen in the absolute and relative body weights across the groups (Figure 4). Suggesting that the the doxicant has not mediated liver hypertrophy, swelling or constriction [29]

4.0 Conclusion

This study revealed that the stem and root bark extracts of *Spathodea companulata* ameliorated DEN-induced toxicity as seen in the results on the body weight and serum transaminase activity in rats. However, in a comparison of the stem and root bark methanol extract at the same dose of 100mg/kg BW *Spathodea companulata* stem bark extract more potently ameliorated the DEN induced toxicity. This study provides scientific validation of the use of this plant in traditional medicine for the management of hepatic disorders

Conflict of Interest

The author declared no conflict of interest exist

Authors contributions

The work was conducted in collaboration of all authors. All authors read and approved the final version of the manuscript.

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