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Protective Effects of *Delonix regia* Leave Extract on Sodium Arsenite-Induced Hepatotoxicity in Rats

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Abstract

Nature provides rich sources of structurally diverse phytochemicals with medicinal relevance and biological activities. Sodium arsenite has been recognized as a worldwide health concern due to its teratogenicity effects on animals. Natural plants are considered as a possible protective agent against arsenic induced toxicity. The aim of this study was to investigate the protective effects of *Delonix regia* leave extracts on sodium arsenite induced hepatotoxicity in rat. Animals were randomly divided into six groups of five per group. Group A (control) received distilled water for fourteen (14) days. Group B received 2.5 mg/kg body weight Sodium Arsenite, group C received 100 mg/kg of leave extract and 2.5 mg/kg of sodium arsenite, Group D received 400 mg/kg of leave extract and 2.5 mg/kg of sodium arsenite, Group E received 100 mg/kg of leave extract, Group F received 400 mg/kg of leave extract only. On the fourteen day all the animals were sacrificed. Biochemical parameters such as plasma Alanine amino transferase (ALT), Aspartate amino transferase (AST) Alkaline phosphate (ALP) and gamma glutamyl transferase (GGT) total protein (TP) were evaluated. Sodium arsenite in rat triggers significant increase in ALT, AST, ALP, TP, and GGT ($p<0.05$ level). Upon treatments with *Delonix regia* leave extract, it decreases the concentration of GGT, AST and ALP compared to the negative control. Group treated with the extract alone showed no adverse effects on the liver parameters. Results from this study suggest that, the administration of *Delonix regia* leave extract confer some protective effects on the liver.

Keywords: Hepatotoxicity; *Delonix regia*; Leaf extract; Sodium Arsenite; Phytomedicine

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Introduction

The use of herbal medicine with phytonutrient or nutraceuticals continues to expand across the world with people resorting to these products for treatment of various diseases [1]. It's been estimated that up to four billion people in the developing world rely on herbal medicinal products as a primary source for healthcare [2-4]. As the global use of herbal medicinal products continues to grow and new products are introduced into the market, public health concerns surrounding their safety are increasingly being discussed. Some herbal medicines have promising potential and many of them remain scientifically important hence the need to examine their toxic effects, enhance and increase knowledge of their potential adverse effects, which hitherto, are limited, establishing the safest and most effective therapeutic dose [5]. *Delonix regia* belongs to the family of *Fabacea* subfamily *caesalpiniodeae*. It is a Legume flowering plant, grown as ornamental tree and also known as flame of the forest. *Delonix regia* leave extract has been reported to exhibit antimicrobial, anti- ulcer and anti-oxidative properties [6,7]. Phytochemical screening of *Delonix regia* leave revealed the presence of alkaloids Flavonoids, steroids, saponins and tannins [8]. The leave is said to posses anti diabetic and anti-cytotoxic activity [9]. *Delonix regia* roots seed and leaves have been used extensively in the treatment of many diseases and ailments including diabetes [10]. Arsenical (chemical compounds that contain arsenic) are used as herbicides, fungicides and rodenticides; hence it may lead to air, soil and water pollution. Human exposure to arsenic

results in its accumulation in the hair, nails, muscles and skin [11]. Such accumulation may result in various diseases like cancers, hepatic damage, diabetes; hypertension [12]. Arsenic toxicity is promoted through reaction of arsenite with sulfhydryl group of enzyme, affecting various tissues including the liver and heart enzymes. Occupationally, arsenic toxicity is linked especially amongst workers in the mining and smelting industries due to ore oxides of sodium which are of arsenic origin [13-15].

Biochemically, the adsorption of arsenic are thought to lead to arsenicosis, enhancing the production of reactive oxygen species that causes DNA methylation, genotoxicity, promoting tumor growth with other carcinogenes [16-19]. *Delonix regia* leaves are thought to have therapeutic effects with minimal adverse effects. Thus, this study was designed to investigate the hepatoprotective effects of *Delonix regia* leaf extract on sodium arsenite induced hepatotoxic rats.

Materials and Methods

Chemicals

Fine chemical of Sodium Arsenite (Sigma-Aldrich) was used in this experiment. Alanine transaminase (ALT), Aspartate transaminase (AST), gamma glutamyl transferase (GGT), total protein (TP), alkaline phosphatase (ALP), analysis were done using agape kit (India).

Plant materials and leaf extraction

Fresh leaves of *Delonix regia* were harvested from the University of Abuja (main campus) and identified at the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria. The leaves were confirmed with a Voucher number NIPRD/H/7105 deposited at the herbarium section of the Institute.

The leaves were allowed to dry at room temperature and blended into fine powder and mixed with 80% ethanol (analytical grade) at ratio of 1:5 for seventy- two hours (72 hours). The mixture was filtered using Whattman filter paper and the filtrate evaporated (9.1% yield) using rotary evaporator. The filtrate was allowed to dry at room temperature.

Phytochemical screening

Preliminary phytochemical screening (18-19) protocol, revealed the presence of saponins, alkaloids, glycosides, tannins, and steroids.

Experimental Procedure

Animals

thirty (30) male wistar rats (100-200g) were used for this study was purchased from the animal department of the National Institute of Pharmaceutical Research Department (NIPRD). The animals were housed in the animal house Department of Medical Biochemistry University of Abuja, and fed with rat pellets from livestock feed (kings veterinary Gwagwalada), for two weeks.

Experimental protocol

Animals were weighed and divided into six groups (group A-F) of 5 rats each. Rats in each group were numbered 1 to 5 and placed in separate cages in the animal house under natural day and night cycles. The animals were housed in standard animal cage, at room temperature with access to water in accordance with international guide for the care and use of laboratory animals (Committee for update of the guide for the care and use of laboratory animals, 2011). They were maintained under controlled environmental condition with a 12 hour dark: light cycle and treated via oral intubation, as follows.

- Group A administered distilled water only (negative control)
- Group B administered with 2.5 mg/kg body weight of sodium arsenite for 14 days (positive control).
- Group C were co-administered with 100 mg/kg body weight of the leave extract and 2.5 mg/kg of sodium arsenite for 14 days.
- Group D were co-administered with 400 mg/kg body weight of the leave extract and 2.5 mg/kg of sodium arsenite for 14 days.
- Group E was administered with 100 mg/kg body weight of the leave extract only for 14 days.
- Group F was administered with 400 mg/kg body weight of the leave extract only for 14 days.

All rats were treated for 14 days. Blood was collected from the rats at three different times. The first was on the third day (3^{rd}) of treatment, the second on the seventh day (7^{th}) of treatment, and the third was on the fourteen day of treatment. The blood samples were collected from the rats through retro orbital bleeding placed in heparin bottles and centrifuged at 1500 rpm for 10 minutes with plasma obtained for liver assays.

Biochemical assay

Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma Glutamyl transferase (GGT), Total protein (TP), Alkaline phosphatase (ALP) were analyzed using commercially available kits prepared by Human Company (Agape kit, India) and an automated analyzer (lab kit, UV spectrophotometer model ST-UV-7558), according to the manufacturer's instructions.

Lethal dose (LD 50) determination

Nine female wistar rats were used and divided into three groups of three animals each. Each group were administered different doses of 1000 mg/kg, 3000 mg/kg 5000 mg/kg. The animals were placed under observation for 24-hours to monitor their behavior and mortality rate. Acute toxicity study (LD50) was determined using the organization for economic cooperation and development OECD method.

Data analysis

All results were expressed as mean ± Standard error of mean

(± SEM). Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical analytical software (SAS) at *P*-values <0.05 which were considered statistically significant.

Results

Preliminary phytochemical screening of the *Delonix regia* leaves extracts were carried out, ascertaining the presence of phytochemicals, those detected are shown in the **Table 1**. The result of the phytochemical constituent of the ethanolic extract of *Delonix regia* leaves is summarized in **Table 1** above. It reveals that tannins, glycosides, saponins, terpenes, flavonoids, phenol steroids are present in both ethanolic leave extract of *Delonix regia*. From the results, on day 3, a significant difference was observed between GGT level of group D compared to group B, the concentration of group D (14.8 ± 4.2) is lower compare to group B. the mean concentration of group D which is (14.8 ± 4.2) is lower compared to negative control group B which is (42.5 ± 7.2). After seven days there was a significant difference between mean GGT level of the mean concentration of group C (22.5 ± 5.8) and group D (18.0 ± 2.5) which is lower compared to the negative control **(Table 2).** Also, for the alkaline phosphate there was a significant difference between group D and negative control group B. the mean concentration of group C (648.2 ± 148.0) is lower compare to the negative control group B (1040 ± 49.8). On day fourteen there was a significant difference between AST levels with the mean concentration of group C (130.3 ± 19.9) and group D (145 ± 8.3) found to be lower compare to negative control group B (187.8 ± 2.2). **Table 3** shows the protective effects of ethanolic leave extract on *Delonix regia* leave extract only on liver parameters. The results showed no significant difference between mean concentration of various group and parameters on day 14 compared to negative control (group A) even with different doses of the leave extract. Also there is a significant difference between mean concentrations of different group compared to the positive control except for total protein.

Discussion

In this study, we investigated the effects of *Delonix regia* leaf extract on sodium arsenic induced liver toxicity in rats. Phytochemical analysis of the leaf extract of *Delonix regia* showed the presence of alkaloids, tannins, and terpenoids, reducing sugar, glycosides and flavonoids. These constituents give value to the therapeutic applications of *Delonix regia* leaf extract.

The ethanolic leaf extract of *Delonix regia* has therapeutic effects on the liver with a Lethal dose (Ld_{50}) of 4000 mg/kg body weight

Table 1 Phytochemical composition of the leaves of *delonix regia*

Phytochemicals	Observation
Tannins	+
Glycosides	+
Saponins	+
Terpenes	+
Resins	-
Flavonoids	+
Phenol	+
Steroid	+

Table 2 Effects of ethanolic leave extract of *delonix regia* on liver enzymes of sodium arsenite treated rats.

Day 3					
Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**TP (g/L)**	**GGT (U/L)**
A (Water Only)	128 ± 8.2	63.8 ± 11.7	858.6 ± 65.4	50.8 ± 6.5	24.2 ± 10.8
B (SA Only 2.5 mg/kg)	152.5 ± 6.5	103.8 ± 10.4	973 ± 459.6		42.5 ± 7.2[a]
C (SA+LE 100 mg/kg	138.6 ± 16.5	90.2 ± 43.7	978.8 ± 430		27.8 ± 23.1
D (SA+LE 400 mg/kg)	152.2 ± 34.5	81 ± 16.6	1011.8 ± 333.2		14.8 ± 4.2[a]
day 7					
A (Water Only)	128 ± 8.2	63.8 ± 11.7	858.6 ± 65.4	50.8 ± 6.5	24.2 ± 10.8
B (SA Only 2.5 mg/kg)	155 ± 3.1	105.3 ± 6.7	1040 ± 49.8[a]	65.5 ± 12.8	50.25 ± 11.2[a,b]
C (SA+LE 100 mg/kg	154.3 ± 5.3	79.3 ± 29.8	719.5 ± 244.5	58.8 ± 12.0	22.5 ± 5.8[a]
D (SA+LE 400 mg/kg	156.4 ± 9.9	66.8 ± 7.1	648.2 ± 148.0[a]	56.2 ± 11.0	18 ± 2.5[b]
Day 14					
A (Water Only)	128 ± 8.2[c]	63.8 ± 11.7a	858.6 ± 65.4	50.8 ± 6.5	24.2 ± 10.8
B (SA Only)	187.8 ± 2.2[a,b,c]	112.0 ± 7.2a	1004 ± 273.2	68.7 ± 5.0	11.0 ± 0.1
C (SA+LE 100 mg/kg	130.3 ± 19.9[b]	84.0 ± 30.5	882.3 ± 87.5	72.3 ± 12.2	34.3 ± 17.4
D (SA+LE 400 mg/kg	145 ± 8.3[a]	80.8 ± 18.8	926.4 ± 338.5	69.4 ± 11.1	34.6 ± 28.9

The results in the Table 2 showed the different liver enzymes and biochemical parameters.
[abc] different letters mean a significant difference at p<0.05 (two way ANOVA) values represented as mean ± SEM. Abbreviations: LE, leave extract; SA, sodium arsenite. Note: the parameters for total protein on the third day of treatment was not recorded because there was shortage of plasma for the analysis of total protein on day 3 and also the low value of GGT at day 14 is as a result of shortage of plasma from the blood of the rats.

Table 3 Protective effect of leave extract only compared to negative control on liver of wistar rats.

Day 14					
Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	GGT (U/L)
A (Distilled Water Only)	128.0 ± 8.2	63.80 ± 11.7	858.8 ± 65.4	50.8 ± 6.5	24.2 ± 10.8
B (SA Only)	187.8 ± 2.2^{ab}	112.0 ± 7.2^{ab}	1004 ± 273.2^{ab}	68.7 ± 5.0	11.0 ± 0.00
E (100 mg/kg leaf extract Only)	127 ± 17.3^{a}	76 ± 9.2^{a}	896 ± 100.7^{a}	55.5 ± 6.9	24.5 ± 11.9
F (400 mg/kg leaf extract)	118 ± 10.7^{b}	75.4 ± 9.4^{b}	904 ± 97.5^{b}	58.8 ± 4.3	21.4 ± 7.2

The result in Table 3 Show the protective effects of the leave extracts as compared against the negative control. Following the treatment as described earlier, the different liver enzymes and parameters were analyzed and quantified.
^{abc} different letters mean a significant difference at $p<0.05$ (Two way anova) values represented as mean ± SEM.
Abbreviations: LE: Leave Extract; SA: Sodium Arsenite. Values represented as mean ± SEM.

considered to be a moderately toxic to mice. The rats treated with 1000 mg/kg body weight for two days did not show any negative effects suggesting that at such acute concentration, administration of the extract is safe.

In this study, liver function test was carried to check the level of damage caused by sodium arsenite in the liver. To help determine the health of the liver, levels of proteins, liver enzymes were measured in the blood. AST, ALT, ALP, total protein and GGT are tests used to check various abnormalities in the liver. ALT and AST test measures enzymes that the liver releases in response to damage or disease. Total protein measures the total amount of albumin and globulin in the body. Alkaline phosphatase can be used to evaluate the bile duct system of the liver, while GGT is measured if there is damage to the bile ducts due to chronic alcohol abuse or certain bone diseases. GGT test is currently the most sensitive enzymatic indicator of liver damage and diseases. These five parameters are part of the routine liver function test carried out; these inform the reason why these parameters were used to determine the extent of liver damage in this study.

Plasma ALT, ALP and GGT are essential enzyme in biological processes. These enzymes are considered as biomarkers of liver injury [20,21]. High levels of ALT (alanine transaminase) are an indication of liver damage from hepatitis infection, liver cirrhosis, liver cancer or other liver diseases. Elevated levels of ALP indicate liver disease or bone disorders, cirrhosis, hepatitis mononucleosis, which can sometimes cause a swelling in the liver. Also, high levels of these enzymes are associated with several pathologies including, myocardial infarction and neoplasm [22]. Further, AST and ALT are enzymes directly associated with conversion of amino acid to keto acid, elevated activities of AST and ALT in the serum is a common laboratory finding in some forms of cancer e.g. colon cancer, especially with liver metastasis. Thus, phyto-compound that targets and regulate these biochemical parameters could be useful in the management of cancer with its multifaceted consequences. Studies show that GGT was usually normal in patient without metastatic carcinomas of the liver but high GGT activity is found in patient with hepatoma and carcinoma of the pancreas [23]. Elevated serum GGT levels have been found in patients with hepato biliary disease and alcohol abuse [24]. The present study showed that sodium arsenite increases the level of these enzymes in the blood. However, the administration of the *Delonix regia* leave extract in rats exposed to sodium arsenite ameliorated the effects of the toxicant, suggesting a possible modulatory role of the extract against sodium arsenite-induced hepatotoxicity [25,26]. This could possibly be attributed to the extract's prevention of intracellular leakage of the enzymes (ALT, ALP and GGT) via membrane stability [27]. However, rats administered with the extract alone showed decrease in the activities of these enzymes, compared with the rat exposed to sodium arsenite alone. Arsenic is a naturally occurring metalloid, which can contaminate water sources and had been found to be a contaminant to underground water. While certain amount of arsenic is present in underground water (>10ug/L) beyond this threshold, arsenic is known to cause health hazards albeit, it has been implicated in the occurrence of various cancers [28]. Various forms such as $NaAsO_2$ (sodium arsenite) are known to be an enzyme inhibitor [29] as it attacks the thiol and sulfhydryl group of some enzymes inhibiting their activities. Earlier it is thought most probable sodium arsenite may share similar mechanism with such enzymes inhibitors as cyanide and polychlorinated biphenyl (PCB) [30]. Biological cell bio-membrane are susceptible to the actions of free radicals via its damage to their active oxygen through some covalent modifications with concomitant binding to cellular macromolecules, formation of lipid peroxidation which is implicated in carcinogenesis, inflammation and aging. The ability for arsenite to increase cell production of free radicals are what may be exerting its harmful effects as this process eventually lead to increase in lipid peroxidation with attendant production of malondialdehyde (MDA) and lipid peroxide. Research show that *Delonix regia* leave extract possess four major bioactive phyto-constituent which are β-sitisterol, lupeol, Flavonoids and phenolic acid including, gallic acid, protocatehuic acid and silycyclic acid. β-sitosterol is a sterol of plants that can reduce cholesterol serum levels, regulate inflammation and possesses some anti-cancer activity. Also, it is thought to provide antioxidant and cardio protective effects by attenuating TNF-alpha induced monocyte adhension on human aortic endothelial cells. Lupeol a triterpenoid compound has anti inflammatory activity and may act as a promising compound to protect heart and liver from injury. Phenol acids exhibit high antioxidant, anticancer and antimicrobial activity with cardio protective activity. Flavonoids show an antioxidant activity both *in vivo* with ability to absorb and neutralize free radicals as well as removes reactive oxygen species. It is however, unfortunate that cellular targets for phyto-active compounds remain largely unclear, although flavonoids and some other phytochemicals have been used to target functionally diverse cellular processes and they do help to modulate the activity of a large number of downstream genes.

Exposure to Sodium Arsenite had been shown to increase activity of liver transaminases in the blood this is an index of hepatotoxicity, which might have resulted from oxidative stress/ reactive oxygen species (ROS) damage to hepatocyte membrane, and leakage of hepatic transaminases into extracellular spaces and ultimately finding their way into the blood from the liver. Further, it is thought that, ROS via its continuous attack on protein, lipids and DNA are thought to be involved in producing the changes in diseases and its associated aging process. They seems to affect cancer cell proliferation through modification of histone acetylation with, its ability to target multiple pathways, including the cell cycle machinery and thus, regulate gene expression and proliferation. The results presented in the **Table 2**, show a significant increase in the activities of AST, ALP, TP, GGT and ALT ($p<0.05$) in the serum of sodium arsenite treated rats when compared to the control. Conversely, the results show a significant decrease in serum activities of AST, ALP and GGT ($p<0.05$) at different doses and days of treatment with *Delonix regia* leave extract and sodium arsenite compared to the sodium arsenite only, suggestive of hepatocyte protection due to *Delonix regia* co-treatment against Sodium Arsenite -induced damage. Research in the field of ROS-biochemistry is slowly evolving with large volume of theory and hypothesis, as it relate bioactive phyto-compounds, yet to be tested. Although, enzymatic tools investigating ROS via xanthine oxidase and its elimination via SOD has helped to facilitate additional research in a number of areas of molecular biology and pathology. Therefore, concerted biochemical/molecular research efforts on these phyto-compounds are required, to drive a successful phytochemical such as *Delonix regia,* intervention on a larger scale, with its potential to boost other immune events due to several feedback mechanisms that tends to be associated with diseases processes.

Our study lead credence to some earlier studies suggesting the use of antioxidants and antioxidant-rich foods and herbal medicinal plants for the management of arsenicosis. The activities of all the liver enzymes (GGT, AST, ALT, TP and ALP) were significantly low compared to the negative control on day 14 confirming the protective effects of the leaf extract.

Conclusion

Conclusively, the results of this study show that ethanolic leaf extract of *Delonix regia* contain certain bioactive agents with therapeutic property, which may give credence to its wide medicinal use across West African countries. However, further detailed studies are needed to characterize and isolate these active compounds/constituents, serving as possible template for drug discovery or food supplements.

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