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Research Article

Antioxidant Actions of Freshly Commuted Hexane Extracts of Alchornea Laxiflora Root on Sodium- Arsenate Induced Oxidative Stress in Male Wistar Rats

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Abstract:

The antioxidant activities of hexane extract of freshly commuted roots (H_R) of *Alchornea laxiflora* on liver damage were examined using *in vivo* sodium arsenate-induced oxidative damage model in male Wistar rats weighing between 150 and 200 g. The animals were grouped into two groups of four animals, group 1 animals were given 1ml of sodium-arsenate (2mg/kg) for 2 days and then treated with graded dosage (0.5, 1.0, 5.0 and 10.0 mg/kg body weight) H_R of *Alchornea laxiflora* for 10days, while group 2 animals were given the same graded dosage H_R extract of *Alchornea laxiflora* for 10 days but in addition, received 1 ml of sodium-arsenate (2mg/kg) from 6th-10th day. At expiration of dosing period, the animals were sacrificed and their blood and organs harvested for estimation of AST, ALT, GGT, Total Bilirubin and Creatinine levels. Pre-treatment with H_R extract of *Alchornea laxiflora* prior to intra-peritoneal administration of 2mg/kg body weight of sodium arsenate reduced significantly ($P<0.05$), the levels of AST, ALT, GGT, Total Bilirubin and Creatinine level in the serum and liver, showing the hepatoprotective and anti-oxidative capacity of *Alchornea laxiflora.* Furthermore, the extract evaluated by its capacity of quenching free radicals induced by the sodium arsenate, showed remarkable scavenging effects on the thyl and singlet oxygen free radicals, at concentrations of 5.0 and 10.0mg/kg. These findings suggest the tendencies of H_R extract of *Alchornea laxiflora* to elicit protective actions in sodium arsenate-induced liver damage in rats hence, a protective role against pro-oxidant induced membrane damage.

Keywords: Alchornea laxiflora, Sodium arsenate, Oxidative stress, antioxidants

Introduction

The folklore knowledge of medicinal plants has significantly contributed in discovering many important drugs of modern system of medicine [*1*]. Many plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardio-protective, anti-bacterial, antiviral and anti-protozoal [*2*]. *Alchornea laxiflora* is one of such plants. It is a deciduous shrub of the family of euphorbiacea [*3*]. The stems of *Alchornea laxiflora* has been used in the treatment of sexually transmitted diseases (STD) with alkaloids as the principal agent [*4*]. Decoctions of the leaves are used in the treatment and management of inflammatory and infectious diseases as well as an important component of herbal anti-malarial formulation [*5*].The leaf and root extracts of *Alchornea laxiflora* contains potent natural antioxidants and may therefore be useful in the preservation of lipid food products which are prone to oxidation and rancidity [6].Phytochemical screening of the powdered leaf sample of *Alchornea laxiflora* revealed the presence of alkaloids, cardiac glycosides, saponins and phenolic compounds [7.]

The presence of terpenoid compounds was also discovered in the root samples of *Alchornea laxiflora* [*6*]. Despite the popular uses of this plant in traditional medicine, there is dearth of information on its antioxidant and hepatoprotective potentials. Animal studies have shown that the liver is a major target organ for arsenic toxicity, being a vital organ for methylation of inorganic arsenate [*7*]. Sodium arsenate can act as a tumour promoter in different animal organs, such as bladder, liver, kidney, thyroid and skin and dose dependence has been confirmed for promoting effects on urinary bladder and liver carcinogenesis [*8*]. Arsenate has been proposed to cause toxicity through increased oxidative stress. In continued search for antioxidant actions and hepatoprotective medicinal plants, this work aims to evaluate antioxidant and hepatoprotective effects of *Alchornea laxiflora* root on sodium arsenate -induced oxidative stress in wistar male rats.

Materials and Methods

Plant Extraction

All procedures were certified by institution research and ethics committee and also by the local government research committee

Samples of freshly commuted roots of *Alchornea laxiflora* was extracted with hexane utilizing a soxhlet apparatus for continuous extraction at 80^0C for 36 hours. In each case, the crude extracts were collected and concentrated with a rotary evaporator to give a hexane root (H_R) extract of *Alchornea laxiflora*

Experimental Animals

Eight Adult male albino rats of Wistar strain weighing 150-200g were obtained from the University of Medical Sciences, Biochemistry Department, Ondo, Ondo-State Nigeria.
The animals were acclimatized and fed with rat pellets from Pfizer feeds, Nigeria and UV irradiated tap water *ad libitum* for 14days. The animals were grouped into two (2) groups, each group having sub-groups containing 4 rats of similar weights and were given specific dose of the H_R extract of *Alchornea laxiflora*.

Group 1 (post-treatment) were given 1ml of sodium arsenate for 2days and then treated with the H_R extract *Alchornea laxiflora* for 10days

Group	Days	Treatment
Control	1-2	Rat chow and water only
A	1-2	Rat chow and water plus 1ml of sodium arsenate (2mg/kg) per day
B	1-2	Rat chow and water plus 1ml of sodium arsenate (2mg/kg) per day
C	1-2	Rat chow and water plus 1ml of sodium arsenate (2mg/kg) per day
D	1-2	Rat chow and water plus 1ml of sodium arsenate (2mg/kg) per day

Group	Days	Treatment
Control	3-12	Rat chow and water only
A	3-12	Rat chow and water plus 0.5mg/kg H_R of *Alchornea laxiflora*
B	3-12	Rat chow and water plus 1.0mg/kg H_R of *Alchornea laxiflora*
C	3-12	Rat chow and water plus 5.0mg/kg H_R of *Alchornea laxiflora*
D	3-12	Rat chow and water plus 10.0mg/kg H_R of *Alchornea laxiflora*

Group 2 (pre-treatment group) were given the H_R extract of *Alchornea laxiflora* for 10 days but in addition were given 1ml of sodium arsenate from 6th -10thday.

Groups	Days	Treatment
Control	1-5	Rat chow and water only
A	1-5	Rat chow and water plus 0.5mg/kg H_R of *Alchornea laxiflora*
B	1-5	Rat chow and water plus 1.0mg/kg H_R of *Alchornea laxiflora*
C	1-5	Rat chow and water plus 5.0mg/kg H_R of *Alchornea laxiflora*
D	1-5	Rat chow and water plus 10.0mg/kg H_R of *Alchornea laxiflora*

Groups	Days	Treatment
Control	6-10	Rat chow and water only
A	6-10	Rat chow + water + 0.5mg/kg H_R of *Alchornea laxiflora* + 1ml sodium arsenate/day
B	6-10	Rat chow + water + 1.0mg/kg H_R of *Alchornea laxiflora* + 1ml sodium arsenate/day
C	6-10	Rat chow + water + 5.0mg/kg H_R of *Alchornea laxiflora* + 1ml sodium arsenate/day
D	6-10	Rat chow and water plus 10.0mg/kg H_R of Alchornea laxiflora plus 1ml sodium

Sodium arsenate (2mg/kg) was administered to all the groups except control. H_R extract of *Alchornea laxiflora* was administered to all groups at varying doses of 0.5, 1.0, 5.0 and 10.0mg/kg respectively.
The animals were sacrificed 24h after the last administration by cervical dislocation. The livers were removed and blotted with 1.5% Kcl. The liver was homogenized and the bloods collected from the heart were used for the estimation of the liver function parameters.

Biochemical studies on liver and serum

Serum and liver homogenates were analyzed for the following parameters: Total Bilirubin (TB) [*9*], L-γ-Glutamyltransferase (GGT) (Szasz, [*10*], Aspartate Aminotransferase (AST) [*11*], Alanine Aminotransferase (ALT) [*11*] and Alkaline Phosphatase (ALP) [*12*] and Creatinine was measured by a RA- 50 Semi Auto analyzer (Bayer). (These were carried out using standard reagent kits from Randox Laboratory Limited, U.K.)

Statistical analysis

All analyses were carried out in triplicates. The data were recorded as mean ± standard deviation and analysed by Microsoft excel (for windows 2007). ANOVA was carried out to test any significant differences between their means. Values of $p>0.05$ were considered significant.

Results

Effects of Hexane extracts of *Alchornea laxiflora* on some liver biochemical indices of rats administered 2mg/kg sodium arsenate (post treatment group). Administration of graded dosage of H_R for 10 days after administration of 2ml of (2mg/kg) of sodium arsenate significantly reduced ($p<0.05$) the levels of AST, ALT, ALP, GGT, Total bilirubin and creatinine levels of the test group and brought it closer to the control (table1)

Effects of Hexane root extracts of *Alchornea laxiflora* on some liver biochemical indices administered 2mg/kg sodium arsenate (pre-treatment group). Administration of 2mg/kg of sodium arsenate for 5 days after pre- treatment with graded dosage of H_R of Alchornea laxiflora significantly reduced ($p<0.05$) the levels of AST, ALT, ALP, GGT, Total bilirubin and creatinine of the test group and brought it closer to the control group (Table 2) more than the post-treatment group

Effects of Hexane root extracts of *Alchornea laxiflora* on some serum biochemical indices administered 2mg/kg sodium arsenate (post-treatment group). Administration of graded dosage of H_R for 10 days after administration of 2ml of (2mg/kg) of sodium arsenate significantly reduced ($p<0.05$) the levels of AST, ALT, ALP, GGT, Total bilirubin and creatinine levels of the test group and brought it closer to the control (table 3).

Administration of 2mg/kg of sodium arsenate for 5 days after pre- treatment with graded dosage of H_R of *Alchornea laxiflora* significantly reduced ($p<0.05$) the levels of AST, ALT, ALP, GGT, Total bilirubin and creatinine of the test group and brought it closer to the control group more than the post-treatment group (Table 4).

Table1: Effects of Hexane extracts of *Alchornea laxiflora* on some liver biochemical indices of rats administered 2mg/kg sodium arsenate (post treatment group)

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total Bilirubin (mg/dl)	Creatinine level (mg/dl)
Control	27.0±0.5	31.7±0.6	234.6±3.8	11.6±1.7	0.04±0.0	0.8±1.3
0.5mg/kg	54.0±2.3	47.8±0.8	289.8±7.5	23.2±1.1	0.08±0.2	8.2±3.3
1.0mg/kg	63.0±0.6	39.2±1.4	240.6±9.7	17.4±0.7	0.02±0.1	6.0±2.2
5.0mg/kg	43.3±0.2	31.2±1.2	231.2±2.1	21.2±0.8	0.02±0.1	1.0±2.0
10.0mg/kg	39.0±0.1	34.1±1.6	338.8±4.3	14.6±1.2	0.01±0.2	2.2±0.6

Results are expressed as meant± standard deviation for four (4) animals in each group

Table2. Effects of Hexane root extracts of *Alchornea laxiflora* on some liver biochemical indices administered 2mg/kg sodium arsenate (pre-treatment group)

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total Bilirubin (mg/dl)	Creatinine level (mg/dl)
Control	27±0.5	31.7±0.6	234.6±3.8	11.6±1.7	0.04±0.1	0.8±1.3
0.5mg/kg	52.0±0.7	35.2±0.7	151.8±4.7	17.4±1.7	0.14±0.1	7.0±1.6
1.0mg/kg	55.5±0.6	38.2 ±0.5	138.0±1.4	14.7±2.5	0.08±0.1	2.0±2.7
5.0mg/kg	31.0±0.9	28.2±0.8	145.6±4.1	12.3±0.8	0.06±0.2	3.1±1.8
10.0mg/kg	37.8±0.4	27.9±2.1	153.2±3.4	14.9±3.3	0.08±0.1	3.0±1.6

Results are expressed as meant ± standard deviation for four (4) animals in each group.

Table 3: Effects of Hexane root extracts of *Alchornea laxiflora* on some serum biochemical indices administered 2mg/kg

sodium arsenate (post-treatment group).

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total Bilirubin (mg/dl)	Creatinine level (mg/dl)
Control	27.0±0.5	31.7±0.6	234.6±1.2	11.60±1.0	0.04±0.4	0.8±1.3
0.5mg/kg	54.0±0.3	53.30±0.7	252.0±1.4	30.16±1.2	0.54±0.1	4.0±1.7
1.0mg/kg	48.5±0.6	51.92±0.1	238.4±1.7	24.74±1.2	0.43±0.1	2.0±1.2
5.0mg/kg	35.4±0.5	35.40±0.5	241.6±6.9	27.90±2.0	0.27±0.1	1.0±1.4
10.0mg/kg	34.0±0.7	36.85±1.0	139.0±6.1	22.85±1.1	0.12±0.0	1.0±0.7

Results are expressed as meant± standard deviation for four (4) animals in each group.

Table 4: Effects of Hexane root extracts of *Alchornea laxiflora* on some serum biochemical indices administered 2mg/kg sodium arsenate (pre-treatment group)

Treatment	AST (IU/L)	ALT(IU/L)	ALP(IU/L)	GGT(IU/L)	Total Bilirubin (mg/dl)	Creatinine level (mg/dl)
Control	27.7±0.4	31.70±0.5	234.6±1.2	11.60±1.0	0.04±0.4	0.8±1.3
0.5mg/kg	43.0±0.2	54.80±0.3	276.0±7.2	31.58±1.3	0.17±0.1	4.0±3.1
1.0mg/kg	49.0±0.2	31.60±0.6	301.2±8.5	32.64±3.2	0.16±0.0	3.0±1.6
5.0mg/kg	37.0±0.5	33.80±0.4	243.6±1.6	24.74±1.6	0.13±0.1	3.0±2.0
10.0mg/kg	29.0±0.3	31.20±0.5	239.0±1.6	21.74±4.5	0.08±0.1	1.8±0.4

Results are expressed as meant± standard deviation for four (4) animals in each group.

Discussion

The liver is a resilient organ, strategically located in the body as the entry port for substances into the bloodstream. It plays a major role in metabolism and excretion of noxious substances, it has a superior role in maintenance, performing and regulatory metabolism [*13*]. Because of its myriads role and strategic location, it is constantly exposed to toxicants from both endogenous and foreign sources which lead to liver disease (a major health concern). Although the liver has natural capacity to regenerate itself after damage, the plant extracts have played a major role in the preservation of its integrity. These plant extracts have abundant secondary metabolites with potent free-radicals scavenging or antioxidant potentials. Drug induced liver toxicity is one of the major route of liver damage, where the drugs induced oxidative stress which if left unabated will ultimately lead to oxidative damage. From the result of this present study, Table 1 and 3 showed that the administration of sodium arsenate increased significantly ($p<0.05$)the levels of AST, ALT, ALP, GGT, Total Bilirubin and Creatinine in the liver homogenate and the serum of the test groups (0.5-10mg/kg) compared to the control. This is to confirm that indeed the administration of sodium arsenate significantly damaged the liver as observed by the increased levels of the intracellular enzymes (AST, ALT, ALP, and GGT). The administration of hexane root extract of *Alchornea laxiflora* after the damage (post -treatment groups) could do nothing to reverse the damage as observed by the increased levels of these enzymes after administration of the extract. However, from the result in table 2 it is obvious that the administration of sodium arsenate after prior administration of hexane root extract of *Alchornea laxiflora* brought the liver levels of AST, ALT, ALP, GGT, Total Bilirubin and Creatinine in the test groups closer to the control. That is, the levels of AST, ALT, ALP, GGT, Total Bilirubin and Creatinine of the pre-treatment group (table 2) are not as high compared to the post-treatment group (table 1). This is suggestive of the fact pre-treatment with hexane root extract of *Alchornea laxiflora* confers some protective measures on the liver against sodium arsenate induced oxidative damage. Table 4 (pre-treatment) also showed the effects of sodium arsenate on serum levels of AST, ALT, ALP, GGT and Total Bilirubin and Creatinine after prior administration of hexane extract of *Alchornea laxiflora* leaf. From the result, there were significant increases ($p<0.05$) in the levels of AST and ALT of the test group compared to the control. The levels of ALP and GGT were also significantly increased ($p<0.05$) compared to the control. However, the levels were not as high as that of the post treatment (group 3). There was also a significant increase ($p<0.05$) in the levels of Total Bilirubin and Creatinine of the test groups compared to the control in a dose dependent manner with the lowest dose (0.5mg) showing the highest increase . From the going, it is obvious that the administration of hexane root extracts of *Alchornea laxiflora* prior to sodium arsenate poisoning confers some protective effects on the liver. The protective effects of the extract could be due to the presence of one or other principles in the plant [*1*]. The presence of these secondary metabolites might be responsible for the pharmacological activities of this plant extract. The anti-oxidative potentials of medicinal plants were reported to be mostly due to their content of phenolic compounds [*14*]. Gutfinger 1981[*15*] discovered that phenolic compounds play an important role in inhibiting autoxidation of oils. Apparently, the contents of phenolic compounds seem to relate to their antioxidant activity. The antioxidant potentials of hexane root extract of *Alchornea laxiflora* shown in (Tables 2 and 4) was greater than that of (tables 1and 3). Therefore, hexane root extracts of *Alchornea laxiflora*

root tested was an electron donor and could react with free radicals to convert them to more stable products and thus inhibit free radical chain reactions. The marked antioxidant activity of the root extract might be connected to their reducing powers. These results indicated that the extract exhibited a potent scavenging effect on thyl and singlet oxygen free radicals and would have effective activities as hydrogen donor and as primary antioxidants by reacting with free radicals. The serum activities of Aspartate amino transferase (AST), and Alanine aminotransferase (ALT) of the pre-treatment group were significantly lowered than the post-treatment group. Increase in the serum enzyme activity signifies damage to the liver membrane. In affected livers, both the levels of ALT and ALP were increased .these results suggests that the plant extract may possess hepatoprotective activity especially at close dose range of (1.0-5.0mg/kg).

Liver enzymes related to sub-cellular functions such as plasma membrane ALP, mitochondrion AST and ALP were affected, not only that the oxidative damage due to either obstruction of the bile ducts or leakage of the cells may be prevented, the plant extract was able to confer some protection on the liver as evident in the reduced levels of serum activities of ALT and AST compared to the control. It has been shown from the result so far, that, hexane root extracts of *Alchornea laxiflora* may confer significant protection on the liver against sodium arsenate induced hepatotoxic, peroxidative and oxidative stress and might be used to prevent liver accumulation of free radicals and inhibit formation of peroxides at a dosage of between 0.5-10.0mg/kg body weight. The extract at low concentration showed little or partial inhibitory effect on sodium arsenate induced free radical

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