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Assessment of Protective Potentials of *Ficus Exasperata* Leaf on Arsenate-Mediated Dyslipidemia and Oxidative Damage in Rat's Brain

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Abstract: This study investigated the protective potentials of methanolic leaf extract of *Ficus exasperata* on sodium arsenate-mediated dyslipidemia and oxidative damage in the brain of rats. Twenty-eight rats were sorted into four groups containing seven rats each. Group A (control) received distilled water while 10 mg/kg bw of arsenic in form of sodium arsenate (As) was administered intraperitoneally to groups B, C and D. Group C and D were treated with oral administration of 100 mg/kg bw and 200 mg/kg bw of F. *exasperata* leaf respectively for 14 days. Arsenate administration resulted in dyslipidemia as shown by significant elevation ($P<0.05$) in total cholesterol, triglycerides, LDL-cholesterol and coronary heart disease risk ratio while it also reduced HDL-cholesterol in the rats. It also causes lipid peroxidation and oxidative damage in the brains of the rat with significant elevation of malondialdehyde level and decrease in levels of reduced glutathione, glutathione s-transferase, catalase and superoxide dismutase. Histology of the cortex region of brain of the rats treated with arsenate showed abnormal neuronal morphology with neuronal degeneration and necrosis. However, treatment with *F. exasperata* significantly reversed and attenuated the arsenate-mediated biochemical alterations. We demonstrated in this study that *F. exasperata* leaf effectively protects against arsenate-induced dyslipidemia and oxidative damage in rat's brain.

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INTRODUCTION

Arsenic poisoning has been a global health concern, affecting millions of people and animals (Tanju and Madhuri, 2013). Exposure of human and animals to arsenic and its compounds is known to induce neurotoxicity. The most typical neurological feature of arsenic toxicity is peripheral neuropathy which may last for several years (Mathew and Vale, 2010). Studies have revealed that exposure of animals to arsenic caused neurological effects which includes changes in levels of neurotransmitters such as dopamine, norepinephrine and 5-hydroxytryptamine (Kannan, *et al.*, 2001).

Chronic arsenic exposure affects the vascular system and causes hypertension and cardiovascular diseases. Acute arsenic toxicity may cause cardiomyopathy and hypotension. The toxicity of pentavalent inorganic arsenic is due to its conversion to trivalent arsenic. The later inhibits pyruvate dehydrogenase by binding to the sulfydryl groups of dihydrolipoamide, resulting in reduced conversion of pyruvate to acetyl CoA. The cumulative effect of these is inhibition of citric acid cycle and reduction in cellular ATP production (Bergquist, *et al.*, 2009). At a more significant and specific level, pentavalent arsenic emulates inorganic phosphate and replaces phosphate in glycolytic and cellular respiration pathways (Hughes, 2002).

Many mechanistic studies of arsenic toxicity have suggested that reactive oxygen species and reactive nitrogen species are generated during inorganic arsenic metabolism in living cells (Shi, *et al.*, 2004). Cascade mechanisms of free radical formation derived from the superoxide radical combined with a decrease in cellular oxidant defence by treatment with glutathione-depleting agents results in an increased sensitivity of cells to arsenic toxicity (Cohen, *et al.*, 2006; Valko, *et al.*, 2005). The metabolism of inorganic arsenic such as arsenite As(III) and arsenate As(V) involves a two-electron reduction of pentavalent arsenic to trivalent arsenic, mediated by glutathione, followed by oxidative methylation to form pentavalent organic arsenic (Hughes, 2002).

Ficus exasperata is a medicinal plant used traditionally as analgesic, anti-arthritic, anti-diarrhea, anti-dysentery, anti-diuretic, abortifacient and also in general debility (Bafor and Igbinuwen, 2009; GRIN, 1994; Burkil, 1985). Its leaf is used in the treatment of many ailments including kidney

disorders, venereal diseases, hemostatic ophthalmia, coughs, hemorrhoids, epilepsy, high blood pressure, rheumatism, arthritis, cancer, intestinal pains, bleeding and wounds (Mshana, *et al.,* 2001; Cousins and Michael, 2002; Irvine, 1961). Experimental evidences have documented its arterial blood pressure reducing effect (Ayinde, *et al.,* 2007), anti-inflammatory, antipyretic and antinociceptive effects (Woode, *et al.,* 2009), anti-ulcer, anti-diabetic and lipid lowering properties (Oyewole, *et al.,* 2013).

The exigent need for novel strategies of treatment of neurodisorders and coronary heart diseases is obvious with the insight that the present therapies are only palliative, thus this study investigated the protective properties of methanolic leaf extract of *F. exasperata* against arsenic induced hyperlipidemia and neurotoxicity in rat's brain.

MATERIALS AND METHODS

Chemicals/ Reagents

Sodium arsenate ($Na_3A_SO_4$) is a product of Sigma-Aldrich Co. St Louis, Missouri, USA. Lipid profile kits used (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol) are products of Randox Laboratories Limited, United Kingdom. All other chemicals are of analytical grade and were obtained from Analar BDH Limited, Poole, England.

Collection of Plant Material and Preparation of Extract

Fresh leaves of *F. exasperate* were collected at Oke Baale Area, Osogbo, South Western Nigeria. The plant was identified at the Botany Unit, Department of Biological Sciences, Osun State University, Osogbo. Dried samples of the plant were deposited in the University herbarium for future reference. The samples were air dried for 2 months after which it was pulverized into powdery form using industrial laboratory grinder. Extraction of the phytochemicals was done by dissolving 700g of the powder in 4.2litres of 98% absolute ethanol for 14 days after which the extract was filtered using a white moslin cloth. Crude extract was obtained by filtration followed by evaporation of the solvent in a rotatory evaporator at 80°C. The paste was weighed and used to prepare stock solution and different doses of the extract.

Experimental animals

Twenty-eight (28) Wistar strain albino rats of weight 150-160 g were used for this study. They were obtained and raised at the Central Animal House, Osun State University, Osogbo, Nigeria. Rats were kept under laboratory conditions (25±2 ^oC and relative humidity of 50±15%) in cages cleaned of metabolic waste twice daily and were allowed to acclimatize for two weeks. They were exposed to 12 hours' daylight and darkness, fed with rat pellet and water *ad libitum.* The experiment was carried out in accordance to current rules and guidelines that have been established for the care of the laboratory animals (NRC, 2011). The rats were acclimatized for two weeks before treatment commenced.

The rats were divided into four groups, seven rats per group: group A (control) received distilled water while 10 mg/kg bw of sodium arsenate was administered intraperitoneally to groups B, C and D. Group C and D were treated with oral administration of 100 mg/kg bw and 200 mg/kg bw of methanolic leaf extract of *F. exasperata* respectively for 14 days.

Preparation of Serum

The rats were sacrificed 24hrs after the last treatment by cervical dislocation and blood sample collected into clean, dry centrifuge tube. The blood was left for 10 min at room temperature to clot after which it was centrifuged at 4,000 rpm for 20 min in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then stored at 4 °C for biochemical analyses.

Preparation of homogenates

The brain was quickly excised from the rat and immediately placed on a blotting paper to remove blood stains. It was then rinsed in 1.15% KCl to remove haemoglobin followed by homogenization in 4 volumes of ice-cold 0.01 M potassium phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenate was centrifuged at 12,500g for 20 minutes at 4^oc to obtain supernatants (post-mitochondrial fractions) which was stored till required for assay.

Measurement of Serum Lipid Profile

Total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol were measured in the serum of individual rats using the appropriate methods. Total cholesterol was determined by the enzymatic endpoint method (Zoppi and Fellini, 1976). Triglyceride was assayed using the GPO-PAP method (Trinder, 1969) while precipitant method (Wieland and Siedel, 1981) was used in the measurement of HDL cholesterol. LDL cholesterol was estimated using the procedure earlier described (Friedewald, *et al.,* 1972). Coronary heart disease risk ratio (CHD risk ratio) was obtained by calculating the ratio of concentrations of total cholesterol to HDL-cholesterol (Oyewole, *et al.,* 2016). Measurement of concentrations was done by the use of Camspec M106 UV spectrophotometer (Ohaus Corporation Pine Brook USA).

Determination of Biochemical Parameters

Catalase (CAT) activity was determined based on the method of Sinha (1972) which measure the

reduction of dichromate in acetic acid to chromic acetate at 570 nm. The method of Habig et al. (1974) was employed in determining Glutathione–s-transferase (GST) activity using 1,2-dichloro 4-nitrobenzene (CDNB) as substrate. Serum globulin was estimated using the procedure of Mokady et al. (1989). The level of superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) based on the ability of the enzyme to inhibit auto-oxidation of epinephrine at pH 10.2 and 30 °C. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Histological Examination

The brains were fixed in 10% formalin and embedded in paraffin wax. Thin sections (7–9 mm thickness) of the tissues were cut and dewaxed in xylene, hydrated in decreasing percentage of alcohol and stained with hematoxylin. They were then dehydrated in increasing percentage of alcohols till 70% and stained with 1% alcoholic eosin. They were differentiated in 90% alcohol and cleared in xylene. These stained sections were observed under the microscope for histopathological analysis.

Statistical analysis

Data of results were expressed as mean ± SD. Comparison was done using one-way analysis of variance (ANOVA) between the control and treatment groups. P values <0.05 were considered statistically significant.

RESULTS

Table 1 show the effect of sodium arsenate and methanolic leaf extract of *F. exasperata* on average body weight gain and brain weight of rats. Administration of arsenate caused decrease in the body weight gain of rats which was boosted following treatment with *F. exasperata*. There was no significant difference between brain weight of control and experimental rats.

Serum lipid profile and coronary heart disease risk ratio in the experimental animals is shown in Table 2. Sodium arsenate administration caused significant elevation (P<0.05) in total cholesterol, triglycerides, LDL-cholesterol and coronary heart disease risk ratio while it also reduced HDL-cholesterol in the rats. These anomalies were however annulled in rats treated with various graded concentrations of methanolic leaf extract of *F. exasperata*.

Data in Table 3 represent the lipid peroxidation and antioxidants status in the brain of experimental animals. Administration of sodium arsenate caused significant (P<0.05) elevation in lipid peroxidation (MDA) by 120% and decrease in levels of GSH (75%), GST (57%), CAT (39%) and SOD (40%). However, treatment with graded concentrations of *F. exasperata* significantly reversed all these changes in a dose dependent manner.

Table 1: Body weight gain and brain weight of rats administered sodium arsenate and leaf extract of *Ficus exasperata*

Experimental groups	Body weight gain	Brain weight
Control	14.50 ± 3.12	1.63 ± 0.10
Na_3ASO_4 only	10.07 ±2.33*	1.52 ± 0.12*
Na_3ASO_4 + 100 mg/kg bw *F. exasperata*	12.82 ± 2.86	1.56 ± 0.09
Na_3ASO_4 + 200 mg/kg bw *F. exasperata*	13.25 ± 3.05	1.59 ± 0.06

Values are means ± SD of 7 rats. * indicates value is significantly different from the control value at P<0.05.

Table 2: Serum lipid profile and coronary heart disease (CHD) risk ratio in rats administered sodium arsenate and leaf extract of *Ficus exasperata*

Experimental groups	Total Cholesterol (mg/dl)	TAG (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	CHD Risk Ratio
Control	114.33±9.31	63.44±5.22	76.34± 6.70	46.82±4.34	1.50 ± 0.23
Na_3ASO_4 only	150.45±8.62*	91.45±7.33*	59.54±5.53*	67.34±6.66*	2.54 ± 0.42*
Na_3ASO_4 + 100 mg/kg bw *F. exasperata*	130.26±7.71	74.72±6.24	65.23±6.22	58.14±5.80	2.05 ± 0.31
Na_3ASO_4 + 200 mg/kg bw *F. exasperata*	126.18±8.22	73.88±5.84	67.55±7.04	56.78±5.33	1.82 ± 0.35

Values are means ± SD of 7 rats. * indicates value is significantly different from the control value at P<0.05.

Table 3: Antioxidants and lipid peroxidation concentrations (unit/mg protein) in the brain of rats administered sodium arsenate and leaf extract of *Ficus exasperata*

Experimental groups	Catalase	SOD	GST	GSH	MDA
Control	14.66±2.10	10.80±1.02	12.21± 3.33	6.38±0.66	4.50±0.45
Na_3ASO_4 only	8.86±0.85*	4.32±0.78*	5.74±2.11*	2.10±0.14*	10.22±1.08*
Na_3ASO_4 + 100 mg/kg bw *F. exasperata*	11.34±0.79	7.58±0.88	9.88±3.21	4.86±0.56	6.32± 0.57
Na_3ASO_4 + 200 mg/kg bw *F. exasperata*	12.48±0.52	8.22±0.64	10.62±3.81	5.23±0.58	5.82 ± 0.62

Values are means ± SD of 7 rats. * indicates value is significantly different from the control value at P<0.05.

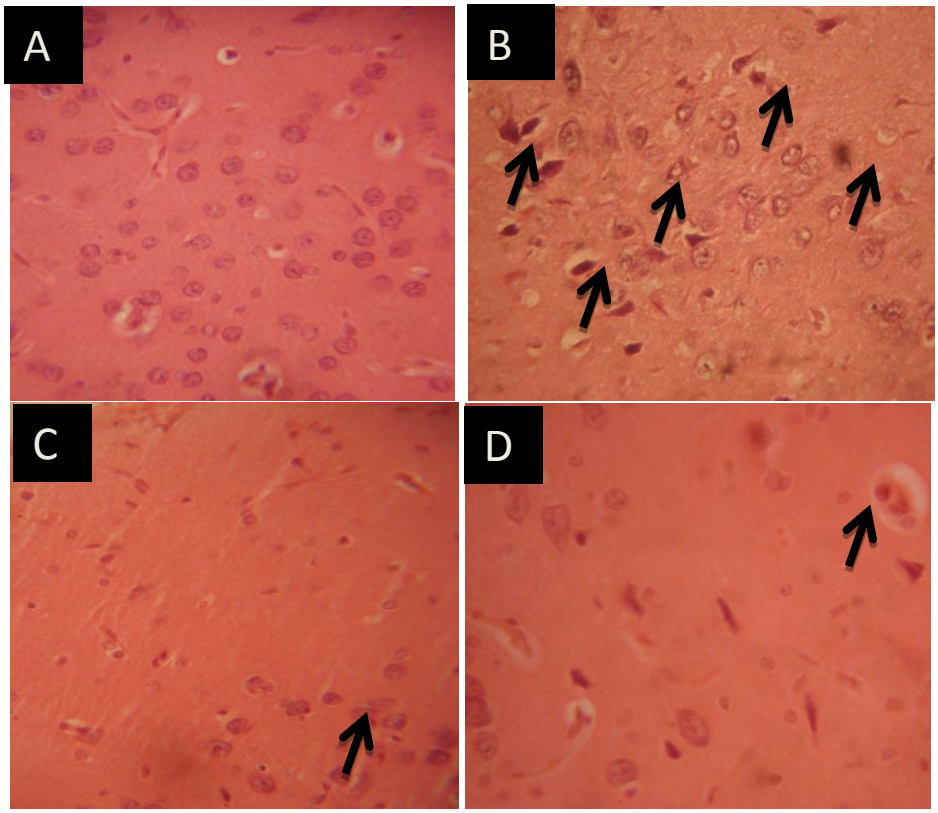

Plate 1: Sections of the cortex region of the brain of rats (Haematoxylin and eosin stained X 200).
A: Control rats showing normal neuronal morphology with no visible lesions seen
B: Rats intoxicated with Na_3ASO_4 showing abnormal neuronal morphology with severe neuronal degeneration and necrosis
C: Rats administered Na_3ASO_4 + 100 mg/kg bw of methanolic leaf extract of *Ficus exasperata* showing normal neuronal morphology with mild visible lesions
D: Rats administered Na_3ASO_4 + 200 mg/kg bw of methanolic leaf extract of *Ficus exasperata* showing normal neuronal morphology with mild visible lesions

DISCUSSION

This present study evaluates the protective properties of methanolic leaf extract of *F. exasperata* on arsenate-induced dyslipidemia and oxidative damage in the brain of rats. Results in Table 1 showed an enhancement of body weight increase in rats treated with methanolic leaf extract of *F. exasperata*. This result suggests that the extract caused stimulation of protein synthesis and growth pathway. The result agree with the previous report of Oyewole and Oladele (2017) who documented that *F. exasperata* increased appetite and feed efficiency, enhanced nutrient metabolism and utilization while it also decreased nutrient loss.

The observed significant increase ($P<0.05$) in the levels of serum total cholesterol, triglycerides, LDL, coronary heart disease risk ratio and a significant decrease ($P<0.05$) in the level of HDL in the serum of arsenate treated rats as compared to normal control is an evidence of hyperlipidemia. This could be due to the peroxidation of membranes and alteration of cellular structure mediated by sodium arsenate. High levels of LDL-cholesterol promote health problems and cardiovascular disease, they are often called "bad cholesterol" as opposed to HDL particles, which are referred to as "good cholesterol" or "healthy cholesterol" (Superko *et al.*, 2002; Barter, *et al.*, 2007). HDL particles are able to remove cholesterol from within the artery and transport it back to the liver for excretion or re-utilization (Lin, *et al.*, 1998). Those with higher levels of HDL-cholesterol seem to have fewer problems with cardiovascular diseases, while those with low HDL cholesterol levels have increased rates of heart disease (Clark and Pierce, 2000). Methanolic leaf extract of *F. exasperata* demonstrated hypolipidemic activities as it significantly reduced triglyceride, cholesterol, LDL, coronary heart disease risk ratio and increased HDL in serum of rats presenting the extract as a candidate drug in the treatment of hyperlipidemia and cardiovascular diseases (Kwiterovich, 2000).

Lipid peroxidation of polyunsaturated fatty acids which resulted into products such as Malondialdehyde (MDA) has been implicated in the pathogenesis of neurodisorders such as Alzheimer's disease, Parkinson's diseases, etc. Significant increase in arsenate-induced lipid peroxidation was reported in this study as measured by elevated MDA formation. However, treatment with methanolic leaf extract of *F. exasperata* attenuated significantly the effect of arsenate and protected against accumulation of toxic lipid peroxidation products in the brain. This protective effect of *F. exasperata* may be due to the

antioxidant constituents of the extract acting as radical chain breaker and subsequently quenches lipid peroxidation, stabilizing membrane integrity thereby preventing the inactivation and depletion of plasma membrane enzymes.

The significant depletion of GSH, GST, SOD, and CAT activities in the brain of rats administered 10 mg/kg bw of sodium arsenate indicate induction of oxidative stress in the rats. Oxidative stress is a result of the imbalance between ROS and antioxidants in the body which can lead to oxidative damage of macromolecules. Brain contains limited amount of antioxidant enzymes which make it vulnerable to oxidative stress. Antioxidant protection of the brain is provided by SOD, catalase and glutathione s-transferase. SOD is a family of metalloenzymes that is known to accelerate the dismutation of endogenous cytotoxic superoxide radicals to H_2O_2, which are deleterious to polyunsaturated fatty acids and structural proteins of plasma membrane. GST is a family of isoenzymes that catalyze the conjugation of GSH with a wide variety of drugs, carcinogens and xenobiotics (including lipid peroxides) to form more water-soluble compound products that are readily excreted from the system.

Treatment with methanolic leaf extract of *F. exasperata* significantly ($P<0.05$) mitigated against all the markers of oxidative stress in brain of rats by significantly increasing GSH content and GST, SOD and CAT activities. This can be attributed to the antioxidant and free radical scavenging properties of the extract, the ability of the extract to enhance the synthesis of these antioxidant enzymes and influence their enzymatic functions.

The neurotoxicity effects of arsenic in the cortex region of brain of the rats treated with sodium arsenate alone was shown by the abnormal neuronal morphology with neuronal degeneration and necrosis. There is also distortion of the layered microanatomy of the neurons with some neurons showing features compatible with pyknosis and karyolysis (Stevens and Lowe, 2000). Administration of the chosen doses of methanolic leaf extract of *F. exasperata* in this study reverse the histological alterations in a dose dependent manner. This histological finding further strengthens the biochemical results.

CONCLUSION

The results of this study shows that arsenate induced dyslipidemia as shown by the increase in serum total cholesterol, triglycerides, LDL, coronary heart disease risk ratio and decrease in the level of HDL in arsenate intoxicated rats. The drug also induced oxidative damage in the brain as revealed by depletion in antioxidants and increased levels of lipid peroxidation. Treatment with *F. exasperata* leaf effectively reduced arsenate-induced dyslipidemia and oxidative damage in rat's brain suggesting its usefulness as a remedy for the treatment of lipid and neurological disorders in man.

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