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Antioxidant Activities of Aqueous and Methanol Extracts of *Flacourtia indica* Fruit (Governor's Plum) on Cadmium induced Toxicity in Albino Wistar Rats

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ABSTRACT

Medicinal plants are widely used by traditional practitioners for curing various diseases. This study was designed to ascertain the antioxidant properties of aqueous and methanol extracts of *Flacourtia indica* fruit (Governor's plum) on cadmium induced toxicity in albino wistar rats. Forty-Five rats of both sexes (140-170g, aged 3months) were divided into nine groups of five rats each. Group A was negative control, Group B positive control while Groups C to I were test groups. Groups B to I were induced with 50, 60, 70, 80, 90,100,110 and 120mg/kg/body weight cadmium oxide respectively for oxidative stress. Groups C to F were respectively treated with oral administration of 120, 150, 180 and 200mg/kg/body weight of methanol extract of *Flacourtia inica* fruit for 7 days while G to I were treated with 150,180 and 200mg/kg/body weight of the aqueous extract. Blood glucose levels and activities of antioxidants such as Superoxide Dismutase, Catalase, Glutathione Peroxidase, reduced glutathione, Vitamin C and Vitamin E were assayed by standard methods before induction, after induction and after administration of extracts. Results after cadmium induction showed significant increase (P<0.05) in blood glucose concentration and significant decrease (P<0.05) in antioxidant levels. These results were reversed after treatment with the extracts with significant decrease (P<0.05) in blood glucose and significant increases (P<0.05) in antioxidants levels. *Flacourstia indica* fruit had the ability to manage hyperglycaemia and mitigated cadmium induced toxicity.
Key words: *Flacourtia indica*, fruit, efficacy, oxidative stress.

INTRODUCTION

Flacourtia indica belongs in the family of flacourtiaceae and is commonly known as "Akpuru" in the Eastern region of Nigeria. It is flowering plant with spiny truck and branches. In shrub form, it grows up to 7.6metres (20 feet). The droping branches bear oval leaves. The Governor's plum itself is a pome, about an inch thick and red ripening purple. It is very flashy with six to ten seed layered carpels. It is eaten raw or made into jelly or jam. It can be fermented to make wine (Nakatani, 2013). *Flacourtia indica* is an indigenous medical plant widely distributed in Bangladesh and India and used for the treatment of a variety of diseases and functional disorder (Tyagi *et al.*, 2010). Cadmium is a chemical element with atomic number 48. It is a soft, bluish- white metal and occurs as minor component in most zinc ores and is a by-product of zinc production. It was used for long time as corrosion resistant plating on steel and cadmium compounds are used as red, orange and yellow fragments to colour glass and stabilize plastics. Cadmium is toxic and listed in the European restriction of hazardous substances. Once it's absorbed, it is efficiently retained in the body in which it accumulates throughout life. It is primarily toxic to the kidney, especially the proximal tubular cells, the main site of accumulation (klassen et al., 2009). Cadmium can also cause bone demineralization through direct bone damage or indirectly as a result of renal dysfunction. In the industry, excessive exposure to air born cadmium can impair lung function and increase the risk of lung cancer (Bambard *et al.*, 2013). In industrialized countries, low environmental exposure to cadmium still prevails.

Antioxidants are substances that prevent, delay or remove oxidative damage to a target molecule and play an important role in the body defense system against reactive oxygen species (Halliwell *et al.*, 2010; Boxen at al., 2012). Antioxidants inhibit the process of oxidation even at relatively low concentration and have diverse physiological role in the body. Antioxidant constituents of plants material act as radical scavengers and help in converting the radical to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables, tea, etc. Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and well-being. Regular consumption of anti-oxidative vegetables and fruits reduces the risk of chronic diseases (Dambriska-kies *et al.*, 2008). Antioxidant-rich diet has a positive impact in the long run (Sin *et al.*, 2013). Sources of antioxidants include oranges, lemons, blueberries, strawberries, grapes, plumes, prunes, red beans, spinach, broccoli, flowers. Others such as vitamin C, vitamin E, α- carotene, selenium, polyphenols, glutathione, peroxidase and cysteine are the main sources of antioxidants. Fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress such as cancer, coronany heart disease, obesity, type 2 diabetes, hypertension and cataract (Halvoorsen et al., 20011).

Fig. 1: *Flacourtia indica* plant.

RATIONALE OF STUDY

Heavy metal intoxication especially by lead, cadmium, arsenic and mercury constitute serious threat to human health (Wenneberg, 2015; Hu 2015). Cadmium is one of the most toxic subsatances in the environment with a wide range of organ toxicity and long elimination half-life (20-30 years) (Jarup *et al.*, 2014). It binds to system residues of protein including metallothionein and reduced glutathione (GSH) in an intracellular complex and is known to be stored primarily in the kidney and liver (Frei, 2010). Metals especially transition metals act as catalysts in the oxidative reaction of biological macromolecules, thus, metal toxicities might be associated with oxidative tissue damage. Cadmium stimulates the population of reactive oxygen species due to its inhibitory effect on mitochondrial electron transport chain and as a result, the electron transport chain becomes highly reduced, that is, electrons are transferred directly to available oxygen to enhance formation of reactive oxygen species (Ochi *et al.*, 2011; Tatrai *et al.*, 2012). Reactive oxygen species lead to cellular damage when the rate of generation surpasses the rate of decomposition by antioxidant defense systems (Datta *et al.*, 2000). One of the most important effects of free radicals is oxidation of polyunsaturated fatty acids. As a result of this free radicals' attack, lipids are oxidized and membranes are damaged (Sakar *et al.*, 2016). Therefore, it is the aim of this study to ascertain the efficacy to aqueous and methanol extracts of *Flacourtia inidca* fruit in ameliorating cadmium induced toxicity in albino wistar rats.

MATERIALS AND METHODS

Preparation of Fruit Extract: Aqueous extract

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Flacourtia indica fruits were obtained from a garden in Enugu, Enugu State, Nigeria, and sun-dried for two hours. 350g of the fruits were ground, 650 ml of water were added and mixed. The mixture was concentrated in a water bath at 70°C for 24hrs.

Methanol Extract: The procedure for the aqueous extract was followed and 650 ml of methanol were used.

Experimental Design

Forty-five albino wister rats (aged 3months, 90-147g) were allowed to acclimatize for two weeks. They were fed with standardized palletized finisher feed and given clean water for the duration of acclimatization and test procedures. The experimental design was in two phases, phase 1 and phase 11. The 45 rats were divided into 9 groups of 4rats each. Group A was negative control, Group B positive control while Group C to Group 1 were test groups.

Phase 1: Induction Phase

Induction with different amounts of cadmium oxide (CDO) was carried out intraperitoneally as follows:
Group A: Negative Control- No cadmium induction, no extract administration
Group B: Positive (test) control: induced intraperitoneally with 50mg/kg cadmium oxide
Group C: Induced intraperitoneally with 60mg/kg CDO
Group D: 70mg/kg CDO
Group E: 80mg/kg CDO
Group F: 90mg/kg CDO
Group G: 100mg/kg CDO
Group H: 110mg/kg CDO
Group I: 120mg/kg CDO
At the end of Phase I (6days), two rats were taken from each group and blood samples collected for biochemical analyses. The rats were sacrificed; the liver and kidneys were preserved in 10% formal saline for histopathology studies.

Phase II: Extract Administration

Groups C to F were given methanol extract while Groups G to I received aqueous extract of *Flacourtia indica* daily for 7days.
Groups C: treated with 120mg/kg methanol extract of *Flacourtia indica* fruit
Group D: treated with 150mg/kg methanol extract of *Flacourtia indica* fruit
Group E: treated with 180/kg methanol extract of *Flacourtia indica* fruit
Group F: treated with 200mg/kg methanol extract of *Flacourtia indica* fruit
Group G: treated with 150mg/kg aqueous extract of *Flacourtia indica* fruit
Group H: treated with 180mg/kg aqueous extract of *Flacourtia indica* fruit
Group I: treated with 200mg/kg aqueous extract of *Flacourtia indica* fruit

METHODS

After 7 days, the animals were anaesthetized with chloroform and blood samples collected by cardiac puncture into fluoride and plain bottles for biochemical assays. The animals were sacrificed and the liver and kidney preserved in 10% formal saline for histopathology.

Plain bottle samples were allowed to clot for 30minutes and serum separated into respective plain specimen tubes. Flouride samples were analysed for blood glucose by glucose oxidase method while the serum samples were assayed for reduced glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Vitamin C and Vitamin E by standard methods and activities measured spectrophotometrically.

STATISTCAL ANALYSIS

Results were expressed as mean ± standard deviation and analysed using one-way analysis of variance (ANOVA). P value (< 0.05) was considered significant.

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RESULTS

Table 1: MEAN VALUES BEFORE INDUCTION OF CDO (CONTROL)

Groups	Blood Glucose (mg/dl)	GSH(nmol/mg protein)	SOD (iu/mg protein)	CAT (KU/L)	GPx (nmol/g)	VIT.C (mg/dl)	VIT.E (mg/dl)
A-I	92 ± 6	0.0018 ±0.001	0.067±0.006	241±3	1.22±0.9	0.17±0.01	0.12 ±0.03

Table 2: Mean values after cadmium oxide induction

Groups	Blood Glucose (mg/ dl)	GSH (nmol/mg protein)	SOD (IU/mg protein)	CAT (KU/L)	GPX (nmol/g)	Vit C (mg/dl)	Vit E. (mg/dl)
A	92 ± 6	0.0018 ± 0.001	0.067 ±0.006	241 ± 3	1.22 ± 0.9	0.17 ±0.01	0.12 ±0.03
B	118 ± 10	0.0021 ± 0.0004	0.016 ±0.002	95 ± 0.0	1.024 ± 0.7	0.018 ±0.0	0.069 ±0.02
C	107 ± 24	0.0027 ± 0.001	0.055 ±0.028	64 ± 62	1.09 ± 0.01	0.015 ±0.0	0.031 ±0.02
D	98 ± 6	0.0014 ± 0.0009	0.010 ± 0.00	217 ±0.00	0.06 ± 0.01	0.002 ±0.0	0.024 ±0.01
E	116 ±12	0.0026 ±0.00141	0.029 ±0.003	200± 10	1.03 ±0.9	0.006 ±0.0	0.04 ±0.01
F	111 ± 14	0.0014 ±0.0011	0.0426 ±0.005	229±0.00	1.04 ± 0.2	0.004 ±0.0	0.035 ± 0.01
G	102 ±5	0.0021 ±0.0011	0.0541 ± 0.006	233± 7	0.06 ±0.01	0.038 ± 0.05	0.033 ± 0.01
H	121 ± 12	0.0017±0.0008	0.0304 ±0.003	234 ± 85	0.08 ±0.24	0.10 ± 0.0	0.008 ±0.00
I	116 ± 20	0.0011±0.000	0.014 ± 0.009	260 ± 32	1.02 ±0.01	0.13 ±0.0	0.011 ±0.00

P (<0.05)

Table 3: Mean values after treatment with Methanol and Aqueous extracts of *Flacourtia indica* fruit

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Groups	Blood Glucose (mg/ dl)	GSH (nmol/mg protein)	SOD (IU/mg protein)	CAT (KU/L)	GPx (nmol/g)	Vit C (mg/dl)	Vit E. (mg/dl)
A	92 ± 6	0.003 ± 0.002	0.067 ±0.006	241 ± 3	1.22 ± 0.9	0.17±0.01	0.12±0.03
B	86 ± 3	0.005 ± 0.0005	11.8 ± 0.8	311± 31	1.02 ± 015	0.17 ±0.06	0.17 ±0.06
C	92± 11	0.0061 ± 0.0015	11.9 ± 1.1	348 ± 70	0.06 ± 0.09	0.11 ±0.06	0.11 ±0.05
D	80 ± 7	0.0058 ± 0.00036	11.4 ± 9	369 ±73	1.12 ± 0.08	0.09±0.00	0.09 ±0.02
E	74 ± 3	0.0077 ±0.0024	11.4 ± 0.4	292±27	1.08 ±0.56	0.06 ±0.00	0.06 ±0.009
F	70 ± 6	0.0058 ±0.0003	11.9 ± 11	382±34	1.11 ± 0.31	0.08 ±0.07	0.08 ± 0.007
G	76 ± 27	0.0046 ±0.0014	11.6± 3	375±24	1.12 ±0.06	0.06± 0.05	0.06 ± 0.005
H	75± 10	0.0032±0.0003	11.8± 0.02	389±93	0.93 ±0.28	0.04 ± 0.02	0.04±0.002
I	70 ± 6	0.0886±0.011	11.8± 0.5	452 ±89	1.07±0.08	0.040 ±0.5	0.04 ±0.005

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DISCUSSION

Antioxidants play an important role in the body's response mechanism against reactive oxygen species. Natural antioxidants are constituents of fruits and vegetables. Phytochemical screening of Flacourtia indica fruit showed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenol, terpenoids, and steriods. The main function of antioxidants is prevention of oxidation. The human body is protected from cardiovascular, neurological and carcinogenic diseases (Antioxidants also delay chronic health problems such as cataracts). Antioxidants ensure the best protection against the development of diseases caused by oxidative stress such as cancer, coronary heart disease, obesity, type II diabetes, hypertension and cataract (Boxin *et al.*, 2012). Table I presented the mean values of control rats, Table 2 indicated blood glucose concentrations and antioxidants activities of Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Vitamin C and Vitamin E after induction with cadmium oxide (CDO) while Table 3 showed the results of rats after treatment with methanol and aqueous extracts of *Flacourtia indica* fruit. After induction, blood glucose concentration of test groups C, D, E, F, G and I increased significantly ($p<0.05$) compared to controls due to the toxic inhibitory effect of cadmium oxide on the islet of langerhands of the pancrease from producing insulin. After the administration of the extracts, there was statistically significant ($P<0.05$) decrease in blood glucose level in all the test groups due to restoration of the pancreas and insulin production. This indicated the hypoglycaemic effect of *Flacourtia indica* fruit.

The human body produces glutathione (GSH) from the synthesis of three amino acids, cysteine, glycine and glutamic acid. It is one of the most abundant tripetide, non-enzymatic biological antioxidant presents in the liver and protects cell from toxins such as free radicals (e.g. superoxide radicals) and maintains protein thoils (Golderg and Spooner, 2013). Glutathione peroxidase (GPx) is an enzyme that occurs in plants, milk and leucocytes and consists of a protein complex with hematic groups that catalyze the oxidation of various substances (Dolas and Gotmara, 2015). Decreased level of GSH is associated with an enhanced lipid peroxidation. Glutathione peroxidase works together with GSH in the decomposition of hydrogen peroxide and other organic hydroperoxides. Under oxidative stress some endogenous factors such as GPx is activated in the defense against injury (Weber *et al.*, 2013). After induction GSH and GPx were decreased as a result of hepatic damage caused by CDo and indicated that the animals were oxidatively stressed. After treatment with extracts, GSH level and GPx activity increased significantly ($P<0.05$) implying efficacy of *Flacourtia indica* fruit in ameriorating lipid peroxidation.

Superoxide dismutase (SOD) is one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishes the effect caused by this radical (landis and Tower, 2015). A significant ($P<0.05$) decrease was observed in SOD after induction with CDO which was indicative of stress. The level of SOD activity after the administration of aqueous and methanol extracts showed significant increase and thus, reduced reactive free radical induced oxidative damage to the liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues with highest activity in red cells and liver. It decomposes hydrogen peroxides and protects the tissue from highly reactive hydroxyl radical (Mueller *et al.*, 2017). A reduction in the activity of CAT was observed in the positive control group after CDO induction which indicated deleterious effect due to the assimilation of superoxide radical and hydrogen peroxide (Chance and Greenstein, 2017). After treatment with methanol and aqueous extracts the results showed significant increase in CAT activity especially with test groups F and I that received 200mg/kg of the extracts, thus revealing hepatoprotective effect of *Flacourtia indica* fruit. Catalase converts hydrogen peroxide to water and oxygen using either an iron or manganese co-factor. It is largely located in most eukaryotic cells in subcellular organelles called peroxisomes (Mueller *et al.*, 2013). It protects cells against oxidative stress.

Vitamin C is a main source of antioxidant and derived from fruit juices, beverages and hot drinks (Gillman et al., 2011; Riman et al., 2013). The levels of Vitamin C in Table I after induction with CDO were significantly decreased in all groups. Vitamin C major role is to neutralize free radicals since it scavenges aqueous peroxyl radicals before these destructive substances damage the lipids. Vitamin C functions along with Vitamin E, a fat soluble antioxidant and with glutathione peroxidase to stop free radical chain reaction (Frei, 2015). The reduced serum levels of Vitamin C in this study reflect levels of oxidative stress. After treatment with both extracts, there were notable increases in the level of Vitamin C signifying antioxidant property of *Flacourtia indica* fruit. It has been suggested that 200mg of Vitamin C daily may reduce the level of stress hormones. Stress suppresses the immune system. Mega doses of Vitamin C increase the level of antibody that fight against germs and viruses in both stressed and unstressed rats with greater antibody increase in the unstressed rats (Block, 2014). Vitamin E is found in all membranes and circulating lipoproteins and one of the most important lipid-soluble primary defense antioxidants (Handan et al., 2017; Abdalla, 2013). It is a generic term used for several naturally occurring tocopherols and tocotrienols. It functions as a chain breaking antioxidant and rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a Vitamin E radical (Bashir et al., 2014). Sources of Vitamin E include wheatgerm, nuts, seeds, whole grains, green leafy vegetables, vegetable oil and fish liver oil. In table 2, there was a reduction in Vitamin E levels after induction with CDO which indicated oxidative stress. The increase in Vitamin E after treatment with methanol and aqueous extracts of *Flacourtia indica* fruit was due to the antioxidant effect. Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting polyunsaturated fatty acids present in cell membrane and also low density lipoprotein (LDL) against lipid peroxidation (Vivek and Surendra, 2016). Possible mechanisms by which antioxidants function to reduce the rate of oxidation of fats and oils include hydrogen and electron donation by antioxidants, addition of lipid to the antioxidants and formation of a complex between lipid and antioxidants. *Flacourtia indica* fruit has a lot of phytochemicals which are plant-derived molecules endowed with steady antioxidant power. The cumulative and synergistic activities of their bioactive molecules present in plant food and fruits are responsible for their enhanced antioxidant properties.

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