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Original Research Article

Assessment of Lipid Parameters and Oxidative Stress Markers in Wistar Rats Treated with Extracts of *Rhizophora mangle*

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Abstract

Medicinal plants are considered more accessible, affordable and also associated with minimal side effects compared to orthodox medicines in most developing countries. These factors in addition to multiple cultural and long term historical relevance have contributed to its increasing popularity around the world. This study was carried out to assess the antioxidative and serum lipid effects of leaf extract of *Rhizophora mangle* on male Wistar rats. Male Wistar rats were divided into four (4) groups of five (5) rats each. Group one (1) served as control and received distilled water. Group two (2) and group three (3) were treated with 200mg/kg bw and 400mg/kg bw of the ethanolic extract of *Rhizophora mangle* respectively. Group four (4) was treated with 600mg/kg of the extract. Extract administration lasted for 30 days. The results obtained showed that the extract did not cause significant changes in superoxide dismutase, catalase, gluthathione reductase enzyme activities but decreased gluthathione peroxidase enzyme activity. The level of malondialdehyde was not altered. The lipid parameters including low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride and total cholesterol concentrations were not significantly affected. This study showed that, extracts of *Rhizophora mangle* did not alter the level of lipid peroxidation and may not confer any benefits against development of lipid induced-cardiovascular disorders.

Keywords: *Rhizophora mangle*, antioxidative, serum lipids, male Wistar rats.

INTRODUCTION

Several contemporary medications were inadvertently discovered and are still being derived from therapeutic plants. Even many food crops, like garlic, have therapeutic properties. Furthermore, medicinal plants have been widely utilized as raw materials for the extraction of the active components that are then used to synthesize various medications. Different medications like blood thinners, antibiotics and some anti-malarials contain plant-based chemicals. Additionally, the active components of taxol, vincristine, and morphine were reportedly extracted from foxglove, periwinkle, yew and opium poppies respectively (Singh, 2015). Indeed, medicinal plants have been crucial to the development of human culture. *Rhizophora mangle* or red mangrove has long had folklore relevance as it has been used in traditional medicine. It is known by different names across different ethnic groups in Rivers State, Nigeria, where the Okrikans, Kalabari, Opobo, and Bonny people refer to it as "Angala,"whereas the Onne, Ogoni, and Eleme groups refer to it locally as "Ngala," which signifies strength (Adedeji *et al*., 2012).

Different parts of this plant have been associated with diverse pharmacologic effects. Its bark has been associated with antiseptic, hemostatic, antifungal and anti-ulcerogenic properties (De-Faria *et al*., 2012). Other reported pharmacologic importance of the plant include, antibacterial (Melchor *et al*., 2001), wound healing (Armas *et al*., 2005) and anti-inflammatory (Marrero *et al*., 2006) effects.

Cardiovascular illnesses and various associated disorders are a leading cause of death in both men and women, globally. Elevated serum concentrations of triglycerides and total cholesterol and more importantly, low-density lipoprotein cholesterol (LDL-c) have been linked to the development of cardiovascular illnesses and are therefore considered to be major risk factors. Numerous disorders, such as certain cancers (Hecht, 1999), atherosclerosis and inflammatory conditions

(Rosenfeld, 1998) and the aging process have been linked to an imbalance between pro-oxidants and anti-oxidants resulting in oxidative stress (Ashok & Ali, 1999). Additionally, lipid peroxidation has been linked to high lipid levels in blood (Ighadoro and Omole, 2012).

Antioxidants play critical roles aimed to disrupt chain reactions that characterize lipid perodixation, by removing the free radical intermediates, and causing inhibition of other oxidation reactions (Oloyede and Afolabi, 2012) The body's antioxidant mechanisms may not sufficiently combat all the free radicals, thereby, increasing the need for dietary intake of antioxidants to boost the antioxidant system in the maintainance of health and prevention of diseases. This study was carried out with the objective to investigate the antioxidant and serum lipid effects of extracts of *Rhizophora mangle*.

MATERIALS AND METHODS

Experimental Animals and Protocols

Adult male Wistar rats weighing 150–200g were purchased from the animal house of Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt, Nigeria. They were acclimatized for two weeks and randomly distributed into 4 groups (n = 5 per group). Group I – served as control and received distilled water. Groups II, III and IV – served as test groups and received 200mg/kg bw, 400mg/kg bw and 600mg/kg bw of ethanolic leaf extract of *Rhizophora mangle* orally, once daily for 30 days. They were allowed free access to water and feeds. Animal handling was in accordance with the National Institutes of Health's guide for the care and use of laboratory animals [National Institute of Health, USA. 1985]. The animals were anesthetized using chloroform and sacrificed at the end of the administration. Blood was collected by cardiac puncture into appropriate sample bottles for estimation of serum lipids and oxidative stress markers.

Collection and Extraction of Plant Material

Fresh leaves of *Rhizophora mangle* were collected from its natural habitat at Eagle Island; an area located in Port Harcourt, Rivers State, Nigeria, and was authenticated by the taxonomist in the herbarium unit, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. It was given an identification code (RSU PB 097). The leaves were washed in tap water to remove dirt, and dried at room temperature (26^{o}C) over a period of 3 weeks. The dried leaves were pulverized using a blender and 500g of the leaves in powder form was obtained. The weighed quantity of the plant was dissolved in 400ml of ethanol for 48 hours in an extraction jar. The mixture was filtered after 48 hours using a Whattman filter paper to separate the filtrate from the residue. The filtrate was poured into a beaker and concentrated using a heating mantle at a temperature of 50^{o}C until a paste (jelly) form was obtained. The dry weight was obtained after heating in an ovum. The yield of the crude ethanolic extract of *Rhizophora mangle* leaves obtained weighed 127g. The extract was stored in a refrigerator at 4^{o}C until it was reconstituted and used for the study.

Serum Lipids and Antioxidant Assay

Blood was taken by cardiac puncture into appropriate sample bottles to determine the serum lipid profile [Total cholesterol (TC), Triglyceride (TG), High density lipoprotein cholesterol (HDL-c), Low density lipoprotein cholesterol (LDL-c), and Very low density lipoprotein cholesterol (VLDL-c)].

The samples were spun for 20 minutes in a serologic manual centrifuge after collection. The biochemical assay was then carried out using 1 mL aliquots of serum. Commercial Labtest Diagnostic kits were used to analyze biochemical parameters, utilizing standard methodologies based on enzymatic and colorimetric methods, spectrophotometry, and in line with the manufacturer's instructions. An automated biochemical analyzer was used to determine the concentrations. The plasma was utilized to calculate oxidative stress indicators according to conventional procedures (Goldberg, 1984; Wasowick *et al.*, 1993; Slaughter and O'Brien, 2000; Vives-Bauza *et al.*, 2007; Condezo- Hyos *et al*., 2013; Peskin and Winterbourn, 2017).

Statistical Analysis

The SPSS software, version 20.0 was used. To calculate the mean and standard error of mean (SEM), the ANOVA was used. In the comparison of the mean level between the control and test groups, we used the post hoc test least significant difference. For all crosses whose probability *(P) value* was less than 0.05 were considered statistically significant.

RESULT

The result for the study is presented in tables 1-6.

Table 1: Mean levels of total cholesterol and triglyceride

Groups (mg/kg)	Total cholesterol (mmol/l)	Sig.	Triglyceride (mmol/l)	Sig.
Control	2.52±0.20		1.07±0.20	
200	2.32±0.04	0.53	1.08±0.12	0.96
400	2.80±0.38	0.38	1.25±0.26	0.48
600	2.84±0.07	0.32	1.28±0.09	0.41

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 2: Mean levels of high density lipoprotein cholesterol and low density lipoprotein cholesterol

Groups(mg/kg)	HDL-c(mmol/l)	Sig.	LDL-c(mmol/l)	Sig.
Control	1.47±0.12		1.51±0.20	
200	1.55±0.17	0.61	1.55±0.18	0.91
400	1.29±0.15	0.27	2.12±0.38	0.08
600	1.52±0.09	0.76	1.86±0.04	0.31

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 3: Mean levels of very low density lipoprotein cholesterol

Groups(mg/kg)	VLDL-c(mmol/l)	Sig.
Control	0.48±0.09	
200	0.44±0.02	0.69
400	0.56±0.12	0.52
600	0.58±0.04	0.40

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 4: Effects of extract on some oxidative stress markers

Groups(mg/kg)	GSH(µg/ml)	Sig.	CAT(µ/g)	Sig.
Control	1.07±0.14		4.06±0.41	
200	1.09±0.13	0.90	3.77±0.20	0.52
400	1.13±0.12	0.75	3.92±0.28	0.75
600	1.16±0.20	0.65	4.10±0.33	0.93

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 5: Effects of extract on some oxidative stress markers

Groups(mg/kg)	SOD(µ/ml)	Sig.	GPx(U/L)	Sig.
Control	0.30±0.05		0.17±0.07	
200	0.25±0.06	0.49	0.04±0.01*****	0.03
400	0.31±0.05	0.87	0.05±0.01*****	0.04
600	0.26±0.05	0.62	0.05±0.01*****	0.04

Values presented as Mean±SEM. n=5. *****Significant at P <0.05

Table 6: Effects of extract on malondialdehyde level

Groups(mg/kg)	MDA(µmol/ml)	Sig.
Control	0.38±0.05	
200	0.51±0.07	0.08
400	0.41±0.03	0.62
600	0.49±0.03	0.13

Values presented as Mean±SEM. n=5. P-value= <0.05

DISCUSSION

The antioxidant and serum lipid effects of *Rhizophora mangle* were assessed in the present study. This assessment was based on the effects of the extracts on some antioxidant enzyme system, lipid peroxidation and general lipid metabolism.

Lipid peroxidation has been hypothesized as an important factor in causal pathway for atherogenesis.

The results of the lipid parameters showed that extracts of *Rhizophora mangle* caused no significant ($p<0.05$) alterations in the serum concentrations of total cholesterol, triglyceride, HDL-c, LDL-c and VLDL-c. The lipid profile tests serves as an initial broad medical screening tool for lipid abnormalities. The test could identify certain genetic diseases and approximate the cardiovascular risk index. There are numerous reports stating that abnormally elevated LDL-c concentration is atherogenic, but a high HDL-c is cardio-protective. In addition, oxidation of LDL-c causes modifications that contributes to the pathogenesis of atherosclerosis (Pittilo, 1990), and also promotes morphologic changes on the vascular endothelium (Kharb and Singh, 2000). There was no significant ($p<0.05$) change in the activities of superoxide dismutase, catalase and gluthathione reductase. But gluthathione peroxidase enzyme activity was significantly decreased in the tests groups. These observations were made when the measured enzyme activities obtained in the test groups were compared to control. The non-alteration in superoxide and catalase enzyme activities implies that extracts of *Rhizophora mangle* may not be capable of boosting the antioxidant production and defense against the negative effects of oxidative stress in the experimental animal models. Both catalase and superoxide dismutase work closely to prevent free radical damage to the body. The superoxide dismutase

enzyme converts the dangerous superoxide radical to hydrogen peroxide which is then converted to harmless water and oxygen by catalase. GSH act as electron donor in various reductive processes essential for synthesis and degradation of proteins, formation of deoxy- ribonucleotides and reduction of H_2O_2 and it's a critical molecule that functions in resisting oxidant stress and maintains the reducing environment of the cell (*Baskin and Salem., 1997).* Furthermore, the level of malondialdehyde was not significantly ($p<0.05$) altered in the extract treated groups. The malondialdehyde is a by-product of lipid peroxidation. Reactive oxygen species generated spontaneously during cell metabolism causes degradation of polyunsaturated lipids leading to the production of malondialdehyde (Del *et al.,* 2005), which serves as a biomarker to measure the level of oxidative stress in an organism [Pryor and Stanley (1975); Moore and Roberts, (1998)]. The extract of *Rhizophora mangle* did not alter lipid peroxidation rates in this study. The change in the oxidative stress markers suggest that the extract of *Rhizophora mangle* did not alter degree of toxic stress in cells, at the dose given.

Although, *Rhizophora mangle* did not significantly alter lipid parameters, it also did not cause changes that help to reduce oxidant induced cellular stress.

CONCLUSION

The extracts of *Rhizophora mangle* did not interfere sufficiently with lipid peroxidation to provide sufficient physiological benefits.by inhibiting the lipid peroxidation process.The extract did not also affect the lipid concentration of the rats.

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