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Effects of *Phyllanthus Amarus* on serum lipid profile and oxidative stress status in *Salmonellae typhi* infested wistar rats

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ABSTRACT

Salmonellae typhi infestation is known to raise cholesterol levels and induce oxidatitive stress secondary to lipid peroxidation. The hypolipidaemic and anti – peroxidative effects of Phyllanthus Amarus on Salmonellae typhi infected rats were investigated in this study. The experimental animals were randomly divided into three study groups. Group I received feed and water and was not induced with typhoid (negative control). Groups II and III received in addition to feed and water a single dose of stock Salmonellae typhi at a concentration of 10^6 CFU/ML. After seven days, widal test confirmed typoid infection and group II were not treated with the extract but rats in group III were treated with 750mg/kg body weight ethanolic extract of Phyllanthus Amarus for ten days at the end of which animals were sacrificed and blood serum obtained for analysis of serum lipids and oxidative stress indices using standard laboratory methods. In group II (positive control), there were significant increases ($p<0.05$) in serum total cholesterol, LDL – cholesterol and triacylglycerol concentration and a decease ($p < 0.05$) in serum HDL – cholesterol relative to the non induced negative control. In group III, the rats recorded a significantly ($p<0.05$) lower serum total cholesterol, LDL - cholesterol and triacylglycerol; and higher HDL – cholesterol ($p<0.05$) when compared to the Salmonellae typhi induced positive control. Similarly, hepatic glutathione peroxidase activity was significantly decreased while malondialdehyde concentration activity was increased significantly ($p<0.05$) in rats in group II when compared to negative control. However, rats in group III showed significant increase ($p<0.05$) in hepatic glutathione peroxidase activity while malondialdehlyde concentration was decreased significantly ($p<0.05$) when compared to positive control. The results suggest that treatment of Salmonellae typhi infection with ethanolic extract of Phyllanthus Amarus reduced hyperlipidaemia and lipid peroxidation induced by Salmonellae typhi infection in rats.

Keywords: *Salmonellae typhi, Phyllanthus amarus*, hypolipidaemia, oxidative stress.

INTRODUCTION

Typhoid fever (also called enteric fever) is an acute life threatening febrile illness caused by the bacterium, *Salmonellae enterica typhi* [1]. It is the second most commonest cause of fever, second only to malaria, particularly in the tropics [2]. An estimated two million cases of typhoid and two hundred thousand related deaths each year have been reported [3,4]. It is contracted through contaminated food and vegetables [5].

In developing countries, *Salmonellae typhi* infection is prevalent and accounts for high rate or mobility and mortality, particularly due to inefficient water carriage method of sewage disposal [3]. Poor sanitary habits and

hygiene have been reported to increase the prevalence of *Salmonellae typhi* infection while reduced incidence in developed countries has been attributed to high level of hygiene [1].

Salmonellae typhi infection causes gastroenteritis in human through bacterial toxin mediation. This increases free radical species in tissues which induce oxidative damage to membrane lipids [6, 7]. ROS induced oxidative damage play an important role in many clinical disorders such as heat disease, diabetes mellitus and cancer [8, 9].

Phyllanthus Amarus is a tropical shrub indigenous to the rainforest of Amazon and other tropical areas of the world [10]. It belongs to the family Euphobiacea and classified as a type of *Phyllanthus nururi* [11]. The plant has been valued in many countries for its medicinal properties and curative potentials for a variety of aliments such as asthma/bronchial infection [12, 13]; Jaundice and hepatitis B and other viral infections [14]. It exhibits inhibitory effects on Human immune deficiency virus (HIV) and reserve transcriptase activity [15]. Its detoxifying and protective effects in the liver during chronic infection and in hepatocellular carcinoma have been reported [16, 17]. [18] and [19] have reported the hypotensive, hypoglyceamic and hypocholesterolemic effects of *Phyllanthus Amarus* extract on hepatocytes of diabetic rats, while [20] and [21] have shown the invitro and antibacterial activity of the plant extract against *Staphylococcus*, *Micrococcus* and *Pasteurella* bacteria.

In rural communities in Nigeria, where *Salmonellae typhi* infection is endemic, people resort to the use of *Phyllanthus Amarus* for the management of typhoid fever related cases. The effect in lipid profile and oxidative stress indices of wistar rats in not known. In view of the foregoing, this study is designed to investigate the lipidaemic and antioxidant effects of *Phyllanthus Amarus* on *Salmonellae typhi* infected Wistar albino rats.

MATERIALS AND METHODS

Plant material: The fresh leaves of *Phyllanthus Amarus* were harvested from the natural habitats in Owerri, Imo State, Nigeria. They were identified and authenticated by Dr. S.C Okeke of the Department of Plant Science and Biotechnology, Imo State University Owerri. The voucher specimen is kept in the University herbarium.

Preparation of Extract:
The fresh leaves of *Phyllanthus Amarus* were washed free of sand and debris. Approximate weight were dried under shade at temperature of 27^0c for two weeks. The dried leaves were homogenized with an electric blender to get powder used for the extraction. A quanity, 400 grams of the powder was macerated in 0.9 liters of 80% (v/v) ethanol. The mixture was allowed to stand for 24 hours after which it was filtered with a chess cloth. The filterate was concentrated in vacuo at reduced pressure and low temperature (34-40^{0}C) to evaporate and solid residue referred to as the extract. Approximate concentrations of the extract were made in distilled water for the experiment.

Salmonellae:
Stock *Salmonellae typhi* was obtained from Federal College of Veterinary and Medical Laboratory Technology of the National Veterinary Research Institute Vom, Jos, Plateau State, Nigeria. The stock *Salmonellae typhi* was subcultured into nutrient agar plates – cesteine lactose electrolyte deficient plate (DCA). Plates were incubated at 37^0C for 24 hours. The plates were examined for growth. Stock culture slants were then prepared using McCartney bottles and nutrients agar. The organism from the subcultrued plate was aseptically inoculated into stock culture slants and incubated for 18 hours.

Animals:
Rats weighing between 170 – 200 grams of both sexes were kept in the Animal House of Faculty of Medicine, Imo State University Owerri. They were housed under standard laboratory condition of 12 hours light/dark cycle. They were allowed to acclamatize for 2 weeks before commencement of the experiment the rats had unrestricted access to water and feeds (product of Pfizer Nig. Ltd)

Induction of Typhoid Fever in the Animals
One (1)ml of *Salmonellae typhi* at a does of 10^6 CFU/ml [22] were orogastrically administrated to the rats to induce typhoid fever.

Experimental design:
Twenty – four rats were randomly assigned into 3 groups of 8 animals each.

Group I: Rats in this group were not induced with typhoid fever and were fed with normal rat chow and had free access to water throughout the period of the experiment. They were used to monitor successful induction of typhoid.

Group II: These rats served as control. They were fed with normal rat chow and orogastically given a single dose of *Salmonellae typhi* at 10^6 CFU/ml but were not treated with the extract.

Group III: Rats in this group were fed with normal rat chow and orogastically given a single dose of *Salmonellae typhi* at 10^6 CFU/ml. After seven days of infection, the animals were orogastically given 750mg/kg ethanolic extract of *Phyllanthus Amarus* extract daily for 10 days.

Analytical Procedure

Twelve hours after the last treatment, the rats were weighed and quickly sacrificed under choloroform anaesthesia. With a sterile syringe and needle, 5ml of blood was collected from each animal by cardiac puncture and allowed to stand for 1 hour to clot, serum was separated from blood cells by spinning at 1000rpm for 10 minutes using Wisperfuge Model 1384 Centrifuge (Samson, Holland) and the resulting supernatant was used for lipid profile analysis. Also from each animal, a portion of the liver was cut, weighed and used for tissue antioxidant estimation. Serum total cholesterol, LDL – cholesterol and HDL cholesterol were estimated by method of [23] as explained in Randox cholesterol Kit (Randox, England). Triacylglycerol was estimated enzymatically as described by [24]. Lipid peroxidation in liver tissue was determined by measuring malondialdehyde (MDA) using the method of [25] with the Bioxytech kits (Oxis-Research, Foster City, USA) Glutathione peroxidase activity was estimated by the method of [26].

Statistical Analysis:

Statistical evaluation of the data was performed using one – way analysis of variance and the students T-test of the SPSS statistical programme of Microsoft excel. $P<0.05$ was considered statistically significant.

RESULTS

Table I shows the effect of ethanolic extract of *Phyllanthus Amarus* on the serum lipid profile of rats infected with *salmonellae typhi.* The result showed a significant ($p<0.05$) increase in total cholesterol, LDL – cholesterol and triacylglycerol and a significant ($p < 0.05$) decrease in HDL-cholesterol concentration in *Salmonellae typhi* infested rats when compared to the control non infested group. Treatment of that rats infested with *Salmonellae typhi* (group III) with ethanolic extract of *Phyllanthus Amarus* showed a significant decrease ($p<0.05$) in total cholesterol, LDL – cholesterol and triacylglycerol, and a significant increase ($p<0.05$) in HDL – cholesterol when compared with the *Salmonellae typhi* induced and non treated (positive control) group as shown in table 1.

Induction of typhoid in rats in group II with *Salmonellae typhi* significantly decreased ($p<0.05$) the hepatic concentration of glutathione peroxidase while the concentration of malondialdehyde was significantly increased when compared to the control non infested group as shown in table II. Treatment of rats infested with *Salmonellae typhi* with ethanolic extract of *Phyllanthus Amarus* significantly increased ($p<0.05$) glutathione peroxidase concentration and significantly decreased malondialdehyde concentration when compared with the rats infested with *Salmonellae typhi* non treated (positive control) group. Table II.

Table I: Mean values of serum lipids in both experimental and control groups

Groups	Total cholesterol µmol/l	HDL – Cholesterol µmol/l	LDL – Cholesterol µmol/l	Triacylglycerol µmol/l
Group I (Negative) control	1.88±0.28	0.69±0.12	0.92±0.26	0.58±0.15
Group II *Salmonellae typhi*	3.53±0.18**	0.13±0.07*	2.93 ±0.34*	1.79±0.14*
Group III *Salmonellae typhi + Phyllanthus Amarus*	1.17±0.41**	0.59±0.08**	0.79±0.35**	0.71±0.12**

Mean ±SD (n=8)

* *Significantly different from negative control (p<0.05)*

** *Significantly different from Salmonellae typhi positive control (p<0.05)*

TABLE II: Mean values of glutathione peroxidase activity and malondialdehyde concentration in both experimental and control groups.

Groups	Glutathine Peroxidoase (mU/g)	Malondialoehyde (mU/g)
Negative control (group I)	4299.65 ±429.22	671.67±110.87
Salmonellae typhi (positive control, group II)	1658.87 ± 405.97*	762.99±71.18*
Salmonellae typhi + Phyllanthus Amarus (group III)	2723.30 ± 429.22 **	639.19±27.70**

Mean ± SD (n=8)

* *Significantly different from negative control (p<0.05)*

** *Significantly different from Salmonellae typhi i.e. positive control (p<0.05).*

DISCUSSION

Some bacterial cells causes hepatic damage to the host organism by the release of toxins. Such toxins alter the process of host metabolism, and in most cases lead to an increase in free radical species [6]. Free radical species or reactive oxygen species (ROS) cause oxidative damage by peroxidation of cellular lipids.

In this study, *Salmonellae typhi* infection significantly increased ($p<0.05$) the serum concentrations of total cholesterol LDL – cholesterol and triacylglycerol. On the other hand, it significantly decreased the concentration of HDL – cholesterol when compared with the negative control (table I). The attendant hyper lipidaemia may be due to the peroxidation of polyunsaturated lipids in cell membrane, phospholipids degradation and increased lipoprotein synthesis and excretion arising from the release of reactive oxygen species [27]. There were significant decrease ($p<0.05$) in hepatic glutathione peroxidase activity and malondialdehyde concentration in the *Salmonellae typhi* infected group compared with the negative control as shown in table II. These as a result of increase in the release of reactive oxygen species which induced oxidative stress and eventual tissue damage [8]. Oxidative stress and hyperlipidaemia have been shown to be prognostic in the development of degenerative disease [28]. The administration or ethanolic extract of *Phyllanthus Amarus* significantly increased glutathione peroxidase activity and decreased hepatic malondialdehyde concentration when compared with positive control as shown in table II, thereby protecting the cells from oxidative damage. Similar results were obtained on the administration of methanolic extract of *Phyllanthus Amarus* against cyuclophosphamide induced toxicity in mice [29]. [30] reported a significant hepato Protection of liver and brain tissues by aqueous extract of *Phyllanthus Amarus* against carbontetrachloride induced liver and train toxicity. The hepatic protection offered by *Phyllanthus Amarus* extract maybe explained on the other hand, by its ability to increase the concentration of both enzymic and non-enzymic antioxidants which reduced intracellular level of reactive oxygen species [31, 32]. On the other hand, flavonoids secondary metabolites have antioxidant property. This may be the biochemical rationale for the observed pattern of results [33].

A significant reduction in serum total cholesterol, LDL – Cholesterol, triacylglycerol and significant increase in HDL – cholesterol, as shown in table 1 may indicate the protective role of *Phyllanthus Amarus* against degenerative disease. The atherogenic risk predictor indices, HDL – cholesterol/ Total cholesterol and LDL – cholesterol/HDL – cholesterol ratios fell within the desirable limits. This may be showing that the ethanolic extract of *Phyllanthus Amarus* has the potential to reduce the risk of development of heart diseases. Similar results have been reported [34, 35, 36]. Saponin fractions are generally known to lower serum lipid levels [19]. The hypolipidamic effects of *Phyllanthus Amarus* extract caused a reduction in serum cholesterol and other fatty acids possibly due to reduction in infiteration of the fatty substances from blood into the arterial walls [37] and its antioxidant property [17]. Antioxidants are known to prevent the oxidative modification of lipoproteins before their incorporation into the fatty streak of the arterial wall [37]. Antioxidant vitamins are also known to inhibit 3 – hydroxy – 3- methyl glutaryl C_OA (HMG C_OA) reeducates activity, thus in inhibiting cholesterol synthesis [35].

CONCLUSION

This study has established the hypolipidaemic and anti Peroxidative roles of *Phyllanthus Amarus* extract on *Salmonellae typhi* infected rats. This may explain why the plant extract is popular in local settings for the treatment of typhoid related infections.

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