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Immuno-modulatory and Anti-Oxidative Potentials of Fermented *Parkia Biglobosa* on Pyrogallol Induced Toxicity on Wistar Rats

Ekoh, C. O[1] ., Dogonzo,I.Y[2]., Bonaventure,M. O[3] and Eji, C. A[4]

[1,2]Department of Biochemistry, Faculty of Biological Sciences, Alex Ekwueme Federal University,Ndufu-Alike, Ebonyi State of Nigeria.

[3]Nigeria Centre for Disease Control and Prevention, Abuja, Nigeria.

[4]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State of Nigeria.

***Corresponding author's email :ekohcharles4law@gmail.com**

Abstract

Background: African locust bean (*Parkia biglobosa*) is a legume of great potential used as foodthe fruit pulp and seeds being rich in sucrose, protein, amino acids, carbohydrates, and lipids, and medcine , due to its anti-hypercholesterolemic, cardio protective, hepato-protective and antioxidant properties . This study was aimed at investigating the Immuno-modulatory and anti-oxidative potential of fermented *Parkia biglobosa on* pyrogallol induced toxicity on wistar rats. **Methodology:**The animals were divided into three groups (n=4). The test group (Group 1) received *Parkia biglobosa* (15mg/body weight) and Pyrogallol (10mg/kg body weight) for 30 days, while the negative control group (Group 2) received pyrogallol (10mg/kg) only. The normal control group (Group 3) received normal feed weight of feed and water for 30 days. Blood samples obtained via ocular puncture were used for the analysis of haematological parameters, White blood cell (WBC), Red blood cell (RBC), Packed cell volume (PCV), platelets, haemoglobin (Hb) results were obtained using haemocytometer (WBC, RBC and Hb), Hawksley haematocrit reader (PCV). Antibodies (primary and secondary antibodies) was also determined and markers of oxidative stress Glutathione (GSH), Glutathione peroxidise (GPx), Vitamin E (Vit.E), Catalase and Malondialdehyde (MDA) was determined using a spectrophotometer (auto analyser). **Results:** The results showed a significant increase (p<0.05) in the total WBC, RBC, and antibodies titre in treated rats (group 1) when compared to the untreated rats (group 2). There was also a significant increase (p>0.05) in the antioxidants, GPx, GSH and catalase in the treated rats (group 1) when compared to the untreated rats (group 2). The result of lipid peroxidation revealed a significant increase (p>0.05) in MDA in the untreated rats (group 2) when compared to the treated rats (group1).**Conclusion:**The findings suggest that *Parkia biglobosa*is has potentials of stimulating an Immunomodulatory and anti-oxidative effect against the toxic effects of pyrogallol.

Keywords: *Parkia biglobosa*, pyrogallol, toxicity-induced oxidative-stress, anti-oxidant, immunodulation,

1 Introduction

African locust bean (*Parkia biglobosa*) is a perennial deciduous legume plant widely distributed over central and west African countries (Millogo-Kone *et al*., 2008). It is put to several uses, which include food, therapeutic and economical purposes (Koura *et al.*, 2011). The fruit pulp and seeds are rich in sucrose, protein, amino acids, carbohydrates, and lipids (Ajaiyeoba, 2002).

When fermented, the seeds are used as spices for seasoning of soups and stews. Among its medicinal uses include for the treatment of leprosy, hypertension, ulcers, burns, skin infections, sterility, and diabetes mellitus, (Odetola *et al.,* 2006; Dedehou *et al.*, 2016). Previous studies by Femi-ola *et al*, (2008), Alinde *et al*, (2014), have associated *Parkia biglobosa* with phytochemicals with anti-hypercholesterolemic, cardio protective, hepatoprotective and antioxidant properties.

Pyrogallol ($C_6H_6O_3$), is a powerful reducing agent with ability to produce free radicals, and it is widely distributed in nature, but can be produced commercially from garlic acid (Budavari, 1996). Application or Ingestion of high amount of this chemical has been associated with oxidative damage, mutagenesis, carcinogenesis and hepatotoxicity (Upadhyay *et al*, 2008). Organs commonly impacted by its toxicity include the liver, lung, kidney and gastrointestinal tract (Kerkar *et al*, 2001).As a result of its free radical generating potential, its often used as an anti-psoriatic drug, and also as a chemical for evaluating the hepato-protective potential of naturally occurring antioxidants. This research is aimed at investigating the immuno-modulatory potential of fermented African locust bean on pyrogallol-induced toxicity in female wistar albino rats.

2. Method

2.1 Collection of plant material

Fermented seed of *Parkia biglobosa* were purchased from Ogwumabiri and Orieugba markets, Umuahia, Abia State of Nigeria, and were identified by Dr. Omosun Garba of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike.

2.2 Study area:

The study was carried out in the animal house of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Experimental Animals

A total of 12 healthy female wister rats purchased from the Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria was used (weight in the range of 80-150g) after being weighed on an electronic scale. The animals were divided into 3 groups (4 rats per cage). All animals had free access to food and water *ad libitum.* They were maintained under standard laboratory conditions (a well-aerated room with alternating light and dark cycles of 12 hours each and at room temperature). All rules applying to animal safety and care were observed.

2.3 Preparation/formulation of feed

Fermented African locust bean (*Parkia biglobosa)* seeds were sun-dried to reduce the moisture content, for four days. It was ground to pellets using a grinding machine (Surgifriend medicals England) at Liduna Pharmacy,Ahiaeke. It was later oven dried for an hour, and weighed using

 weighing balance (Ohaus). The feed was formulated by measuring out 55g of commercial pelletized feed and 15g of *Parkia biglobosa.*It was mixed and fed to the wister albino rats in group 1 once daily for 30days

2.3 Preparation of pyrogallol

Pyrogallol reagent was prepared by dissolving 25g of pyrogallol in 100mL of distilled water. It was shaken vigorously for uniform mixing, and this reagent was used for the study. It was administered orally using a gavage syringe.

2.6 Experimental design

The animals were randomly distributed into three groups of four animals each. Goup3 served as the normal control, while rats of group 1 served as the test group, and group 2 as the negative control. Animals of group 3 was given water and food, group 1 pyrogallol and fermented *Parkia biglobosa*, while group 2 which served as the negative control was fed pyrogallol (10mg/kg) body Weight for thirty days.

2.7 Blood collection

After administration, the animals were starved overnight, and blood samples were collected through ocular puncture, after the rats were sacrificed by cervical dislocation. The blood samples were dispensed into two different types of tubes. The first part was dispensed into sample tubes containing an anti-coagulant, for haematological analysis. The second part was dispensed into a clean plain sample tubes and allowed to clot for about15 min and centrifuged at 3000 rpm for 10 min. The serum was separated from the clot by simple aspiration with Pasteur pipette, and dispensed into clean tubes for serum biochemical analysis.

2.8 Biochemical analysis

Serum biochemistry was carried out on each of the samples. The activities of catalase, glutathione peroxidase, reduced glutathione, and malondialdehyde were determined spectrophotometrically, using the respective test kits,while the concentrations of the haematological indices, erythrocytes count, total leucocytes count, packed cell volume and haemoglobin concentrations were done using a haematological analyser (haemocytometer).

2.9 Statistical analysis

The statistical analysis was done, using one way ANOVA.

3. **Results**

The activities of GPx, CAT and concentration of GSH in groups 1 and 3 increased significantly ($p<0.05$) when compared with group 2 (Table 1). Similarly, the concentrations of Vit E in groups 2 and 3 were slightly elevated though not statistically significant, when compared with Group 2. However, the MDA concentrations in groups 1 and 3 were significantly reduced ($P<0.05$) when compared with group 2.

Table 1: Effect of Parkia biglobosa on antioxidant enzymes and lipid peroxidation

Parameters	Group 1 (PG+PB)	Group 2 (PG)	Group 3 (Water)
Glutathione Peroxidase (GPx) (U/L)	405.15 ± 15.83*	287.73 ± 11.39*	350.60 ± 29.60
Glutathione reductase (GSH) (mg/dl)	2.82 ± 0.19 *	2.64 ± 0.26*	3.17 ± 0.08
Vitamin E (Vit. E)	1.45 ± 0.09	1.36 ± 0.04	1.45 ± 0.08
Malondialdehyde (MD)A (mg/dl)	18.60 ± 0.27 *	24.34 ± 0.29*	18.14 ± 0.18
Catalase (CAT) (U/ml)	5.40 ± 0.29*	1.52 ± 0.24*	2.96 ± 0.16

PG: Pyrogallol, PB: *Parkia biglobosa* Result represents mean ± standard deviation. Asterisk indicates statistically significance between groups.

The primary antibodies level in groups 1 and 3 (Table 2) showed a significant increase ($P<0.05$), when compared with group 2. Similarly, the secondary antibodies in group 2 reduced significantly ($p<0.05$) ,when compared with groups 1 and 2.

Table 2 Effect of *P*arkia *biglobosa* on Antibodies

Parameters	Group 1	Group 2	Group 3
Primary Antibodies	8.00 ± 0.00	4.00 ± 0.00*	8.00 ± 0.00
Secondary Antibodies	20.00 ± 8.00	8.00 ± 0.00*	16.00 ± 0.00

Result represents mean ± standard deviation. Asterisk indicate statistical significance between groups.

WBC concentration in group 1 showed a significant increase ($P<0.05$), when compared with groups 2 and group 3. Group 3 showed a significant decrease ($P<0.05$), when compared with group 2. The RBC, PCV and Hg concentrations in groups 1 and 3 showed a significant increase ($P<0.05$), when compared with group 2. Group 3 showed no significant decrease ($P>0.05$), when compared with group 1. There was no significant difference ($P>0.05$) in platelet concentration across the three groups.

Table 3 Effect of fermented Parkia biglobosa on haematological parameters

Parameters	Group 1	Group 2	Group 3
White blood cells(WBC)(10^9/L)	7800.00 ± 365.15*	5500.00 ± 1569.50 *	2550.00 ± 129.10
Red blood cells (RBC)(10^{12}/L)	212.50 ± 0.58	202.75 ± 0.96 *	215.25 ± 3.77
Packed cell volume(PCV)%	44.50 ± 1.29	34.50 ± 4.12%*	43.50 ± 1.29
Haemoglobin (Hb)g/Dl	16.25 ± 0.42	13.05 ± 0.57*	15.88 ± 0.42
Platelets%	140.00 ± 0.00	140.00 ± 0.00	142.50 ± 5.00

Result represents mean ± standard deviation. Asterisk shows statistically significance between groups.

4.Discussion

This study investigated the possible immunological and anti-oxidative effect of fermented locust bean seed (*Parkia biglobosa)* on Wistar Rats. *Parkia biglobosa* has been shown to possess haematinic and anti-oxidative abilities as it stimulates the activity of the bone marrow and mitochondria redox status (Salminen *et al.,*2008, Komolafe *et al*, 2014). Our findings showed a consistent significant ($P<0.05$) increase in haematological indices of total white blood cells (WBC), red blood cell (RBC) count, packed cell volume (PCV), humoral antibodies and haemoglobin (HB) concentration in test rats, compared to the positive control and the normal control in some cases. The elevation in the total white blood cell count and antibody titre observed in the treated rats (group 1), could be due to the potentials of PG to provide protection from the destructive effect of pyrogallol by stimulating increase production of T-lymphocytes. Similarly, the lower WBC count observed in the untreated rats (group 2), could be due to their suppression by the destructive effect of pyrogallol on different tissues affected. This finding is consistent with the report of Ojewumi (2016), who had earlier observed an increase in haematological indices of rats fed with fermented locust bean seed (*Parkia biglobosa).*

The result of red blood cell count showed a significant increase in the test group and normal control, when compared with the positive control. Pyrogallol, which was administered to the positive control, was an antagonist of oxygen,hence, did not permit the binding of heme proteins to oxygen, thereby shortening the lifespan of the red blood cells in the process. The fermented seeds of African locust bean (*Parkia biglobosa*) are a good source of important minerals such as phosphorus, calcium, thiamine and iron required in the body , and which aid in alleviating iron or non-iron deficiency anaemia. This finding agrees with the report of Ijarotimi and Keshinro (2012), who had earlier observed that fermented seeds of *Parkia biglobosa* was effective in the management of anaemia as it increased haemoglobin, red blood cells, white blood cells and packed cell volume.The difference in concentration of Malondialdehyde (MDA) was used to confirm the level of lipid peroxidation. Our findings revealed a significant increase ($p<0.05$) in MDA level in the untreated rats (group 2), compared to the treated rats (group1) and the normal control (group 3). The increase in MDA concentration has been reported to be due to the toxic effect of Pyrogallol on the membrane phospholipids. This finding is in agreement with previous studies by Saleh *et al*, (2021).

The result of this study revealed a significant increase ($p<0.05$) in the activities of GPx, Catalase and the concentration of GSH in the treated rats (group 1) and normal control (group 3), when compared with the untreated rats. Anti-oxidants including GPx, GSH, and Catalase have been shown to play a key role in neutralizing the effects of free radicals and oxidative stress (Nwaehujor *et al.,* 2010). Anti-oxidants including GPx, GSH, and Catalase have been shown to play a key role in neutralizing the effects of free radicals and oxidative stress (Nwaehujor *et al.,* 2010).However, there was no significant difference in the concentration of the anti-oxidants in the treated and normal groups. This finding is in agreement with that of Tokoudagba *et al.* (2010) and Ogunyinka *et al* (2016), who reported that *Parkia biglobosa* enhances antioxidant defence system and also protects the brain tissues against oxidative stress.

Conclusion

In conclusion, the observed increases in the concentration of the haematological indices (WBC, and Antibodies) and anti-oxidative markers (GPx, GSH, and Catalase) in the treated rats was an indication that *Parkia biglobosa* possesses both immune-modulatory and anti-oxidative properties.

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Ethical Issues/Clearance

The present research work was permitted by the Animal Ethical Committee of Michael Okpara University of Agriculture, Umudike, Abia State of Nigeria.

Conflict of interests

Authors declare there was no conflict of interest.

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Authors contribution

Ekoh C.O and Dogonzo I.Y designing the project, experiment, analysis and writing out of the final draft.
Bonaventure M.O and Eji C.A were involved in the experiment and writing out the first draft.

All the authors read the final draft and approved it before submission for publication.