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The Effect of Ethanol Extract of *Acalypha wilkesiana* on the Oxidative Stress Indices of Paracetamol-induced Hepatotoxicity in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author CCM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors CLO and SIO managed the literature searches, analyzed the study and performed the spectroscopy analysis. Author CCM managed the experimental process and author CLO identified the species of plant. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Objective: The effect of *A. wilkesiana* on the oxidative stress indices/biomarkers of wistar albino rats with paracetamol induced hepatic injury was investigated in this work.
Study Design: Animal experimental study.
Place of Study: Department of Biochemistry, Faculty of Biological Science, University of Port Harcourt, P.M.B 5323, Port Harcourt, Nigeria.
Methods: Fifty-four wistar albino rats weighing 150-200 g were randomly allotted into six experimental groups of nine rats each: (Group 1) normal control, (Group 2) positive control, treated with only paracetamol (PARA), (Group 3) treated with paracetamol (PARA) and 100 mg per kg body weight of *A. wilkesiana* (A.W) ethanol extract, (Group 4) treated with paracetamol and 200 mg per kg body weight of *A. wilkesiana* (A.W) ethanol extract, (Group 5) treated with paracetamol and 300 mg per kg body weight of the *A. wilkesiana* ethanol extract while group 6 animals were treated with paracetamol and 25 mg per kg body weight of standard drug silymarin. The experiment lasted

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for 21 days. The blood samples were collected at 7 days interval for analyses of oxidative stress biomarkers.
Results: Paracetamol induction resulted in the increase of malondialdehyde (MDA) level, reduced the plasma glutathione levels, catalase activity and superoxide dismutase (SOD) activity. Treatment with different doses of the ethanol extract of *A. wilkesiana* (A.W) leaves increased the plasma level of glutathione, catalase activity, and the SOD activity while the malondialdehyde level was reduced.
Conclusion: The study revealed that treatement with ethanol extract of *A. wilkesiana* (A.W) leaves may have therapeutic effect against paracetamol induced oxidative stress.

Keywords: Acalypha wilkesiana; oxidative stress; paracetamol.

1. INTRODUCTION

A. wilkesiana also known as wilkes copper leaf, fire dragon, Jacob's coat or catch-if-you-can [1] is an ever green shrub from the family euphorbiacaece. The name of the species was given in honour of USA Commander Charles Wilke who was the founder of the plant. It is mainly planted as an outdoor plant. This plant is used in folk's medicine for the treatment of skin infections. *A. wilkesiana* is found all over the world especially in the tropical [2] and subtropical countries. It grows naturally in Fiji and nearby Islands in the south pacific [3]. The weeds are wild and can be found growing almost everywhere. Research has shown that this plant is rich in phytochemicals which are plant chemicals implicated in disease prevention and treatment. The plant has been used as ointment in treatment of fungal skin infection and microbial infections.

Oxidative stress or oxidative damage can occur due to the presence of reactive oxygen species (ROS). Disruptions in the cell's redox reaction could lead to the formation of free radicals and peroxides such as superoxide anion, hydrogen peroxide, hypochlorous acid and peroxinitrate ions. These free radicals and peroxides are responsible for cell damage, cell death also known as (apoptosis), mutations leading to cancer, development of heart diseases, infections and many other diseases [4]. Reactive oxygen species also alter biological macro-molecules which include proteins, carbohydrate, lipid and DNA [5]. Phytochemicals which are chemical compounds found in plant are known for their potentials in reducing the effects of these free radicals. Phytochemicals such as alkaloids, flavonoids, tannins are known for their antioxidant effects. Research has shown that most plants and animals possess natural antioxidants which help them fight against these reactive oxygen species (ROS).

Superoxide dismutase, catalase, glutathione peroxidase are natural antioxidants which act as a defence against reactive oxygen species. Superoxide dismutase is an enzyme which catalyses the dismutation of toxic superoxide (O_2^-) radical to molecular oxygen (O_2). Catalase and glutathione peroxidase are enzymes which catalyse the decomposition of hydrogen peroxide to water and oxygen. Some natural antioxidants such as vitamin C and E found in fruits and vegetables acts as scavengers, they reduce the effects of reactive oxygen species in the body [6]. In an earlier study, phytochemical analysis of the leaves of *A. wikesiana* showed the presence of alkaloids, flavonoids cardiac glycosides, phenols, terpenoids and steroids. Another study demonstrated the presence of alkaloids, flavonoids, saponins and cardiac glycosides in the leaves of *A. wilkesiana* [7] Based on this data we evaluated the effect of ethanol extract of the leaves of *A. wilkesiana* on paracetamol induced oxidative stress.

2. METHODOLOGY

2.1 Experimental Animals

A total of fifty-four (54) male and female wistar albino rats of about of 16 weeks old were used for this study. The rats were purchased and housed in the Pharmacology Department Animal House at Ofrima, Abuja Park of the University. The animals were acclimatized for seven (7) days, during which they were fed with normal feed (Top feeds- grower's mash) and clean water. The wistar albino rats weighed 160 -200 g and they were marked for easy identification. The fifty-four (54) rats were grouped into six (6) various experimental groups with nine (9) rats in each group.

2.2 Plant Material

The *A. wilkesiana* leaves used for this study were obtained from Abuja park of University of Port Harcourt.

The plant samples were identified at the Herbarium of the Plant Science and

Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria as *A. wilkesiana muell Arg*. by Dr. Nwosu Edwin.

2.3 Equipment and Reagent

2.3.1 Drugs, diets, chemicals and reagents

They include the following: paracetamol (Emzor Pharmaceuticals Ltd, Nigeria), chloroform (BDH chemicals Ltd.), ethanol 95% (SIGMA chemicals), formalin 10%, and finisher feed (Top Feed Ltd.).

2.4 Preparation of Extract

2.4.1 *A. wilkesiana* extract

Leaves of *A. wikesiana* were washed and shade dried at room temperature, after which the leaf powder was prepared using home grinder/blender. 1000 g of the powdered *A. wilkesiana* leaves was weighed and soaked in 3000 ml of 95% Ethanol for 48 hours after which it was sieved using a muslin cloth and afterwards filtered with Whatmann filter paper size 1. The filtrate was concentrated using Rotary Evaporator at 45℃, the weight of the concentrates were taken and the percentage yield calculated and kept at 4℃ until usage.

2.5 Experimental Design

Research was carried out according to the rules and regulations guiding the use of animals. The animals were sorted into six groups with nine (9) animals each. The animals were grouped as in the Table 1.

Table 1. Group description

Groups	Treatment
1	Control (without PARA, untreated)
2	Disease control (PARA 2000 mg/kg body weight only)
3	PARA 2000 mg/kg body weight + *A. wilkesiana* 100 mg/kg body weight
4	PARA 2000 mg/kg body weight + *A. wilkesiana* 200 mg/kg body weight
5	PARA 2000 mg/kg body weight + *A. wilkesiana* 300 mg/kg body weight
6	PARA 2000 mg/kg body weight + sylimarin 25 mg/kg body weight

Three rats in group 1 served as control (without paracetamol (PARA), untreated), group 2 served as disease control (PARA + Distilled water). Rats in group 3, 4 and 5 received paracetamol (PARA) and oral administration of ethanol leaf extract of *A. wilkesiana* (A.W) 100, 200 and 300 mg/kg body weight respectively for three weeks. Treatment started 48 hours after they received paracetamol and lasted for 21 days. Distilled water was used as vehicle for drug and extract administration. Mode of administration was adopted from the work done by Ikewuchi [8]. Three (3) rats (n=3) were sacrificed from each group at seven (7) days interval during treatment for three (3) weeks. The animals were anaesthesized using chloroform. The animals while under anaesthsia were painlessly sacrificed and the blood sample were collected into Heparin bottles for oxidative stress biomarkers assay.

2.6 Determination of Oxidative Stress Biomarkers

The plasma Superoxide dismutase activity was determined using auto-oxidation method [9] while Malondialdehyde [10], Glutathione (GSH) and Catalase were determined estimated using spectrophotometric method [11,8].

2.7 Ethical Approval

The protocol of this study was approved by the research ethical approval committee of the institution. We certify that all the rules and regulations guiding the use of animals were followed during the course of this research.

2.8 Statistical Analysis

Means and SEMs were calculated for all data. Significant differences between means were evaluated using Post Hoc Turkey. A difference was considered significant when p was less than 0.05. Data analysis was carried out using Microsoft Excel (2010) Microsoft Corporation, Seattle, WA, (USA) and Statistical Package for Social science (SPSS) version 16 Inc., Chicago, IL USA.

3. RESULTS AND DISCUSSION

The effect of ethanol extract of *A. wilkesiana* is as shown in the Tables 2-4.

3.1 Discussion

Oxidative stress is due to the presence of reactive oxygen species in the body. These reactive oxygen species disrupt the integrity of

Table 2. The effect of ethanol extract of *Acalypha wilkesiana* on the oxidative stress indices of paracetamol induced oxidative stress in wistar albino rats in week 1 of the experiment

Treatment group	SOD U/ml	MDA umol/ml	CATALASE U/ml	GSH ug/ml
Group 1	2.35±0.08^{bcd}	0.82±0.02^{df}	0.18±0.02^{ef}	1.35±0.12b^{cdef}
Group 2	1.49±0.07^{aef}	0.86±0.09^{df}	0.11±0.03^{ef}	0.42±0.01^{a}
Group 3	1.83±0.06^{ae}	0.62±0.12	0.19±0.01^{f}	0.41±0.00^{a}
Group 4	1.72±0.24^{aef}	0.41±0.02^{ab}	0.19±0.08^{f}	0.51±0.03^{a}
Group 5	2.31±0.32^{bcd}	0.59±0.28	0.27±0.39^{ab}	0.45±0.00^{a}
Group 6	2.21±0.08^{bd}	0.49±0.04^{ab}	0.31±0.02^{abcd}	0.49±0.01^{a}

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c, d, e, f) are significantly different at p<0.05

Table 3. The effect of ethanol extract of *A. wilkesiana* on the oxidative stress indices of paracetamol induced oxidative stress in wistar albino rats in week 2 of the experiment

Treatment group	SOD U/ml	MDA umol/ml	CATALASE U/ml	GSH ug/ml
Group 1	2.32±0.09^{b}	0.82±0.02^{df}	0.17±0.02	1.33±0.12^{bcdef}
Group 2	1.51±0.14^{aef}	0.78±0.24^{df}	0.09±0.02^{def}	0.50±0.01^{af}
Group 3	1.92±0.29	0.77±0.15	0.15±0.06	0.43±0.00^{afd}
Group 4	2.01±0.35	0.37±0.06^{ab}	0.19±0.03^{b}	0.50±0.03^{af}
Group 5	2.14±0.15^{b}	0.43±0.13^{a}	0.19±0.03^{b}	0.50±0.00^{af}
Group 6	2.19±0.08^{b}	0.26±0.04^{ab}	0.19±0.01^{b}	0.75±0.07^{abcde}

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c, d, e, f) are significantly different at p<0.05

Table 4. The effect of ethanol extract of *Acalypha wilkesiana* on the oxidative stress indices of paracetamol induced oxidative stress in wistar albino rats in week 3 of the experiment

Treatment group	SOD U/ml	MDA umol/ml	CATALASE U/ml	GSH ug/ml
Group 1	1.99±0.39	0.85±0.06^{cdef}	0.13±0.05	1.93±0.45
Group 2	1.74±0.20	0.72±0.23	0.12±0.06	0.50±0.02
Group 3	1.95±0.17	0.46±0.06^{a}	0.18±0.57	0.49±0.02
Group 4	2.27±0.25	0.44±0.02^{a}	0.19±0.02	0.55±0.03
Group 5	2.16±0.04	0.44±0.14^{a}	0.19±0.03	0.49±0.01
Group 6	2.15±0.03	0.44±0.03^{a}	0.19±0.02	0.74±0.09

Values are represented as mean ± SD, n=3 per group /week. Values in the same column with common superscript letters (a, b, c, d, e, f) are significantly different at p<0.05

different macromolecules, such as lipids, proteins, DNA etc [12]. Oxidative stress is involved in the reduction of natural antioxidant such as glutathione, in paracetamol overdose the liver's glutathione is depleted as a result of excess production of N-acetyl-p-benzylquinon imine. Natural antioxidants abound which help to stop the effect of these reactive oxygen species. Some of the antioxidants which has been provided by nature to combat the effects of free radicals include gluthathione, vitamin C, vitamin E, superoxide dismutase which catalyses the dismutation of superoxides, catalase, phytochemicals including flavonoids.

In the present research, the role of paracetamol induced toxicity in the degeneration of the body's antioxidant system was investigated. Paracetamol toxicity due to its overdose significantly reduced the concentration of glutathione, the activities of catalase and superoxide dismutase (0.50±0.01 ug/ml, 0.11±0.03 U/ml and 1.49±0.07 U/ml respectively) compared to control in week one of the experiment as can be observed in the Table above. Similar trend was observed in week 2 and 3 of the experiment. There was an increase in the levels of malondialdehyde 0.86±0.09 umol/ml when compared to normal (Group 1). Increase in malondialdehyde shows the presence of lipid peroxidation which could lead to loss of membrane fluidity and elasticity.

In the present research the effect of ethanol leaf extract of *A. wilkesiana* on the oxidative stress biomarkers were also investigated. The result obtained shows a significant increase in the activities of the superoxide dismutase and

catalase activity of the paracetamol treated groups after oral administration of ethanol leaf extract of *A. wilkesiana*. A similar trend was observed in the plasma glutathione level of the groups treated with paracetamol and different doses of ethanol leaf extract of *A. wilkesiana* (Tables 2 - 4). A decrease which was statistically significant at (p<0.05) was observed in the plasma level of malondialdehyde of the groups treated with paracetamol and different doses of ethanol extract of *A. wilkesiana* leaves. It is possible that lipid peroxidation reaction was inhibited by the extract, thereby leading to a decrease in the production of malondialdehyde. The overall antioxidant effect could be as a result of the rich phytochemical content of *A. wilkesiana.* Research has shown the presence of gallic acid, corilagin, geranin, quercetin, ratinoside and kaempferol in the leaves of *A. wilkesiana* [13]. Akinde [13] reported the presence of besquiterpenes, monoterpenes, triterpenoids and polyphenols in leaves of *A. wilkesiana.* Ogbuehi et al. [14] also demonstrated the presence of tannins, flavonoids, saponins, steroids, alkaloids, anthroquinones, cardiac glycosides and carotenoids in the leaves of *A. wilkesiana*. The presence of Terpenoid, phytate, and protein has also been demonstrated in the leaves of *A. wilkesiana* [15]. The presence of these phytochemicals in the leaf extract of *A. wilkesiana* as mentioned above supports the antioxidant effects of the plant extract. Phytochemicals are implicated in the treatment of various infections and diseases which are caused by reactive oxygen species. Thus ethanol leaf extract of *A. wilkesiana* may have the potential to reduce oxidative stress due to paracetamol overdose.

4. CONCLUSION

In conclusion, this research has shown that paracetamol overdose can results in oxidative stress. The result of this research has also shown that ethanol leaf extract of *A. wilkesiana* has potentials in reducing oxidative stress caused by paracetamol toxicity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sagun VG, Levin GA, Welzan PC. Revision and phylogeny of *Acalypha* (Euphorbiaceae) in Maleysia. Blumea. 2010;55(1):2-6.
2. Madziga HA, Sanni S, Sandabe UK. Elemental analysis of *Acalypha wilkesiana* leaf. Journal of American Science. 2010; 6(11):510-514.
3. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology. 2007;39(1):44–84.
4. Evans MD, Cooke MS. Factors contributing to the outcome of oxidative damage to nucleic acids. Bio Essays. 2004;26(5):533–42.
5. Ikewuchi JC, Uwakwe AA, Onyeike EN, Ikewuchi CC. Hepatoprotective effect of an aqueous extract of the leaves of *Acalypha wilkesiana* 'godseffiana' muell arg (euphorbiaceae) against carbon tetrachloride induced liver injury in rats. Excli Journal. 2011;10:280-289.
6. Amelia MG, Ana-Maria B, Mihnea-Alexandru G, Aurel P. The role of oxidative stress and the effects of antioxidants on the incidence of infectious complications of chronic lymphocytic leukemia. Oxidative medicine and cellular longevity. Hindawi Journal. 2014;6.
7. Davey MW, Stals E, Panis B, Keulemans J, Swennen RL. High-throughput determination of malondialdehyde in plant tissues. Analytical Biochemistry. 2005; 347(2):201–207.
8. Aebi H. Catalase. In: Bermeyer HV, editor. Methods in enzymatic analysis. New york: Academic press. 1974;2:674-84.
9. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972;247(10):3170-5.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;95:351-358.
11. Seedlak J, Lindsay RH. Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry. 1968;25:1192-1205.
12. Irshal M, Chaudhuri PS. Oxidant-antioxidant system: Role and significance in human body. Indian Journal of Experimental Biology. 2002;40(11):1233-9.

13. Akinde BE. Phytochemical and microbiological evaluation of the oils from the leaves of *Acalyphya wilkesiana*. The state of medicinal plant research in Nigeria. University of Ibadan Press. 1986; 362–3.
14. Ogbuehi I, Adikwu E, Oputiri D. Effect of *Acalypha wilkesiana* muell arg leaf extract on the oxidative indices, liver enzymes and liver integrity of rats infected with *Plasmodium berghei.* British Journal of Pharmacology and Toxicology. 2014;5(2): 68-74.
15. Onuah CL, Monago CC, Omeodu SI. Ethanol extract of *Acalypha wilkesiana* muel arg leaves ameliorated paracetamol-induced hepatotoxicity in rats. International Journal of Biochemistry Research and Review. 2016;12(2):1-7.

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