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THE EFFECT OF ETHANOLIC LEAF EXTRACT OF *NEWBOUILDA LAEVIS* ON THE ANTIOXIDANT STATUS FOLLOWING TOXICITY OF CARBON TETRACHLORIDE ON MALE WISTAR RATS

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Abstract: **The effect of *Newbouilda laevis* on the Antioxidant status was studied using Wistar rats. The N*ewbouilda laevis* extraction was done using 70% ethanol. 30 male wistar rat weighing 130g to 200g were randomly divided into 5 groups; Group A served as the positive control, Group b served as the negative control receiving CCL_4 intrapertoneally for one week, Group C to E received 150, 300, and 600mg/kg of the extract of *Newbouilda laevis* for 21 days. The animals were sacrificed under anesthesia and blood sample collected and MDA, SOD, CAT, GSH, GPx, and TAC levels were determined. The results showed that E.L.N has antioxidant effect as there was significant increase in the level of MDA P< 0.005, however, it negatively affected the antioxidant levels as it significantly decrease the levels of Superoxide-dismutase and Catalase across the low dose, medium dose and high dose groups this shows that E.L.N had a negative impact by bring down the levels of SOD and Catalase P< 0.005. An significant increase in the level of Glutathione was observed in the group D P<0.005 while a significant decrease was observed in Group E. the increase observed in the low dose was not significant P>0.005. The level of Glutathione peroxidase was significantly increased in Group D however no significant increase was noted in other Groups P>0.005. The level of Total antioxidant was significantly increased in Group C however no significant increase was noted in other Groups P>0.005. The study shows that the use of E.L.N has a mild to moderate positive effect in increasing the antioxidant level and hence should be used with caution.**

Keywords: ***Newbouilda laevis*****, Malondialdehyde, Superoxide-dismutase, Catalase, Glutathione, Glutathione Peroxidase, Total antioxidant.**

1. INTRODUCTION

The use of heavy chemicals and drugs in cellular toxicities has been on the rise in recent times affecting cellular activities, resulting in production of reactive oxygen species (ROS) thereby altering metabolic process such as liver (Muhammad *et al.*, 2015). Carbon tetrachloride (CCl4) is called also tetracholoromethane, Halon-104, and Refrigerant-10 (Yoshioka *et al.*,

2016). Its toxicity depends on the generation trichloromethyl radical (CCl_3), which further translates trichloromethyl into trichloromethyl peroxyl radical (CCl_3O^2-) in the presence of oxygen that is more toxic than trichloromethyl radical (El-mohsen and Abdelaziz 2014). The generated free radicals of CCL4 metabolites results in peroxidative degeneration of distinct tissues by binding with lipids, proteins, and DNA. Report had it that CCl4 is the best model for the generation of reactive oxygen species (ROS) in many tissues (Kamisan *et al.*, 2014).

The antioxidants system is an important system that plays a major role in the replenishing of the oxidative damage cause by lipid peroxidation of chemicals. Medical and nutritional specialists have of late given attention to the antioxidant characteristics of food constituents because of the potency of oxidative stress-mediated lipid peroxidation to induce a variety of pathological conditions such as atherogenesis, aging, and liver diseases etc.(Dryden *et al.*, 2005). Malondialdehyde (MDA) as a biomarker is widely used for examining oxidative stress in biomedical field where Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in cellular damage.

Newbouldia laevis also called the "Boundary Tree" is a medium sized angiosperm in the Bignoniaceae family, which is native to tropical Africa, and grows to a height of about 10 m (Okeke 2003).

2. MATERIALS AND METHOD

Location of the study: This study was carried out in the Department of Physiology, College of Health Science, Nnamdi Azikiwe University, Nnewi Campus.

Materials: Thirty-(30) Inbreed male wistar rats, Randox Reagent Kits, *Newbouilda Laevis*, electronic weighing balance (M-Metlar, M311L, China), Whatman Qualitative filter paper no. 1, Distilled water, Ethanol (JHD Chemicals, Guangdong China), Normal laboratory chow, Rotatory Evaporator (TT-52, Techmel & Techmel, USA), Heparinized capillary tube, Standard Cages, S. Pyrex beakers (Techmel, USA), Oral cannula, Chloroform, Spectrophotometer, and Centrifuge (England).

3. METHODOLOGY

Preparation of Plant Extract: Leaves of *Newbouildae laevis* (N.L) were plucked fresh from the School Farm, washed under running tap water to remove dirt, cut into pieces and air dried under ambient temperature. The dried *Newbouildae laevis* leaves were milled into coarse powder using Local grinder. 250g of the coarse *Newbouildae laevis* leaves were macerated in of 1000mls 95% ethanol for 48hours. It was then filtered using a porcelain cloth and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator, which were further dried using a laboratory oven at 45°C into a gel-like form. The extracts were preserved in a refrigerator for further usage, and were prepared according to the method described by Al-Attar and Abu Zeid (2013) with modifications.

Experimental Animals: Inbreed 30 male wistar rat weighing 130g to 200g were used for the study, and housed in the animal house in the Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The animals were kept in standard cages with good ventilation at ambient temperature, and fed with normal laboratory chowed and distilled water *ad libitium* for 2 weeks. Animals were kept on 12-hours light and dark cycles, and Rat handling and treatment were conform to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Experimental Design: Experimental Animals were grouped into five-(5) groups of six animals each; group A served as positive control received food and distilled water; group B received CCL4 Only served as negative control; group C received CCL4 for 1 week and treated with 150mg/kg of Ethanolic leaf extract of *Newbouildae laevis* (E.N.L); group D received CCL4 for 1 week and treated with 300mg/kg of E.N.L; and group E received CCL4 for 1 week and treated with 600mg/kg of E.N.L. The leaf extract of N. *laevis* was administered orally for 21 days.

Induction of Reno-toxicity: Induction Reno-toxicity by CCL4 was done by injecting 1ml of CCl4 and Olive oil in the ratio of 1:1 through intraperitoneally route twice a week (Adewale *et al.*, 2014).

Sample Collection: Animals were anaesthetized with chloroform in an enclosed container 24 hours after the last administered dose of the E.N.L, blood was collected using heparinized capillary tube and put in a plain container, and centrifuge using centrifuge (England) and serum were retrieved and used for serum MDA, SOD, CAT, GSH, GPx, and TAC.

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Measurement of MDA was achieved according to the method of Buge and Aust (1978), in which lipid peroxidation was achieved by mixing 1ml of sample serum and 2ml of Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), and HCL solution thoroughly for 15-minutes in boiling water bath. After cooling, the precipitate was removed and centrifuge at 300rpm for about 10 minutes. The absorbance was determined at 535nm against reagent blank, which having all reagent excluding the serum. MDA level was calculated as: Absorbance/1.56 x10.

CAT activity was assayed by measuring the degradation rate of H2O2 using Beutler's method. The rate of disappearance of H2O2 was monitored spectrophotometrically at 230 nm. The assay medium consisted of 50 µl 1 M Tris HCI buffer (pH 8), 930 µl 10 mM H2O2, 930 µl deionized water, and 20 µl serum sample. One unit of CAT activity is defined as the amount of enzyme causing about 90% destruction of the substrate in 1 minute in a volume of 1 ml. CAT activity in the serum was expressed as U/ml (Beutler 1984).

SOD activity were determined as described by Beyer and Fridovich (1987). This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetra-zolium chloride) to form a red formazon dye. SOD activity is then measured by the degree of inhibition of this reaction.

GSH activity was determined by the method of Fitri et al., (2016). A total of 200µL of standards and samples were added to the cuvettes. Then 200µL of chromogen was added to each cuvette, and 200µL of the enzymes was added to each of the cuvette, mixed, and then incubated at room temperature for 5 minutes. A total of 200µL of NADPH was added to each cuvette. Changes in absorbance at 412nm for 3 minutes were recorded and observed.

GPx activity was determine by the method of Rush and Sandiford (2003). 0.2ml each of EDTA, sodium azide, GSH, H202, serum sample were mixed and incubated at 37°C for 10-minutes. The reaction was arrested by addition of 0.5ml of TCA and tubes were centrifuged. To 0.5ml of supernatant, 3ml of phosphate solution, and 1 ml of DTNB were added and the color developed was read at 420nm immediately using spectrophotometer. GPx activity was expressed U/ml.

TAC activity was estimated according to the method described by Rubio et al., (2016).

Data analysis: Data obtained were subjected to SPSS version 25. ANOVA was used to analyzed the result (serum MDA, SOD, CAT, GSH, GPx, and TAC) followed by multiple comparism using post HOC Turkey HSD. Values were presented as MEAN□STD, and data were considered significant at $p<0.05$.

4. RESULTS

Table 4.1 Effect of E.N.L on Malondialdehyde, Superoixde-dimutase, and Catalase following CCL4 induced oxidative stress

Groups	Malondialdehyde (nmol/ml)	Superoxide-dismutase (U/ml)	Catalase (U/ml)
	MEAN±STD	MEAN±STD	MEAN±STD
Group A (control)	1.51±0.01$_{NS}$	14.92±0.57**	79.27±0.73$_{NS}$
Group B (CCL4 Only)	1.60±0.01	13.37±0.06	79.12±0.24
Group C (CCL4 for 1 week and treated with 150mg/kg of E.N.L	1.71±0.01**	9.95±0.27***	54.91±5.63***
Group D (CCL4 for 1 week and treated with 300mg/kg of E.N.L	1.92±0.04$_{NS}$	6.790±0.64***	56.78±0.44***
Group E (CCL4 for 1 week and treated with 600mg/kg of E.N.L	1.93±0.03**	6.45±0.58***	51.48±1.17***

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$*

E.N.L = Ethanolic leaf Extract of *Newbouildae laevis*

Table 4.1 result showed a non-significant ($p>0.05$) increase in MDA level in-group B when compared to group A; group C and E had a significant ($p<0.05$) increase in MDA level when compared to group B, and group D had a non- significant ($p>0.05$) increase in MDA level when compared to group B.

Superoxide-dismutase result showed a significant ($p<0.05$) decrease in-group B when compared to group A; group C, D, and E had a significant ($p<0.05$) decrease in SOD level when compared to group B.

Catalase result showed a non-significant ($p>0.05$) decrease in-group B when compared to group A; group C, D, and E had a significant ($p<0.05$) decrease in Catalase level when compared to group B.

Table 4.2 Effect of E.N.L on Glutathione, Glutathione Peroxidase, and Total antioxidant capacity following CCL4 induced oxidative stress

Groups	Glutathione (U/ml)	Glutathione Peroxidase (U/ml)	TAC (U/ml)
	MEAN±STD	MEAN±STD	MEAN±STD
Group A (control)	17.67±0.55***	0.84±0.06**	779.62±9.59*
Group B (CCL4 Only)	12.27±0.15	0.47±0.01	499.18±0.84
Group C (CCL4 for 1 week and treated with 150mg/kg of E.N.L	13.83±3.72$_{NS}$	0.63±0.26$_{NS}$	741.12±150.00**
Group D (CCL4 for 1 week and treated with 300mg/kg of E.N.L	16.23±0.25**	0.75±0.06*	633.38±56.84$_{NS}$
Group E (CCL4 for 1 week and treated with 600mg/kg of E.N.L	12.04±0.54$_{NS}$	0.58±0.01$_{NS}$	679.26±1.15$_{NS}$

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$*

E.N.L = Ethanolic leaf Extract of *Newbouildae laevis*

Table 4.2 result showed a significant ($p<0.05$) decrease in glutathione level in-group B when compared to group A; group C had a non-significant ($p>0.05$) increase, while group D had a significant ($p<0.05$) increase and group E had a non-significant ($p>0.05$) decrease in glutathione level when compared to group B.

Glutathione Peroxidase result showed a significant ($p<0.05$) decrease in-group B when compared to group A; group D had a significant ($p<0.05$) increase, and group C and E had a non-significant ($p>0.05$) increase when compared to group B.

Total antioxidant capacity result showed a significant ($p<0.05$) decrease in-group B when compared to group A; group C had a significant ($p<0.05$) increase, and group D and E had a non-significant ($p>0.05$) increase when compared to group B.

5. CONCLUSION

The antioxidants system is an important system that plays a major role in the replenishing of the oxidative damage cause by lipid peroxidation of chemicals.

At the medium dose of 150mg/kg and 600mg/kg, it shows that E.L.N has antioxidant effect as there was significant increase in the level of MDA. And also it negatively affected the antioxidant levels as it significantly decrease the levels of Superoxide-dismutase and Catalase across the low dose, medium dose and high dose groups (150mg/kg, 300mg/kg and 600mg/kg), this shows that E.L.N had a negative impact by bring down the levels of SOD and Catalase.

The administration of E.L.N at medium dose showed that it promoted the level of Glutathione while at low dose its increase was not significant. Also in the high dose group (group E), there was a significant decrease in the level of glutathione.

At medium dose (Group D), Administration of E.L.N showed significant increase in the level of Glutathione Peroxidase when compared to group B but the increase recorded in group C and E was not significant.

Administration of E.L.N at low dose (150mg/kg) had significant increase in the level of Total antioxidant while the increase recorded in groups D and E was not significant.

The study shows that the use of E.L.N has a mild to moderate positive effect in increasing the antioxidant level and hence should be used with caution.

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