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Antioxidant Properties of *Carica papaya* on Cadmium Toxicity on Prefrontal-Cortex of Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HBA and ODO designed the study and wrote the final manuscript. Authors MNN and ARO performed the statistical analysis. Authors EO and AAA wrote the first draft of the manuscript. Authors FEO and PKO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cadmium is a carcinogenic agent in humans, and therefore, a cancer causing substance. In the body it is extensively circulated, but it is bound mainly to erythrocytes. Though cadmium binds to various macromolecules and proteins in the body, it is not well metabolized. Cadmium intensifies oxidative damage in the nervous system (and various other organ systems) thereby causing harm. The damage results from an increase in production of reactive oxygen species and inhibition of antioxidant enzymes as shown by previous researches. This study investigated possible effects of *Carica papaya* as an antioxidant in the damage of the brain caused by cadmium.

Methods: Thirty Wistar rats weighing between 120 g-180 g of both sexes were divided into six groups of five animals each and used in this study. Six groups were induced intraperitoneally with a single dose of 3.5 mg/kg body weight of cadmium sulphate. Two out of these six groups were treated with high and low doses of *Carica papaya*, 250 mg/kg and 400mg/kg body weight respectively for the period of four weeks. After sacrifice, enzyme assays were carried out on the tissue supernatant homogenates.

Results: The investigation showed that MDA level of activities decreased in the prefrontal cortex supernatant of groups 1, 2 and 6 animals and an increase in the MDA level of activities were observed in groups 4 and 5 rats which was significant at $p<0.05$. There was a decrease in catalase activities in groups 2 and 3 rats, also, an increase in CAT activities in groups 4 and 5 rats, the decrease was significant in groups 2 and 3 animals as compared with the control group 1 rats. GST activities decreased in groups 2, 3, 4, 5 and 6 rats and the decreased activities were significant at $p<0.05$ when compared with control group 1 animals. Progressive increase in the superoxide dismutase was observed in groups 1, 2, 3, 4, 5 and 6 rats and this increase in activities was found significant at $p<0.05$ in groups 4, 5 and 6 rats when compared with the control group 1 rats.

Conclusion: The present study has justified the ameliorative properties of *Carica papaya in* scavenging the free oxygen radicals and supplement the cellular antioxidant systems.

Keywords: Carica papaya;cadmium;prefrontal cortex;antioxidant.

1. INTRODUCTION

The brain is the control center for registering sensations, correlating them with one another and with stored information, making decisions, and taking actions. The adult brain consists of four major parts: brain stem, cerebellum, diencephalon, and cerebrum. The brain stem is continuous with the spinal cord and consists of the medulla oblongata, pons, and midbrain. Posterior to the brain stem is the cerebellum. Superior to the brain stem is the diencephalon, which consists of the thalamus, hypothalamus, and epithalamus. Supported on the diencephalon and brain stem is the cerebrum, the largest part of the brain [1,2].

The prefrontal cortex (frontal association area) is an extensive area that lies anterior to the precentral area in the anterior portion of the frontal lobe that is well-developed in primates, especially humans. It includes the greater parts of the superior, middle, and inferior frontal gyri; the orbital gyri; most of the medial frontal gyrus; and the anterior half of the cingulate gyrus (Brodmann areas 9, 10, 11, and 12). Large numbers of afferent and efferent pathways connect the prefrontal area with other areas of the cerebral cortex, the thalamus, the hypothalamus, limbic system, cerebellum and the corpus striatum [3]. It is an area of the cortex that is capable of associating experiences that are necessary for the production of abstract ideas, judgment, emotional feelings, and personality [4].

Cadmium is a heavy metal, a member of group IIb in the periodic table of elements. It is a non-essential trace element, naturally occurring in Zinc and Lead ores and in some rock phosphate fertilizers [5,6,7]. Industrial uses of the metal and agricultural activities have now led to its widespread dispersion at trace levels into the environment and human foodstuffs [8, 9, 10]. Cadmium is a major health problem, mostly because of its long half-life (15 to 20 years) and persistence in the environment and a variety of tissues [11]. Generally, cadmium binds strongly to organic matter where it will be immobile in soil and be taken up by plant life, eventually, entering the food supply [12]. It has been reported, that Cd mostly increases inhibition of complexes II and III of the mitochondrial electron transfer

chain in the liver, brain and heart tissue. It has been concluded that an early exposure of Cd may produce alterations in the development of different lipids, which may produce CNS dysfunctions with a possibility of being manifested even in later life [13].

Inhibition of the cellular antioxidant defense causing oxidative stress is a mechanism of action of cadmium. Short-term exposure to cadmium has been revealed to decrease the activities of almost all of these antioxidant enzymes in vitro and in vivo, whereas with more elevated doses and extended exposure also enhancement of activities was found, probably because of adaptive induction of genes. The decrease in the activity and/or intracellular levels of antioxidants caused by cadmium, together with the generation of radicals that are produced during normal metabolism, may explain the increase in lipid peroxidation and DNA damage in cells [14].

Membranes of neurons contain numerous phospholipids, made up of polyunsaturated fatty acid esters, which are very responsive to attacks by ROS, resulting in a chain reaction which produces lipid peroxidation and causes extensive damage to neuronal membrane. It is well established that oxidative stress is closely associated to the pathology of a range of neurodegenerative diseases, including age-associated disorders [15,16,17].

Carica papaya (family Caricaceae) originated in Central America. Papaya is a rapid-growing, semi-woody plant found in tropical regions. The means of reproduction of pawpaw is quite complex. The plants are male, hermaphrodite, or female [18]. Pawpaw fruits are rich in antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, panthotenic acids and minerals such as potassium and magnesium, the fruit is also a good source of fibre. The extract of *Carica papaya* is also known to have antioxidant properties [19]. It aids in the prevention of colon cancer. Beta carotene which helps to mop up free radicals that cause various forms of cancer, thereby preventing, is found in papaya. Studies have shown that it also helps to prevent the occurrence of diabetic heart disease [20].

In Australia, it is believed in some quarters that several cancer diseases can improve after drinking papaya leaf extract. Papaya is used in tropical folk medicine. According to Reed [21], papaya latex is very much useful for curing dyspepsia and is externally applied to burns and scalds. A diet that includes papaya fruit has been observed to lessen oxidative stress and change lipid profiles [22]. Consequently, the threats of diseases such as cancer and cardiovascular diseases, which result from the activities of free radicals, are reduced [22]. The nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and E and an excellent source of vitamin C (ascorbic acid). Many of the metabolites from these medicinal plants especially flavonoids exhibited potent antioxidant activity *in vitro* and *in vivo* [23, 24, 25]. The objective of this current study was to evaluate the antioxidant properties of *Carica papaya* extract in cadmium induced prefrontal cortex toxicity through a critical measurement of antioxidant enzymes activities in the brain tissue such as superoxide dismutase, catalase, MDA and GST.

2. METHODS

2.1 Preparation of Extract

A fresh mature unripe *Carica papaya* (pawpaw) fruit weighing 1518 g was collected in April 7th, 2014 and was purchased from Kuchikau town, Nasarawa state, Nigeria, and taken to the Biology Department of Bingham University, Nigeria for authentication. It was peeled and the seeds discarded. The regimen was cut into pieces and 1000 g was weighed. The weighed regimen powderwas then percolated in 1000ml of distilled water. The mixture was left to soak for 72 hours (3 days) at room temperature. After the 72 hours, it was sieved and the residue was weighed. The weight of the residue was 570 g, and the supernatant was 1150 ml. The supernatant was then stored in the refrigerator till when it was needed at three (3) days after regimen preparation [26].

2.2 Experimental Animals

30 male Wistar rats weighting between 120 g-180 g were used for this research work. The rats were randomly selected into six groups as follow 1, 2, 3, 4, 5 and 6, each group containing five rats. They were kept in the animal house of Bingham University, Nigeria and given water and feed twice daily. Their beddings were changed routinely to maintain a conducive environment for the rats. The treatment for the various groups was administered orally. All experimental investigations were done in compliance with

humane animal use as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub. No NIH 86–23 [27] and in accordance with ethical approval of the Anatomy Department, Bingham University, Karu, Nigeria.

2.3 Administration of Chemical and Treatment

The cadmium stock solution was made by dissolving 10 mg of Cadmium sulphate salt in 5 ml of 0.9% w/v phosphate buffer. The cadmium stock solution was administered intraperitoneally in doses corresponding to the weight of the rats using 1 ml insulin syringes.

2.4 Procedure of Animal Sacrifice

The rats were sacrificed through euthanasia via stonig gun (it is used to make wild animals unconscious for observation or treatment as the case may be, mostly in the field of zoology and vetinary medicine). The skulls were dissected and the brain tissues were removed. The brain tissues were submerged in sucrose solution. The brain tissues for biochemical analysis were placed in 5% sucrose solution, centrifuged at 4000 rpm for 10 minutes and the supernatant collected. The supernatants obtained were used to carry out by first principle methods with the use of appropriate salts for enzyme activities such as superoxide dismutase (SOD) [28], Glutathione S-transferase (GST) [29], Malondialdehyde (MDA) [29] and Catalase [30] spectrophotomerically with the use of spectrophotometer.

2.5 Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (S.E.M), One- Way ANOVA using Medical software packages to determine the level of significance $P < 0.05$ (95% CI) was taken as the significant level.

3. RESULTS AND DISCUSSION

This present study shows that cadmium caused a significant decrease in malondialdehyde (MDA) activity in Groups 2, 3 and 6 animals' brain tissue which was significant at $P < 0.05$ and an increase in MDA activities was also observed in groups 4 and 5 rats but were not significant at $P < 0.05$. It also produced a resultant decrease glutathione S-transferase (GST) in groups 2, 3, 4, 5 and 6 animals and catalase (CAT) activities was observed to decrease in groups 2 and 6 rats, which was increased in groups 3, 4 and 5 animals, while superoxide dismutase (SOD) activity increased across groups 2, 3, 4, 5 and 6 rats, though these increments were only significant at $P < 0.05$ for animals in group 5 and 6. Ognjanović [31] reported a significant increase in lipid peroxidation in animals exposed to cadmium. Superoxide dismutase (SOD) and catalase (CAT) activities were also reported to be decreased ($P < 0.05$ and $P < 0.005$) in the same experiment, whereas glutathione S-transferase (GST) increased.

The enzyme activities of GST and Catalase increased in group 3, treated with vitamins C and E, compared to their activities in groups of rats induced only with cadmium. This suggests that the vitamins have protective action against free radicals. Previous researches have shown that non enzymatic antioxidant components which consists of molecules such as alpha tocopherol (Vit. E), ascorbic acid (Vit. C), glutathione and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions [32]. Whereas, there was a significant decrease ($P < 0.05$) in MDA activity and there was a significant increase at $P < 0.05$ in SOD activity.

In group 4 animals treated with high dose of *Carica papaya*, there was significant increase in the activities of MDA, SOD and CAT, while there was a significant decrease in enzyme activity of GST. This is in agreement with previous studies which reported that, *Carica papaya* contains antioxidant phytochemicals, such as vitamin C, betacarotene, lycopene and vitamin E all of which act as antioxidants and subsequently decrease the need forexternal consumption of these antioxidant enzymes to combat oxidative stress [33,34,35,36,37]. In an earlier study, the presence of alkaloids, flavonoids, saponin, tannin, anthraquinones, and anthacyanosides in *Carica papaya* extract was reported [38]. Also, previous independent studies have reported that the protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities [39]. The presence of these active biological principles may thus be accounting for the biological effect of *Carica papaya* extract and could be via antioxidant and/or free radicals scavenging activities. Group 5 rats treated with

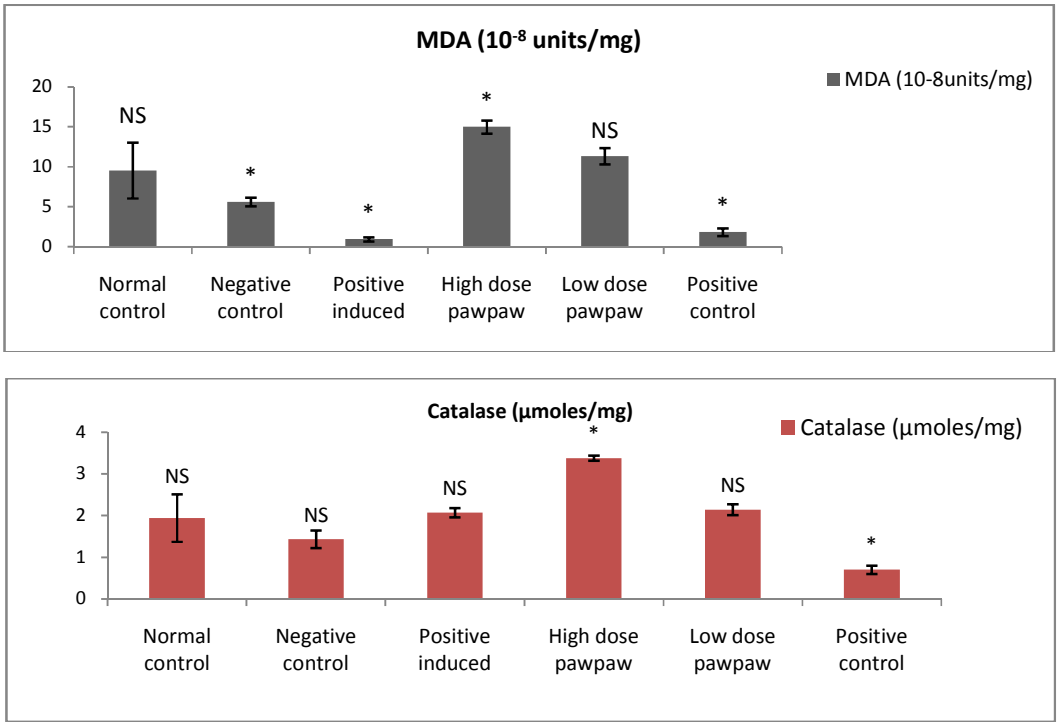

Fig. 1. Bar chart showing MDA and catalase enzyme activity in the prefrontal cortex of wisterrats

**indicates statistically significant mean difference when the value is compared to the control at p<0.05. NS indicates no significance*

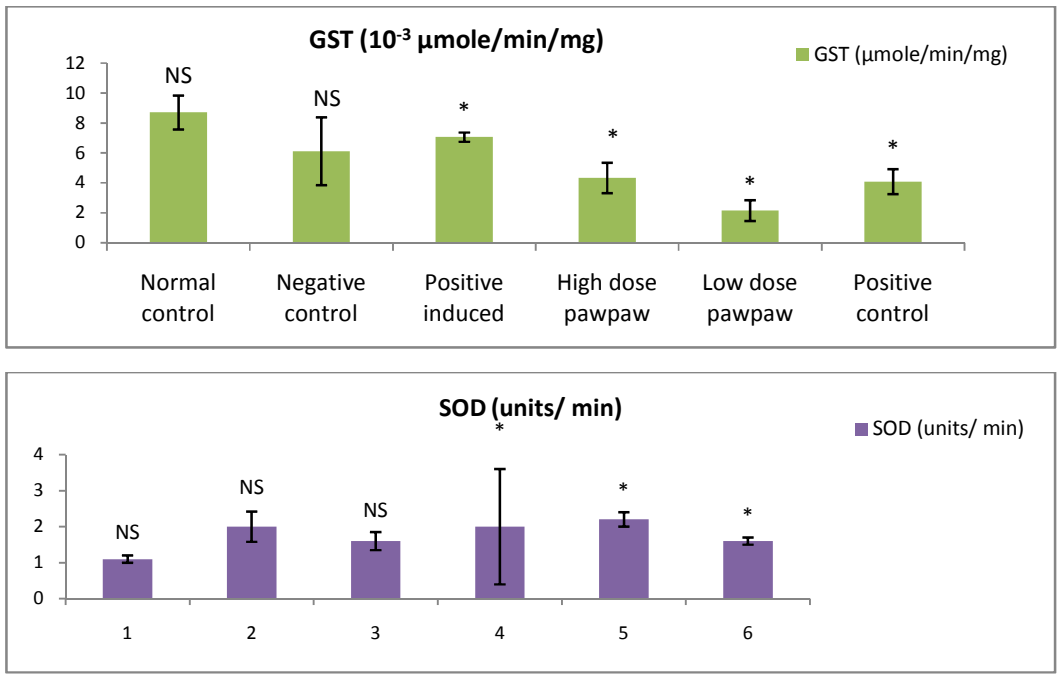

Fig. 2. Bar chart showing GST and superoxide dismutase enzyme activity in the prefrontal cortex of wistarrats

**indicates statistically significant mean difference when the value is compared to the control at p<0.05. NS indicates no significance*

low dose of *Carica papaya* also showed significant increase in SOD, also a non-significant increase in MDA and CAT activities and a significant decrease in GST activity.

Metabolites and enzymes scavenging reactive oxygen species (ROS) are part of the protective mechanism employed by living organisms to protect themselves from deleterious effects of ROS. Studies have shown that the activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase have been affected by cadmium [40,41,42,43,44]. Under normal conditions, free radicals are formed in minute quantities and are rapidly scavenged by natural cellular defense mechanisms comprising of enzymes like low dose of *Carica papaya* also showed significant increase in SOD, also a non-significant increase in MDA and CAT activities and a significant decrease in GST activity.

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Phenolics found in plants and their products have established significant interest over the years because of their probable ability to prevent lipid peroxidation, characterized by MDA production, and diseases associated with it [45].

There was significant decrease in MDA, SOD, GST and CAT enzyme activities of animals in group 6 animals.

4. CONCLUSION

It can be ascertained that *Carica papaya* contains antioxidant constituents that might help to supplement cellular enzyme complex activities in brain tissue. It can then be said that *Carica papaya* has bioactive constituents that can help to scavenge reactive oxygen species generated by cadmium, and as a result prevent oxidative stress and cellular damage.

CONSENT

Not applicable because experiments were carried out on animal models andall experimental investigations were done in compliance with humane animal use as stated in the “Guide to the care and use of Laboratory Animals Resources”. National Research Council, DHHS, Pub. No NIH 86 – 23 [27] and in accordance with ethical approval of the Anatomy Department, Bingham University, Karu, Nigeria.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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