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The Role of Aqueous Extracts of *Annona Muricata* Linn in Regulating the Levels of Oxidative Markers in Caffeine-induced Brain Toxicity in Wistar Rats

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Abstract:

*This is an ethno pharmacology study in which the effect of leaves of soursop (*Annona muricata* Linn) was undertaken on caffeine –induced brain toxicity in wistar rats. Forty-wister rats of both sexes weighing 90- 250g were procured for the study. The rats were divided into 8 groups of 4 rats per gp, in which gp 1 served as the control, gps 2-7 served as the study or treatment gp while gp 8 received standard drug (lorazepam). All drug administration was by oral gavage and study lasted for 15 days after which the rats were sacrificed under chloroform anesthesia. The brain tissues were dissected and homogenized for estimation of superoxide dismutase (SOD) and malondialdehyde (MDA) levels and the rest for histological studies. Results showed that there were alterations in the histoarchitecture of the brain tissues i.e., distorted pyramidal cells with vacuolated cytoplasm, irregular pyramidal cells with darkly stained nuclei. Furthermore, *Annona muricata* demonstrated a significant difference in the oxidative stress makers (SOD and MDA) between the control and the drug treated groups. We conclude that *Annona muricata* leaves extract can efficaciously and effectively counter oxidative stress in caffeine –induced brain toxicity in rats.*

Keywords: Caffeine brain toxicity, antioxidant, SOD, MDA

1. Introduction

Traditional African herbs contain many useful compounds that can be used in the treatment of many chronic diseases. Numerous reports suggest that traditional herbs have the potentials for preventing pathological outcome of some neurodegenerative diseases, cancers, drug- induced brain disorders etc., as most of the active principles of some useful drugs have been initially isolated from plants. Furthermore, most of the herbal drugs are a mixture of a number of plants or parts of the same plant ingredients whose cumulative effects increase their efficacy in curing diseases as well as reducing toxicity (Manomani et al, 1995). One such plant is *Annona muricata* Linn, called soursop due to the sweet and sour flavor of the large fruit, is a low and tropical fruit-bearing tree in the Annonaceae family, with long history of traditional use. It is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world (Dalziel, 1955). This plant is a species of the family Annonaceae, order Magnoliales and division Magnoliophyta (Farombis, 2003). The genus *Annona* comprises over 70 species among which *Annona muricata* is the most widely used (Hutchison and Dalziel, 1963; Arbonier, 2004)

Soursop is native to tropical central and South America and the Caribbean but is now widely cultivated in tropical areas world-wide, including Southern Florida and Southeast Asia (Farombis, 2003).

Soursop is a slender, small and cold-intolerant tree, generally reaching heights of 4-6m, it flowers and bears fruit 3-5yrs after planting. The leaves are glossy, dark green, and generally evergreen, with a distinctive odour (Dalziel, 1955).

Its fruits are the largest of the *Annona* species, 20-30cm long often weighing 4.5kg. The fruits have green incredible skin, with many soft, curved spines, but the white juicy pulp is edible (Farombis, 2003). It has an aroma of pineapple but a sour or musky flavor, it is eaten fresh or used for making juice and other beverages, custards, etc., (Ekaluo et al, 2016).

Soursop, also referred to as graviola has numerous traditional medicinal uses in South America and the Caribbean, and has become nutritional medicinal supplement. The fruit, seeds, leave bark and roots have been used to treat intestinal parasites, coughs, asthma, bronchitis, liver ailments, inflammation, diabetes, hypertension, etc., (Ekaluo et al, 2016). The seeds are also insecticidal and the preparation from the leaves has been used to kill head-lice and bedbugs (Ekaluo et al, 2016). Further studies have shown that the extracts of graviola have antiviral, antiparasitic, antirheumatic, anti-inflammatory and antihypertensive properties (Adewole and Ojewole, 2009). In addition to the health benefits of the Annonaceae family, soursop also contains small amounts of neurotoxic alkaloids such as annonacin which appear to be linked to atypical parkinsonism and other neurological effects when consumed frequently and in large quantities ((Gyamfi et al, 2010)

Soursop leaves are rich in several compounds including proteins, calcium, fructose, vit A and B hence the leaves have excellent medicinal properties making them important ingredients in ethnomedicine. The leaves are employed in treating cystitis, diabetes, headaches and insomnia. Internal administration of the decoction exhibits antirheumatic and neurologic effects while the boiled leaves are applied topically to treat abscess and rheumatism (Mishra et al, 2013, Ekaluo et al, 2016). The fruit is used as natural medicine for arthritic pain, neuralgia, arthritis, diarrhea, skin rashes, worm infestation and milk let down after delivery (Ekaulo et al, 2016).

Caffeine (1, 3, 7-trimethyl xanthine) is the most commonly ingested pharmacologically active and psychoactive substance, found in beverages, in products containing cocoa and in some medications. Caffeine consumption alters circulating androgen levels in man (Ekaluo et al, 2016). It is a central nervous system stimulant (Graham, 2001), and once it reaches the blood stream, spreads throughout the entire system: respiratory, musculoskeletal, cardiovascular, renal and the central nervous system (Battram et al, 2008). Caffeine increases adrenergic discharge, elevating the heart rate, constricting blood vessel and thereby increasing the blood pressure (Battram et al, 2008). In the central nervous system, caffeine stimulates the release of excess serotonin in the brain which increases sympathetic activity and decreases neuronal inhibitor activity (Battram et al, 2008).

The purpose of this study is to explore the possibility of *Annona muricata* leaves extracts' effectiveness in countering caffeine- induced brain toxicity in the society which has reached an alarming rate. The orthodox treatment of this malady has not produced the desired results due to non-availability of drugs, poor economic presentation, treatment failures, etc. The resort to ethnomedicine becomes an alternate imperative using aqueous extracts of the leaves of soursop on caffeine-induced brain toxicity model in rats.

2. Materials and Methods

Fresh leaves of *Annona muricata*, harvested from the same tree were used for the study. The leaves were identified by the Taxonomist in the Botany Department of ESUT where a voucher specimen ESUT/BOT/168 is deposited in the herbarium. The fresh leaves of *Annona muricata* were washed with tap running water, dried in the laboratory at room temperature for two weeks before being ground into powder.

500g of the powdered *Annona muricata* sample was soaked in 2 litres of distilled water for 24hr. The mixture was stirred every 8hr using sterile glass rod. The soaked sample was filtered using a Whatman No 1 filter paper at room temperature. The filtrate was concentrated using rotary evaporator at 40° C, and finally dried in a water bath until the extract became completely solid. It was weighed and stored in refrigerator until required for the study and the yield was 65%.

2.1. Drugs

The following drugs were used: Caffeine (Sigma Chemical Co, USA), Lorazepam, Wyeth USA, all were of reagent grade.

3. Experimental Animals

Forty adult albino rats of either sex, weighing 90-250 (mean 100.0 \pm 1.5g) were used for the study. The rats were procured from the Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria and quarantined for 2 weeks for acclimatization before use

The animals were housed in conventional wire mesh cages under controlled temperature condition of 28° C, 50% humidity and (12hour light/12hour dark) cycle. The rats had free access to water and were fed on standard livestock pellets from (Guinea feed Nig Ltd).

Experimental design:

The rats were weighed again and divided into 8 experimental groups of 4 rats each.

(a) Control

GP1- served as the control receiving only water and standard food pellets

GP2 - received 200mg/kg caffeine per oral using cannula

(b) Prophylactic or protective effect of the extracts

GP 3- received 200mg/kg extract + 200mg/kg caffeine per oral using cannula

GP4- received 300mg/kg extract +200mg/kg caffeine per oral using cannula

GP -5received 400mg/kg extract +200mg/kg caffeine per oral using cannula.

(c) Effects of the extract on rats pretreated with Caffeine

GP 6- received 200mg/kg caffeine + 300mg/kg extracts per oral using cannula.

GP7 received 200mg/kg caffeine +300mg/kg extracts per oral using cannula

GP- 8 received 200mg/kg caffeine +1mg/kg lorazepam per oral using cannula

All the rats in the various cages had free access to food and water *ad libitum* and were kept for the next fifteen days.

On the 15th day of the study the rats were weighed again and sacrificed under chloroform anesthesia. The brains were cut open and tissues for SOD (Super Oxide Dismutase) and MDA (Malondialdehyde) analysis were homogenized and preserved in sucrose solution.

4. Results

Gp	Superoxide dismutase SOD g/mol	Malondialdehyde MDA g/mol	Remarks
1	479.1 ± 3.3	0.29 ± 0.05	Only water and food
2	117.9 ± 2.76	12.66 ± 0.07	Only 200mg/kg caffeine
3	208.73 ± 3.03	2.44 ± 0.07	200mg/kg extract and 200mg/kg caffeine
4	313.87 ± 3.07	1.52 ± 0.35	300mg/kg extract and 200mg/kg caffeine
5	332.67 ± 3.07	0.91 ± 0.07	400mg/kg extract and 200mg/kg caffeine
6	170.47 ± 4.26	8.91 ± 0.07	200mg/kg caffeine and 300mg/kg extract
7	121.27 ± 3.95	10.16 ± 0.14	200mg/kg caffeine and 300mg/kg extract
8	191.83 ± 3.10	4.03 ± 0.04	200mg/kg caffeine and 1mg/kg lorazepam , standard drug
			<u>Note:</u> all the animals had free access to food and water <i>ad libitum</i> drug administration was per oral via canular.

Table 1: Levels of SOD and MDA in the Frontal Lobe of Caffeine Induced Toxicity in Rats

Caffeine alone caused a fall in SOD of 75.40% and a rise in the MDA of 4272.4% to cause toxic stress. In the same manner lorazepam produced a rise in SOD by 62.7% and fall in MDA of 68.17%. On the other hand, *Annona muricata* produced similar results in caffeine-treated rats i.e., SOD increased by 44.59% and a reduction in MDA of 29.62%.

MDA levels in rats treated with a constant dose of caffeine (200mg/kg) and varying doses of *Annona muricata* (200, 300, 400mg/kg) had fall in the levels of MDA: 2.44, 1.32 and 0.91mg/ml respectively. When the level of caffeine is constant (200mg/kg), the level of SOD falls in a progressive manner from 271 to 157 gm/mol. (see Table 1).

5. Discussion

Caffeine (1,3,7 –trimethyl xanthine) is the most widely consumed stimulant drug in the world (Shannon, 2007). It is present in a variety of forms: medications, coffee, tea, soft drinks and chocolate. It is commonly used to keep awake and alert. It is technically a drug and the recommended daily intake for healthy adults is 400mg, (Shannon, 2007). Caffeine is a fast acting stimulant that acts on the central nervous system to increase the blood pressure, heart rate and boost energy (Marx et al, 2010). Caffeine acts to increase the activity of reticular formation hence its ability to maintain wakefulness (Hitner and Nagle, 1994).

Antioxidants attack free radicals, waste products created when the body converts food into energy. Free radicals cause harmful chemical reaction that can damage cells in the body making it difficult for the body to fight infections. They also damage organs and tissues (Shannon, 2007)

In the present study, before caffeine administration superoxide dismutase (SOD) activity dropped by 75.4% while malondialdehyde, (MDA), activity increased by more than 4000% causing toxic stress. Superoxide dismutase (SOD) enzyme, acts as antioxidant and protects cellular components from being oxidized by reactive oxygen species formed as a result of toxic stress. During increased levels of oxidative stress SOD concentration increased with the degree of stress conditions. SOD out-competes damaging reactions of superoxide thus protecting the cell from superoxide toxicity. The main reactions are with itself (dismutation) or with another biological radical such as nitric oxide.

Results showed that MDA contents of caffeine-induced toxicity were significantly higher than those of the control at the same time point, while the SOD activities were significantly lower than those of the control group. For the extract-treated group (*Annona muricata*), MDA contents were significantly reduced and SOD activities increased significantly in caffeine-induced toxicity.

MDA is the end product of lipid peroxidation, while SOD is a biologically active substance that could eliminate harmful substances generated during metabolic processes. Their changes indicated that *Annona muricata* administration could effectively counter oxidative stress in caffeine-induced brain toxicity in rats.

Lorazepam, a benzodiazepine acts on the limbic system to produce its effects i.e., increasing the SOD level by 62.71% and reducing the MDA level by 68.17% in the caffeine-treated rats. On the other hand, *Annona muricata* extract produced similar results in caffeine treated rats i.e., SOD increased by 44.59% and a reduction in MDA of 29.62%. The difference between the effect of the standard drug lorazepam and the extract is not significant showing that *Annona muricata* extract might be acting on the limbic system. The limbic system is involved with emotional and behavioural response of the body associated with reward and punishment (Hitnar and Nagle, 1994), sexual behavior, anger, ruga, fear, anxiety and is important for mutual health. Lorazepam exerts a selective effect on the limbic system and is used for the treatment of certain behavioral and emotional disorders.

6. Conclusion

Annona muricata Linn has a role in regulating the levels of oxidative markers in caffeine-induced brain toxicity in wistar rats which can be explored in human

7. Competing Interests

The authors declare that they have no competing interests.

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