Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(3): 849-861, June 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Effect of *Stachytarpheta cayennensis* essential oil on liver function and oxidative stress markers in Calabar, Nigeria

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Received: 06-03-2024 Accepted: 29-04-2024 Published: 30-06-2024

ABSTRACT

The effect of essential oil from the leaves of *Stachytarpheta cayennensis* (a potential bio-pesticidal agent) on some biochemical indices of Wistar rats was tested to determine the safety of this oil on non-target organisms. Twenty-eight (28) albino Wistar rats weighing between 150-180g were divided into seven groups of four rats each. Group A served as negative control, B_1, B_2, and B_3 were exposed to 500 mg, 300 mg and 150 mg/kg body weight of a synthetic insecticide (BNC) while S_1, S_2, S_3 were exposed to 5000, 3000 and 1500 mg/kg body weight of *Stachytarpheta cayennensis* essential oil. After twenty-eight days, blood samples were collected and tested for liver function markers: aspartate transaminase, alanine transaminase, alkaline phosphatase, and oxidative stress markers: superoxide dismutase, catalase, glutathione peroxidase, and hydrogen peroxide. Liver enzyme activities were higher ($p < 0.05$) in the BNC exposed rats compared to the control and essential oil exposed groups. Superoxide dismutase (SOD) and catalase (CAT) activities decreased significantly ($p < 0.05$) while glutathione peroxidase and hydrogen peroxide concentrations increased in the BNC exposed groups relative to the control group. The essential oil was milder in its effects on tested biochemical indices, and may hold better promises than the synthetic pesticide in terms of safety.

Keywords: Biopesticide, Toxicity, Insecticide, Botanicals, Environment. Sustainable.

INTRODUCTION

Recent attention on essential oils have focused on their broad range of bioactivities on pests, and disease-causing organisms, thus their potential use as alternatives to synthetic chemical insecticides for crop protection and other pest management practices. It is interesting to note that the molecules within the oils that cause these effects are usually non-toxic for mammals (Adanlemegbe et al., 2023). Essential oils also have medicinal applications due to their therapeutic properties, as well as

DOI : https://dx.doi.org/10.4314/ijbcs.v18i3.10

agro-alimentary uses because of their antimicrobial and antioxidant effects. Recently, the value of essential oils in human medicine, as phytopharmaceuticals and in aromatherapy has increased and together with their widespread use in foods and beverages have defined their relative safety through both empirical practice and more rigorous experimental evaluations using animal models (Isman, 2020).

Insecticides are chemicals used to kill insects. They can cause poisoning, if swallowed, inhaled, or absorbed through the skin (Sousa et al., 2020). The properties that make insecticides deadly to insects can sometimes make them poisonous to humans. Most serious insecticide poisonings result from the organophosphate and carbonate contents of insecticides. Pyrethrins and pyrethroids which are other commonly used insecticides derived from flowers, are usually not very poisonous to humans. Synthetic insecticides have been associated with short term and long-term effects on human health, including elevated cancer risks and potential disruption of the body's metabolic function as well as the reproductive, immune, endocrine, and nervous systems. (Nicolopoulou-Stamati et al., 2016).

Human poisoning by pesticides has long been seen as a severe public health problem. As early as 1990, a task force of the World Health Organization (WHO) estimated that about one million unintentional pesticide poisonings occur annually, leading to approximately 20,000 deaths (Dunn et al. 2021). The scientific literature published between 2006 and 2018, supplemented by mortality data from WHO revealed that about 385 million cases of Unintentional Acute Pesticide Poisoning (UAPP) occur annually world-wide including around 11,000 fatalities. Based on a worldwide farming population of approximately 860 million. About 44% of farmers are poisoned by pesticides every year (Dunn et al., 2021). These insecticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin (USEPA, 2023). Therefore, the introduction of alternative; plant-based insecticides, has become a serious issue of national interest.

Pesticides are among the most extensively used chemicals in the world today and they are also among the most hazardous compounds to humans. A report from Aljazeera (2017) indicates that over 200,000 people are killed due to the toxicity of these dangerous chemicals every year. Although pesticides are intended to harm only the targeted pests, if not used correctly, they can also cause harm to people or the environment. Pesticide intoxication may be caused either by swallowing accidently, or by inhalation of fumes or by skin contact or accidental eye exposure (Makris et al., 2019). This has led to the search for more botanical pesticides with comparatively less toxicity than the synthetic insecticides conventionally used in the management and control of pests (Samb et al., 2023).

Sustainable chemistry advances innovations across all chemistry fields to design new and safe chemicals through efficient production processes, which address the challenges of protecting public health (Hao et al., 2021). Modern agriculture is negatively affected due to the rapid exploitation of natural resources, indiscriminate use of pesticide application, and frequent weather events influenced by climate change. Bio-control action is a significant tool for integrated pest management (IPM), it offers alternative and positive management techniques that are safer for humans and the environment (Rani et al., 2021). Natural pesticide products are available as alternatives to synthetic chemical formulations, but their safety or toxicity to humans is one issue that the current research seeks to investigate. Botanical pesticides are efficacious in managing different crop pests, inexpensive, easily biodegraded, have varied modes of action, their sources are easily available and have low toxicity to non-target organisms (Lengai et al., 2020). Moreover, conventional insecticides are based on a single active ingredient, while the botanicals contain mixtures of chemical compounds that can affect both behavioral and physiological processes. Thus, the chance of pests developing resistance to such botanicals is very low. It seems that seeking bio-pesticides which are efficient, as well as being suitable and adaptive to ecological conditions, is essential in obtaining sufficient pest control (Quattara et al., 2023). This has led to this research on botanical insecticides with comparatively less toxicity than the synthetic insecticides conventionally used in the management and control of pests.

The specie *Starchytarpheta cayennesis* is an erect, branching somewhat angular fibrous sub-shrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, a slightly wrinkled appearance, green color terminal inflorescence with linear stalks, sessile flower and a gamosepalous calyx (Adeneye et al., 2006). It is distributed throughout Brazil, Africa and Asia (Adeneye et al., 2006). It belongs to the *verbena* family and known by many common names, including blue snakeweed, and rough leaf vervain (English name). In Nigeria, it is known as *Truamure, (Yoruba), Aranumon (Ibibio/Efik), Wulsigi (Hausa), Okeanwundeohia (Igbo), Anano (Obudu)* (Encyclopedia, Science News and Research Reviews, 2023). Okonkwo and Onyeji (2018) amongst other researchers, had reported the insecticidal efficacy of this plant against some insect pests. The current research sought to analyze the effect of essential oil extracted from the leaves of *Stachytarpheta cayennensis* on some liver enzymes and oxidative stress markers of albino Wistar rats.

MATERIALS AND METHODS

Materials

All reagents and chemicals used were of analytical standard. BNC insecticide was manufactured by Golden dream commodity FZE, Lekki Free trade zone Lagos, Nigeria, with NAFDAC number: A5-0739 was used as a positive control. The active ingredients are: esbiothrin-0.26%, permethrin 0.28%, beta-cypermethrin 0.1% and lemon 0.31%. While distilled water (100% pure) was used as a negative control. Chemicals and reagents were procured from Agappe Diagnostics Limited, India.

Fresh leaves of *Stachytarpheta cayannesis* were harvested from a field in Esuk Atu village behind the University of Calabar Teaching Hospital Latitude: 4° 57′29.6″N and Latitude 8° 20′48.4 ″E Calabar, Cross River State Nigeria, following local, regional and global regulations on the 4th of February 2023. The plant was identified by Dr Effa, Effa. of the Department of Botany, University of Calabar Nigeria, and specimen deposited at the herbarium unit of the Department of Botany with voucher number BOT/HERB/UCC/351. Fresh leaves (1000 g in 2000 ml of distilled water) were pulverized and subjected to hydro distillation with Clevenger apparatus, according to the protocol described by Soonwera and Sittichock (2020). The oil was then filtered out into a clean 100 ml amber bottle and stored at 4°C until used for animal studies. Essential oil yield was estimated by volume and weight using the following equations by Ashokkumar et al. (2021):

$$\text{Essential oil (\%V/W)} = \frac{\text{Volume of oil collected (ml)}}{\text{Weight of the sample (g)}} \times 100$$

All protocols for the use of animals in research where strictly followed according to the guidelines by the National Research Ethics Committees (2019). This study was approved by the animal ethics committee of the Faculty of Basic Medical Science, College of Medical Sciences, University of Calabar Nigeria with Reference number; FAREC/PA/[UC/049]. A total of thirty-one (31) albino Wistar rats of both sexes weighing between 150 and180 g were obtained from the animal house of the Department of Biochemistry, University of Calabar under normal environmental conditions of room temperature and relative humidity. Acute toxicity of essential oil was carried out on 3 animals to determine the dose levels of exposure for the experimental animals using Lorke's method (1983), LD_{50} was observed to be greater than 5000 mg/kg, this influenced the choice of treatment doses for the subacute toxicity test. Subsequently**,** twenty-eight (28) rats were divided into seven (7) groups of four rats each. The experimental design is as shown in Table 1. Group A served as the negative control which was exposed to distilled water only, groups B1, B2 and B3 were exposed to 500, 300 and 150 mg/kg body weight of BNC insecticide, while groups S1, S2, S3 were exposed to 5000, 3000 and 1500 mg/kg body weight of essential oil. Animals were exposed to treatments in a closed chamber for 5 hours, once a day for 28 days.

On the 28th day, animals were fasted overnight, but with access to water. The rats were thereafter anaesthetized with ketamine (50 mg/ml) injection at 2 mg/kg body weight and sacrificed. Blood samples were collected via cardiac puncture using sterile 2 ml syringes and needles. Blood was put into plain bottles and allowed to stand for 2 hours, before it was centrifuged at 3000 rpm for 10 minutes to prepare serum. Serum was separated using a Pasteur pipette and stored in a refrigerator at 4 °C until used for biochemical analysis. The following methods were used for analysis Alanine amino transferase (ALT) and Aspartate amino transferase (AST) activities were analyzed by Reitman and Frankel's method (1957). Alkaline Phosphatase (ALP) Schlebusch et al (1974). Superoxide dismutase activity as described by Sun and Zigma (1978). Catalase activity (Sinha,1972). Estimation of glutathione peroxidase (GPx) (Ahmed et al., 2021).

Statistical analysis

Data obtained was analyzed for statistical significance using the one-way analysis of variance (ANOVA) with a post-hoc Dunnet (to compare intra and inter differences between groups) at $p<0.05$ using SPSS statistical package (2020). Results were expressed as mean ± SEM for n = 4.

Table 1: Experimental design.

Group	Number of animals	Treatment	Dose/body weight
A	4	Distilled water	5000 mg/kg
B_1	4	BNC Insecticide	500 mg/kg
B_2	4	BNC Insecticide	300 mg/kg
B3	4	BNC Insecticide	150 mg/kg
S1	4	*S. cayennesis* oil	5000 mg/kg
S2	4	*S. cayennesis* oil	3000 mg/kg
S3	4	*S. cayennesis* oil	1500 mg/kg

RESULTS

Essential oil yield of 0.75% was recorded. Comparative effect of esssential oil and BNC Insecticide on some serum liver enzymes (AST, ALT and ALP) are shown in Figures 1, 2, 3 respectively. BNC Insecticide treated groups had significantly higher activities of liver enzymes when compared with the groups treated with the essential oil of *S.cayennensis* leaves. A significant ($p<0.05$) increase in AST activity was observed in all treated groups, relative to the control. BNC treated groups had significantly higher AST activities relative to the S. *cayennesis* treated groups. There was no significant change ($p>0.05$) in ALT and ALP activities in S. *cayennesis* treated groups relative to the control. However group B_1 which was exposed to the highest concentration of the BNC insecticide, had significantly higher ALT and ALP activities relative to the S *cayennesis* treated and control groups. Results of antioxidant enzymes and oxidative stress makers are presented in Figures 4, 5, 6 and 7 . The result showed a significant ($p<0.05$) decrease in the activities of superoxide dismutase (SOD), and catalase (CAT) especially in group B_1 relative to the control and S_2 group. Glutathione peroxidase (GPX) increased significantly ($p<0.05$) in B_1 and B_2 (Figure 6) relative to the control and extract treated groups. No significant difference ($p>0.05$) was observed in the H_20_2 concentration (Figure 7) of groups S_2 and S_3 relative to the control. However, there was a significant ($p<0.05$) decrease in H_20_2 concentration in all the groups treated with synthetic pesticides relative to the control group.

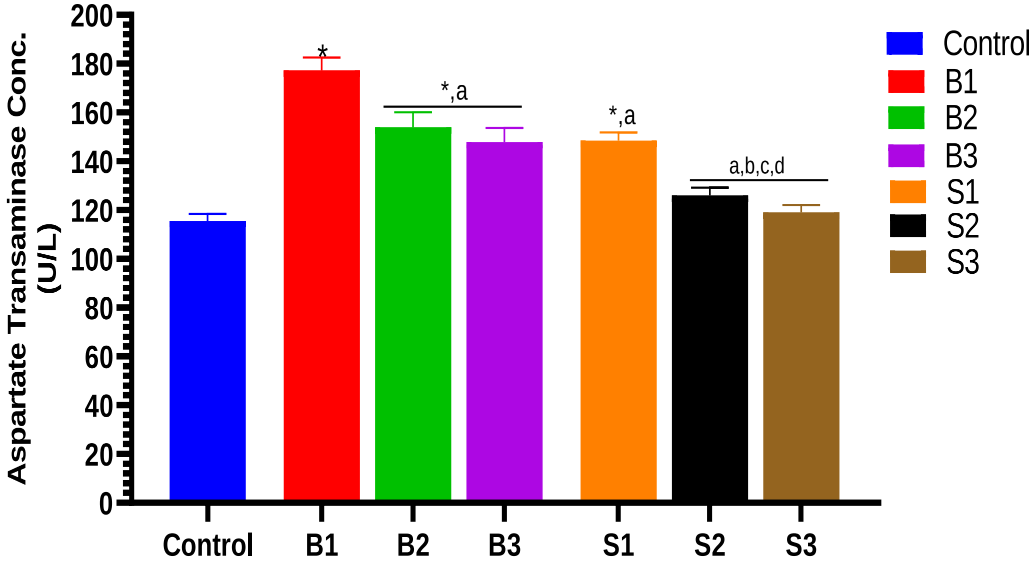

Figure 1: Comparison of Aspartate transaminase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at p<0.05
a= Significantly different from B1at p<0.05
b= Significantly different from B2 at p<0.05
c= Significantly different from S1 at p<0.05
d= Significantly different from S2 at p<0.05
e= Significantly different from S3at p<0.05

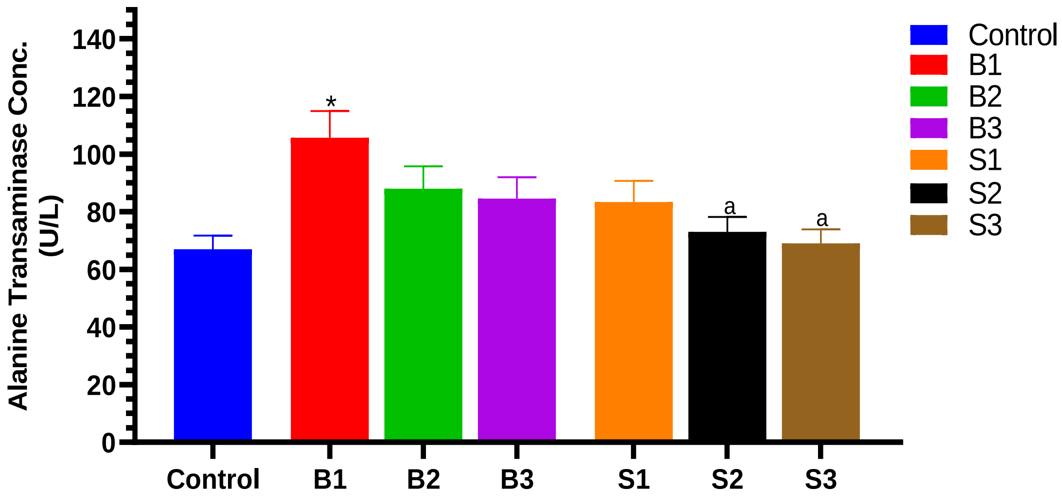

Figure 2: Comparison of Alanine transaminase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at p<0.05
a= Significantly different from B1 at p<0.05
b= Significantly different from B2 at p<0.05
c= Significantly different from S1 at p<0.05
d= Significantly different from S2 at p<0.05
e= Significantly different from S3 at p<0.05

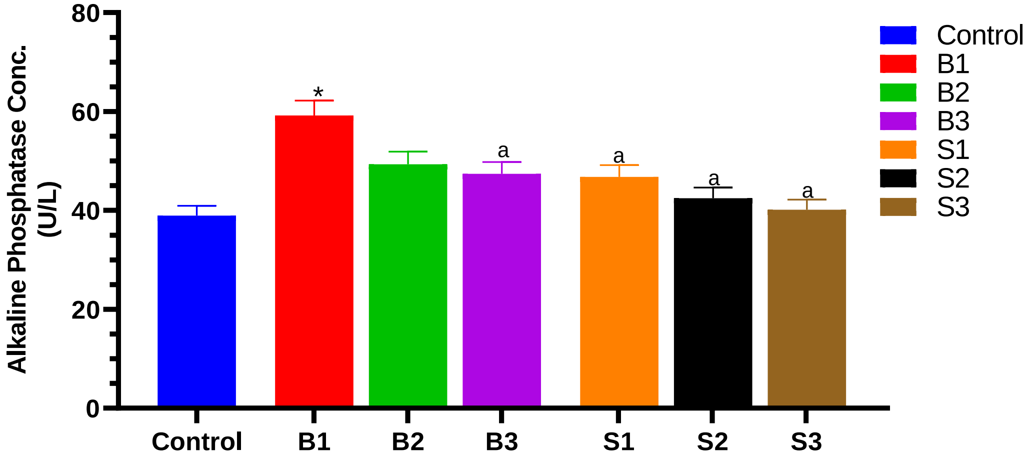

Figure 3: Comparison of Alkaline Phosphatase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at p<0.05
a= Significantly different from B1 at p<0.05
b= Significantly different from B2 at p<0.05
c= Significantly different from S1 at p<0.05
d= Significantly different from S2 at p<0.05
e= Significantly different from S3 at p<0.05

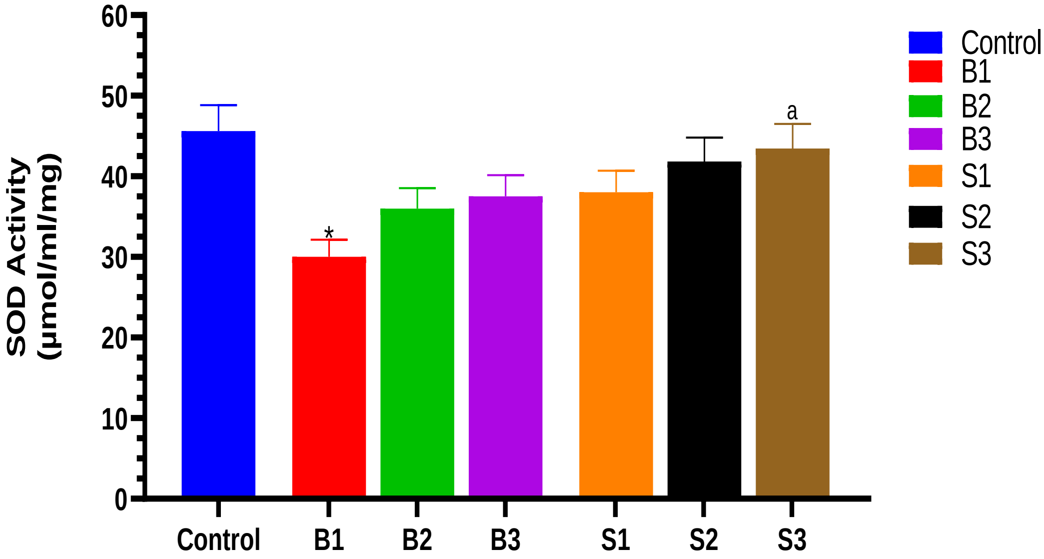

Figure 4: Comparison of superoxide dismutase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at p<0.05
a= Significantly different from B1 at p<0.05

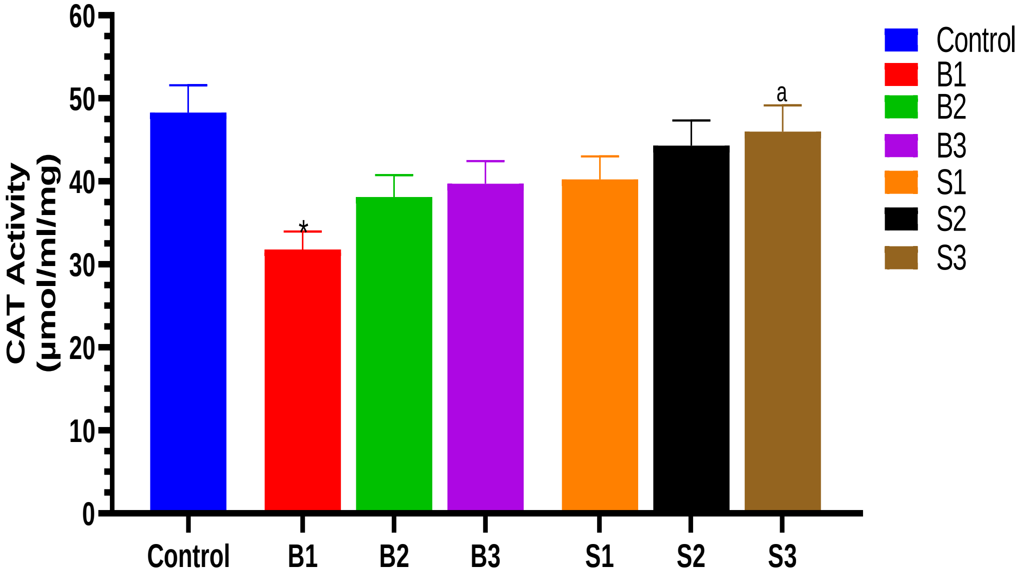

Figure 5: Comparison of catalase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at p<0.05
a= Significantly different from B1 at p<0.05

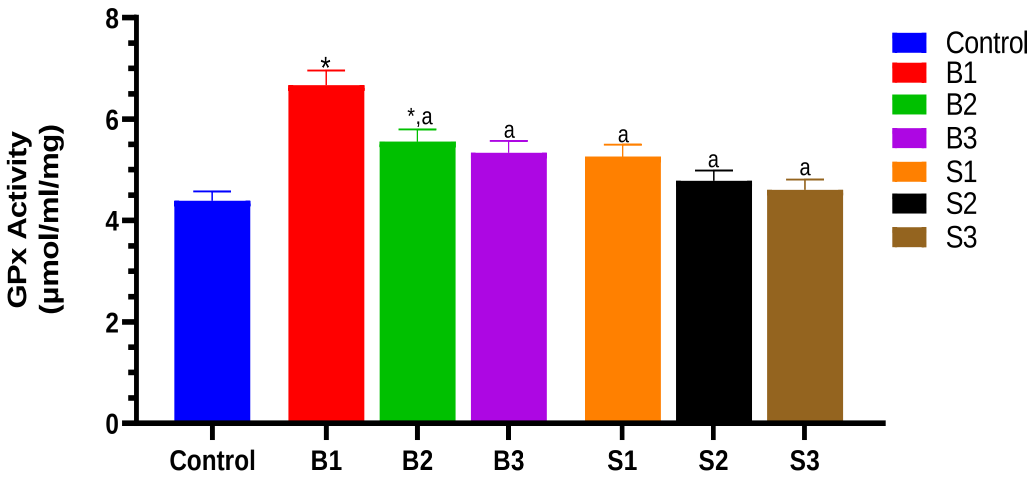

Figure 6: Comparison of Glutathione peroxidase activity in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at $p<0.05$
a=Significantly different from B1 at $p<0.05$
b=Significantly different from S1 at $p<0.05$
c= Significantly different from S2 at $p<0.05$
d= Significantly different from S3 at $p<0.05$

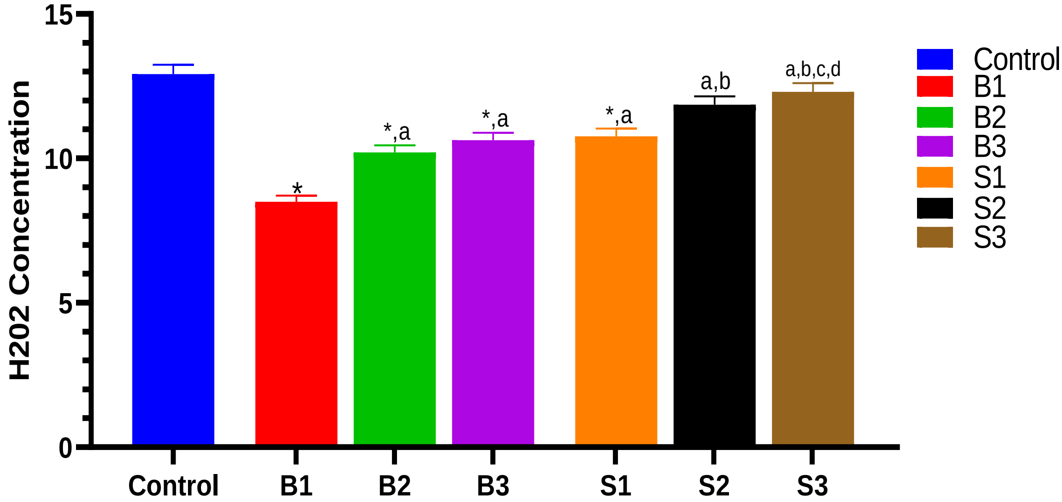

Figure 7: Comparison of H_2O_2 concentration in the different experimental groups.
Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).
*= Significantly different from Control at $p<0.05$
a=Significantly different from B1 at $p<0.05$
b=Significantly different from S1 at $p<0.05$
c= Significantly different from S2 at $p<0.05$
d= Significantly different from S3 at $p<0.05$

DISCUSSION

Antioxidants are compounds that delay or inhibit oxidative damage to target molecules and important tissues and organs in the body. The activities of the antioxidant enzymes; superoxide dismutase (SOD) and catalase (CAT) decreased significantly following exposure to the highest dose of BNC insecticide (group B1) (figures 4 and 5). Studies have shown that excessive free radical production resulting in oxidative stress could be an important mechanism of insecticide toxicity (Sule et al., 2022). The decrease in these parameters following exposure to insecticide has also been reported by Sharma and Singh, (2012); Ojo et al. (2014). However, in this study, these alterations were observed to be lower in the groups exposed to *S. cayennensis* essential oil. Superoxide dismutase (SOD) is the primary key factor in the defense mechanism of the antioxidant system against oxidative stress by catalyzing the dismutation of two superoxide radicals (O^{2-}) into molecular oxygen (O_2) and H_2O_2. On its own part, hydrogen peroxide is neutralized by the combined action of catalase and glutathione peroxidase in vertebrates. These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the activity of these enzymes. In the present study, a significant decrease in the activity of SOD and CAT (Figures 4 and 5) was observed in the BNC insecticide treated groups, suggestive of an increased superoxide radical production and other reactive oxygen species. The antioxidant enzyme CAT protects SOD against inactivation by hydrogen peroxide, reciprocally SOD protects CAT against inhibition by superoxide anion that could be formed during exposure to toxins. Similarly, glutathione provides protection to the tissues by catalyzing conjugation reactions that occur with xenobiotics (Labarrere and Kassab, 2022). There are many cellular and molecular targets of ROS, and as such, measurements of oxidative stress and damage in humans are quite complex. Sapbamrer et al. (2019) found no difference in 8-Oxo-dG among pesticide applicators pre- and post- pesticide application seasons. In contrast, both Kahl et al. (2018) and Lozano-Panigua et al. (2018) found that agricultural workers occupationally exposed to pesticides had significantly increased levels of oxidative stress biomarkers. In the current study, a significant increase in the activity of glutathione peroxidase (GPx) and concentration of H_2O_2 was also observed in group B1. Cattelan et al. (2018) observed that farmers who used pesticides had significantly reduced levels of SOD, GPx, and glutathione reductase (GSH) compared to farmers who did not use pesticides, this agrees reasonably with our findings with SOD, and may represent a reduced antioxidant defense system in response to an increase in ROS. Other recent studies suggest that occupational pesticide exposure is associated with elevations, rather than reductions, in antioxidant enzyme activity. Sapbamrer and colleagues (2019) found significantly increased SOD activity post-pesticide application relative to pre-pesticide application season. In addition, Lozano-Paniagua et al. (2018) found an elevated but non-significant increase in paraoxonase-1(PON1) activity in greenhouse workers compared to controls.

The significant ($p<0.05$) decrease in the activities of SOD and catalase especially in the groups exposed to the synthetic insecticide, is indicative of the fact that BNC insecticide may have generated free radicals that reacted with membrane lipids and induced a breakdown of membrane structure thus decreasing the enzymatic and non-enzymatic antioxidant activities, as well as H_2O_2 concentration. Conversely, the exposure of Wistar rats to *S. cayennensis* essential oil did not cause any significant changes ($p<0.05$) in the activities of SOD, CAT and GPx, promoting it as a better alternative and more environment friendly than the synthetic pesticide, this agrees with the report of Okonkwo and Onyeji (2018).

The liver is the organ responsible for the metabolism of endogenous and foreign compounds. Blood is transported to the liver through the portal vein which carries blood containing digested nutrients from the gastrointestinal tract, and the hepatic artery which carries oxygenated blood from the lungs (Kanu et al., 2016). Liver enzymes AST and ALT are frequently used as biomarkers of liver injury because they are released by hepatocytes into the extracellular space in higher concentrations when there is a threat or injury to the liver.

Results from this study indicate that exposure to BNC Insecticide significantly elevated the activities of liver enzymes; AST, ALT and ALP in rats (Figures 1, 2 and 3). The elevated concentration of serum ALT is indicative of likely hepatotoxicity. In the same way, Moriles and Azer (2022) observed that the elevation of ALT enzyme in the plasma is not unconnected to the fact that the enzyme may have leaked into the blood stream from the liver cytosol which is a reflection of liver dysfunction. According to Lowe et al. (2023), ALP is employed many a times for the assessment of the integrity of the plasma membrane. Elevation of serum ALP activity in this study following exposure to these pesticides, may be associated with liver plasma membrane distortion. However, these observable alterations in liver function parameters were not significant ($p>0.05$) for serum ALT and ALP activities in the groups exposed to the different doses of *S. cayennensis* essential oil, and significant ($p<0.05$) only in serum AST levels of rats exposed to the highest dose of the essential oil indicative of mild hepatic impairment at very high concentration. On the contrary, the synthetic pesticide caused significant ($p< 0.05$) increase in the activities of liver enzymes at all dose levels tested, showing more acute toxicity patterns than the *S.cayennensis* essential oil.

Conclusion

The results obtained from this study suggest that the oil may possess mild effects on metabolic and oxidative stress markers of rats at very high concentrations (5000mg/kg body weight). Exposure to synthetic pesticides as observed in this study caused adverse effects on biochemical indices especially the liver as evidenced by the increased activities of liver enzymes in groups B_1, B_2 and B_3. The decrease in activities of antioxidant enzymes (SOD and catalase) may be indicative of oxidative stress induced by the synthetic pesticide BNC, while biopesticides which are natural occurring compounds presented an eco-friendlier alternative, that was safer to non-target organisms.

Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential-oil-based pesticides owing to the complex mixtures of constituents that many of these oils contain. Ultimately, it is in developing countries which are rich in endemic plant biodiversity that these pesticides may ultimately have their greatest impact in the near future. Integrated pest management (IPM) programmes which combine natural and synthetic pesticides for the protection of crops is an option which has come to stay and have helped to reduce the application and circulation of synthetic pesticides in the environment.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by COO, and GAU. The first draft of the manuscript was written by GAU. SNO and CIOU commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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