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HEPATOPROTECTIVE EFFECT OF *HARUNGANA MADAGASCARIENSIS* AGAINST SUBCHRONIC 2,2-DICHLOROVINYL DIMETHYL PHOSPHATE (DDVP) INDUCED TOXICITY IN MALE WISTAR ALBINO RATS.

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Abstract
This study investigated the hepatoprotective potential of *Harungana madagascariensis* (HM) extract against sub chronic toxicity induced by 2,2-dichlorovinyl dimethyl phosphate (DDVP) in Wistar albino rats. Wistar albino rats were randomly assigned to five groups of Eight (8) rats each; control (group 1), 'DDVP exposed' alone (group 2), and treatment groups (groups 3, 4, 5 'DDVP exposed' + 50 mg/kg, 100 mg/kg, 150 mg/kg *H. madagascariensis* extract, respectively). The study spanned for 30 days, during which the male rats were treated with DDVP (10 mg/kg b.w) except for the positive control while organ weights (liver, kidney, heart, testes/epididymis), serum liver enzyme biomarkers (ALP, AST, ALT, total bilirubin, total protein), and percentage weight gain were assessed at the end of the study. The negative control group exhibited significant reductions in organ weights, elevated liver enzyme biomarkers, and decreased weight gain, indicative of DDVP-induced hepatotoxicity. The treatment groups demonstrated varying degrees of recovery in organ weights and serum biomarkers, with higher doses of HM extract showing more pronounced effects. *H. madagascariensis* extract exhibited hepatoprotective potential against sub-chronic DDVP-induced toxicity, as evidenced by improvements in organ weights, serum biomarkers, and weight gain. The observed dose-dependent responses highlighted the significance of HM as a potential therapeutic agent in achieving optimal hepatoprotection. These findings contribute to the understanding of natural remedies like plant-based supplements for control of pesticide-induced toxicity with HM having suggestive evidence of therapeutic potential for treatment of liver-related disorders.

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1.0 Introduction

According to Okwulu and Abatan (2023), the liver is an essential organ that produces bile, aids in metabolism, and filters the blood, among many other important physiological functions. Toxic substances are just one of several environmental factors that might harm the liver, and one organophosphate that can cause serious harm to the liver is 2,2-Dichlorovinyl dimethyl phosphate (DDVP) (Oluwadunni *et al.,* 2019).

The authors also confirmed World Health Organization claims that hepatic disorder has been classified as a high priority area of health care and estimated that over 500 million people suffer from it; in both acute and severe (chronic) forms. The agents that cause these dysfunctions are known as hepatotoxicants or hepatotoxins, according to Fredman *et al.* (1996), and other causes include the inhalation of xenobiotics, such as industrial pollutants or smoking, excessive consumption of medicinal medications, herbal remedies, or dietary supplements, and alcohol consumption (Adeneye *et al.,* 2008).

Even at therapeutic dose levels, the reactive metabolites or immunologically mediated reactions of some medicines can harm hepatocytes, biliary epithelial cells, and/or the liver vasculature, leading to liver and kidney damage (Nku-Ekpang *et al.,* 2021; Asogwa *et al.,* 2023). However, a species of tree known as dragon's blood tree, *Harungana madagascariensis*, is monotypic and has been claimed to offer cures to jaundice, dysentery, typhoid fever, constipation, liver issues, anemia, malaria, river blindness, ulcer, asthma, hepatitis, dysmenorrhea, and toothache, according to Okwulu and Abatan (2023).

Multiple scientific studies have thus "documented" the traditional usage of this plant's leaves and stem bark to cure conditions such as anemia, diabetes, bacterial infections, diarrhea, and gastrointestinal ailments, as researchers have found bioactive chemicals in the bark, roots, and leaves of the *Harungana madagascariensis* plant, including anthracene derivatives and flavonoids with antiplasmodial and antibacterial properties (Ndjakou *et al.,* 2007; Moronkola *et al.,* 2015). According to Adefegha and Adetuyi, A. (2010), traditional Malagasy medicine uses the medicinal herb *H. madagascariensis* to alleviate various symptoms, including liver disorders, and the ability of HM to reduce toxicant-induced liver damage has been shown in earlier research as the bioactive chemicals found in abundance in HM include tannins, flavonoids, and saponins, which are well-known for their ability to protect the liver, reduce inflammation, and function as antioxidants (Biduaya *et al.,* 2020).

As opined by Kengni *et al.* (2016), sub-chronic 2,2- dichlorovinyl dimethyl phosphate (DDVP) can cause oxidative stress, inflammation, and hepatocellular damage in the liver when exposed to it subchronic, and lipid peroxidation, protein denaturation, and DNA damage can result from oxidative stress, which is caused by an excess of reactive oxygen species (ROS) relative to antioxidants. This imbalance disrupts cellular equilibrium, and by drawing in immune cells and encouraging tissue damage, inflammation, which is set off by the secretion of pro-inflammatory mediators, can worsen liver damage; this damage to the liver cells, known as hepatocellular injury, can reduce liver function and, in extreme circumstances, cause the liver to fail (Happi *et al.,* 2020).

A brief history of this plant shows that the tropical shrub *H. madagascariensis* belongs to the *Hypericaceae* family. It is found all over the edges and banks of tropical rainforests, given that it originally hails from Madagascar, South Africa, Central Africa, the Democratic Republic of the Congo, Sudan, Ethiopia, and Lesotho, with the English names such as "blood tree," "orange-milk tree," "dragon's blood tree," "Haronga," and "Harungana" (Biduaya *et al.,* 2020). While the Igbo people of southeastern Nigeria call it "oturu," the Yoruba people of southwestern Nigeria call it "amuje," and the Bini people of southwestern Nigeria call it "itue-egbo" because it has a cylindrical trunk and a spreading, golden-green crown, allowing the plant to reach a height of 4-7 meters, and on rare occasions 10-25 meters. It is said that the pounded bark is used to treat leprosy, and the red

sap extracted from the stem bark is drunk as a medicine for tapeworm infection, craw-craw, or to cover wounds among the Ghanaians (Kuete and Seukep, 2023).

In the same vein, the Ondo people of southwestern Nigeria use the plant's extract as a remedy for jaundice, scabies, and acute enteritis; and another remedy for malaria that has gained reputation is a boiling infusion of the plant's leaves, as there have been recent reports of various plant stem bark extracts having antibacterial and antifungal properties (Llorent-Martinez *et al.*, 2020).

These characteristics point to the possibility that HM could prevent liver damage caused by DDVP. As such, despite its traditional' use to treat liver diseases, scientific evidence supporting its hepatoprotective effects against DDVP-induced toxicity is limited. Therefore, this study aimed to investigate the hepatoprotective potential of HM against subchronic DDVP-induced toxicity in rats. By evaluating the effects of *H. madagascariensis* on serum liver enzyme levels, total bilirubin, total protein levels, and histopathological changes in liver tissues, this study aims to provide insights into the potential therapeutic role of HM in liver protection.

2.0 Methodology

2.1 Study Design

According to Peters *et al.* (2018), this study was a randomized, controlled experiment with five groups of Wistar albino rats. The rats were housed in individual cages in a temperature-controlled room with a 12-h light/dark cycle. They were given free access to food and water. Rats in the DDVP exposure groups were administered DDVP at a dose of 10 mg/kg body weight per day through oral gavage. Rats in the *H. madagascariensis* extract groups were administered HM extract at specified doses through oral gavage. The rats in the control group were not administered any treatment.

2.1.1 Animal and experimental design

Male Wistar albino rats were obtained from the central animal house, University of Port-Harcourt. Subchronic 2,2-dichlorovinyl, dimethyl phosphate (DDVP) administration was performed through oral gavage to mimic real-world exposure. HM extract was prepared and administered in accordance with established protocols, considering the specified dosage for each treatment group. The groups were as follows:

Group 1: Control group (no DDVP exposure and no *H. madagascariensis* extract)
Group 2: DDVP exposure only
Group 3: DDVP exposure + 50 mg/kg *H. madagascariensis* extract
Group 4: DDVP exposure + 100 mg/kg *H. madagascariensis* extract
Group 5: DDVP exposure + 150 mg/kg *H. madagascariensis* extract

2.2 Preparation of *the Harungana madagascariensis* stem bark extract

H. madagascariensis stem bark was obtained from the pharmacognosy botanical garden, University of Port-Harcourt, Choba, River State. To prepare the stem bark for extraction, it was shade-dried for weeks, crushed into a fine powder, and then subjected to Soxhlet extraction with 70% methanol. The extract was then condensed at decreased pressure and kept at 20 °C until it was needed.

2.3 Data Validity and Reliability

To enhance data validity and reliability, rigorous control measures were implemented, including standardized animal housing conditions, consistent administration protocols, and careful calibration of the equipment used for measurements.

2.4 Statistical Analysis

At the end of the study, the absolute and relative weights of the liver, kidney, heart, and testes/epididymis were recorded, the levels of liver enzyme biomarkers (ALP, AST, ALT), total bilirubin, and total protein were

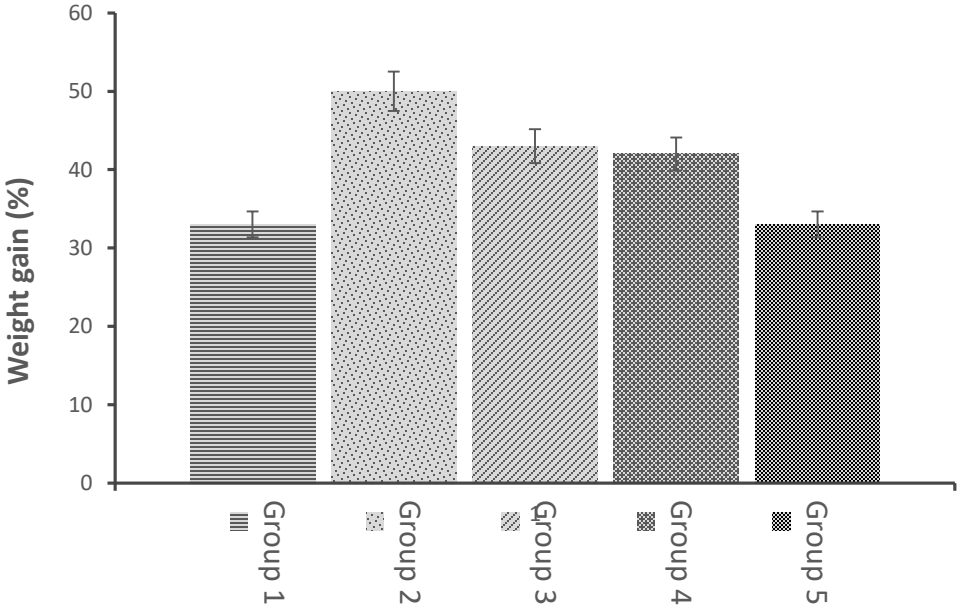
measured, the initial and final weights were recorded for each rat, and the percentage weight gain was calculated. After 30 days, the rats were euthanized and their organs were weighed. The absolute and relative weights of the liver, kidneys, heart, and testes/epididymis were compared between the groups using descriptive statistics such as mean and standard error for each parameter. Oneway ANOVA followed by Tukey's post hoc test was conducted to determine significant differences between the groups. Statistical significance was set at $p < 0.05$.

2.5 Ethical Considerations

This study adhered to ethical standards for animal research. Approval was obtained from the Institutional Animal Care and Use Committee, University of Port-Harcourt, Nigeria, and all animal care and procedures were performed in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

3.0 Results

3.1 Weight gain in Wistar albino rats



Data were expressed as percentage (%) of n = 5 determination. [abcde] represent groups with different superscript letters, and values in the same column having the same superscript letter were not significantly different at p<0.05. Group 1: Normal or control (healthy rats), Group 2: DDVP exposure rats at 10 mg kg1 b.w through ingestion by oral gavage with no intervention (negative control), Group 3: DDVP exposure + 50 mg kg1 H. madagascariensis extract (treatment group), 4: DDVP exposure + 100 mg kg1 H. madagascariensis extract (treatment group), 5: DDVP exposure + 150 mg kg1 H. madagascariensis extract (treatment group).

In figure 1.1 above, group 1, representing normal and healthy rats, exhibited baseline weight gain over the 30-day period. This group serves as a reference for typical weight gain in the absence of experimental interventions, while the rats exposed to DDVP alone (group 2) showed a significant reduction in weight gain compared with the control group, hence showing noticeable decrease in weight gain. This suggests that DDVP exposure has a detrimental effect on the overall health and growth of the rats. Groups 3, 4, and 5 receiving different doses of HM extract alongside DDVP exposure exhibited variations in weight gain. Therefore, if the treatment groups show improved weight gain compared with the negative control (group 2), this implies that HM extract may have a protective effect against the negative impact of DDVP. A dose-dependent relationship may be inferred because there is a gradation in weight gain improvement across the treatment groups. Therefore, the significant decrease in weight gain in the negative control group (Group 2) indicates the adverse effects of DDVP exposure on the

growth of rats, whereas variations in weight gain among the treatment groups suggest that HM extract may counteract the negative impact of DDVP. The results highlighted the importance of HM extract in mitigating the toxicity induced by DDVP, emphasizing its potential as a protective agent for overall rat health.

3.2.1 Absolute and relative Liver weight of organs of Wistar albino rats

Table 1.1: Absolute and relative weights of organs of Wistar albino rats

GROUPS	ALW (g)	RLW (%)	AKW (g)	RKW (%)	AHW (%)	RHW (%)	A(T/E)W (%)	R(T/E)W (%)
GROUP 1	7.99±0.32^{c}	4.78±0.24^{a}	1.62±0.07^{b}	0.96±0.05^{b}	1.29±0.14^{b}	0.78±0.11^{b}	3.55±0.22^{a}	2.12±0.13^{a}
GROUP 2	3.36±0.13^{a}	1.76±0.10^{b}	0.82±0.03a^{a}	0.44±0.02^{a}	0.53±0.20^{a}	0.28±0.11^{a}	9.23±0.76^{c}	4.80±0.22^{b}
GROUP 3	4.31±0.21^{ab}	2.28±0.07^{a}	0.91±0.02^{a}	0.48±0.02^{a}	0.44±0.04^{a}	0.22±0.02^{a}	8.61±0.63^{c}	3.74±0.79^{ab}
GROUP 4	3.77±0.11^{ab}	1.66±0.41^{a}	0.86±0.01^{a}	0.56±0.02^{a}	0.49±0.01^{a}	0.30±0.00^{a}	6.31± 1.02^{b}	1.96±0.61^{a}
GROUP 5	4.58±0.42^{b}	2.56±0.27^{a}	0.86±0.0^{a4}	0.78±0.02^{a}	0.51±0.01^{a}	0.30±0.00^{a}	5.71±0.22^{b}	2.62±0.59^{a}

Data were expressed as mean±SE (Standard Error) of n = 5 determination. ^{abcde} represent groups with different superscript letters and values in the same column having the same superscript letter were not significantly different at p<0.05. Group 1: Normal or control (healthy rats), Group 2: DDVP exposure rats at 10 mg kg1 b.w through ingestion by oral gavage with no intervention (negative control), Group 3: DDVP exposure + 50 mgkg1 H. madagascariensis extract (treatment group), 4: DDVP exposure + 100 mg kg1 H. madagascariensis extract (treatment group), 5: DDVP exposure + 150 mg kg1 H. madagascariensis extract (treatment group). Absolute liver weight (ALW), Relative liver weight (RLW), Absolute kidney weight (AKW), Relative kidney weight (RKW), Absolute heart weight (AHW), Relative heart weight (RHW), Absolute testes/epididymis weight (AT/EW), and relative testes/epididymis weight (RT/EW).

The normal or healthy rats (group 1) in table 1.1 above had significantly higher absolute and relative liver weights than the other groups (7.99g and 4.78%, respectively), whereas DDVP exposure only (group 2) showed a considerable reduction in both absolute and relative liver weights (3.36g and 1.76%, respectively) when compared with the control. Results from the treatment groups appear to mitigate the decrease in liver weight induced by DDVP exposure. For the treatment groups, administration of HM extract at different doses (50mg/kg, 100mg/kg, and 150mg/kg) appeared to mitigate the decrease in liver weight induced by DDVP exposure.

3.2.2 Absolute and relative weight of organs of Wistar albino rats

The control group in the table above exhibited higher absolute and relative kidney weights than other groups, whereas DDVP exposure alone led to a reduction in both absolute and relative kidney weight, and this effect was partially alleviated in the treatment groups. Similar to liver and kidney, the control group in table 1.1 had higher absolute and relative heart weights, whereas DDVP exposure alone led a reduction in both absolute and relative heart weight, and the treatment groups showed varying degrees of weight gained compared to the control. The testicular weight parameters are relevant to reproductive health in male rats. The control group from the table above generally exhibited higher organ weights compared to DDVP exposed alone, which showed a significant decrease in absolute and relative testes/epididymis weight, whereas treatment with HM extract appeared to counteract the negative effects of DDVP to some extent. The treatment groups (3,4,5) showed a trend toward mitigating the adverse effects of the exposure, with varying degrees of effectiveness depending on the dosage of the HM extract. These findings suggest that HM extract may have a hepatoprotective effect against the subchronic toxicity induced by DDVP exposure, as evidenced by the improvements in organ weights observed in the treatment groups compared with the negative control or the DDVP group.

3.3.0 Effect of *H. madagascariensis* extract on serum liver enzyme biomarkers

Table 1.2: Effect of *H. madagascariensis* extract on serum liver enzyme biomarkers

GROUPS	ALP (U/L)	AST (U/L)	ALT (U/L)	TB (g/l)	TP (g/l)
GROUP 1	18.35±1.26^{a}	29.40±0.87^{a}	7.42±0.49^{a}	5.28±0.15^{a}	65.80±2.01^{c}
GROUP 2	27.00±1.14^{b}	45.20±1.52^{b}	12.36±0.55^{b}	9.34±0.53^{b}	39.00±2.00^{a}
GROUP 3	20.42±1.34^{a}	34.78±2.15^{a}	10.48± 0.10^{ab}	6.32±0.07^{a}	55.40±0.75b^{b}
GROUP 4	18.20±1.34^{a}	32.50±2.64^{a}	9.96±1.06^{ab}	6.22±0.17^{a}	56.40±0.87^{b}
GROUP 5	18.20±2.94^{a}	31.48±1.20^{a}	7.90±0.90^{a}	5.58±0.31^{a}	58.40±1.54^{b}

Data were expressed as mean±SE (Standard Error) of n = 5 determination. ^{abcde} represent groups with different superscript letters and values in the same column having the same superscript letter were not significantly different at p<0.05. Group 1: Normal or control (healthy rats), Group 2: DDVP exposure rats at 10 mg kg1 b.w through ingestion by oral gavage with no intervention (negative control), Group 3: DDVP exposure + 50 mg kg1 H. madagascariensis extract (treatment group), 4: DDVP exposure + 100 mg kg1 H. madagascariensis extract (treatment group), 5: DDVP exposure + 150 mg kg1 H. madagascariensis extract (treatment group).

3.3.1 Alkaline Phosphate (ALP)

The normal or healthy rats in table 1.2 exhibited a baseline ALP level of 18.35 U/L, whereas the DDVP exposed alone group showed a significant increase in ALP levels (27.00 U/L), indicating liver dysfunction. Treatment groups with *H. madagascariensis* extract appeared to mitigate the elevation in ALP levels induced by DDVP exposure. Same on the above table, the control group showed a baseline AST level of 29.40 U/L and the DDVP exposed alone showed a substantial increase in AST levels (45.20 U/L), suggesting liver damage. Treatment groups with HM extract administration reduced the elevated AST levels. Also, table 1.2 above had a baseline ALT level of 7.42 U/L for the control, whereas the DDVP-exposed group alone induced a significant increase in ALT levels (12.36 U/L), indicating liver injury. Treatment groups with HM extract demonstrated potential in reducing elevated ALT. The normal healthy group serving as the control showed a baseline total bilirubin level of 5.28 g/l, and the DDVP-exposed group alone showed a substantial increase in total bilirubin levels (9.34 g/l) in table 1.2, indicating impaired liver function, whereas treatment groups with HM extract administration reduced the elevation in total bilirubin caused by DDVP exposure.

Accordingly, the healthy or control group had a baseline total protein level of 65.80 g/l, and the DDVP-exposed group alone showed a significant decrease in total protein levels (39.00 g/l), suggesting compromised liver function, while treatment groups showed potential of the extract in mitigating the decrease in total protein levels caused by DDVP exposure. The result indicated that the negative control group (group 2) exhibited abnormalities in liver biomarkers, indicating hepatotoxicity. However, the extract when administered in different doses (Groups 3, 4, 5), appears to exert a protective effect by mitigating the alterations induced by DDVP exposure. Therefore, the results suggested a potential hepatoprotective role of the extract against DDVP-induced liver damage, as evidenced by the modulation of various serum liver enzyme biomarkers and other parameters.

3.4 Effect of *H. madagascariensis* extract on liver smear for histopathological

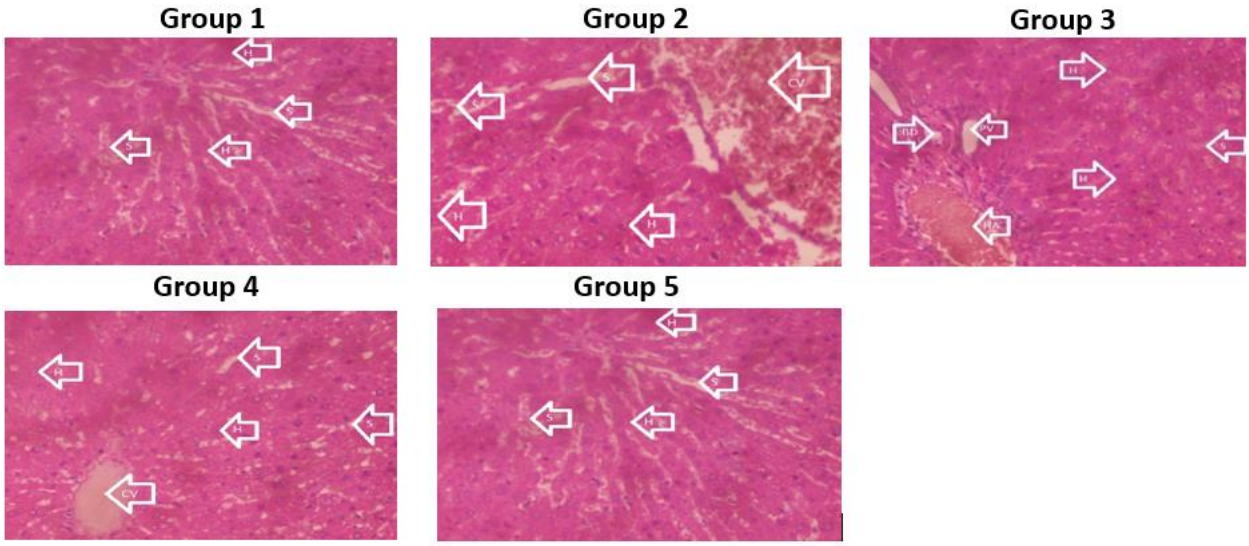


Plate 1.1: Photomicrograph of liver smear for histopathological examination of male Wistar albino rats. Group 1: Normal or control (healthy rats), Group 2: DDVP exposure rats at 10 mgkg^{-1}b.w through ingestion by oral gavage with no intervention (negative control), Group 3: DDVP exposure + 50 mgkg^{-1} H.madagascariensis extract (therapeutic group), 4: DDVP exposure + 100 mgkg^{-1} H.madagascariensis extract (therapeutic group), 5: DDVP exposure + 150 mgkg^{-1} H.madagascariensis extract (therapeutic group).

In plate 1.1 above, the photomicrograph was taken at 4×100 magnification on the liver smear for histopathological examination of male Wistar albino rats. This showed hepatocyte lines with the hepatic artery, bile duct, portal vein, and central veins, as indicated among the various groups. However, group 2 showed high level of histological lesions, indicative of the toxic damage caused by DDVP in the liver of the experimental rats compare to the control which showed normal tissue cells. Succinctly the result of the treatment groups 3,4,5 showed moderate tissue lesion when compared to the control, this may be attributed to the effect of the administered extract which helped in mitigating the negative impact of DDVP exposure in the rats and restoring the normal tissue architecture.

4.0 Discussion

The results of the study in table 1.1 showed that the control group (group 1) exhibited significantly higher absolute and relative liver weight, indicating normal liver development, while the negative control (group 2) exhibited a notable reduction in both absolute and relative liver weight, suggesting hepatotoxic effects induced by DDVP; and the treatment groups (groups 3, 4, 5): Showed varying degrees of recovery in liver weight, implying a potential hepatoprotective effect of HM extract against DDVP-induced liver damage. On the other hand, in table 1.2 the negative control group exhibited significant elevations in these liver enzyme biomarkers, indicative of liver damage induced by DDVP while treatment groups 3,4 and 5 showed a trend of reducing these elevations, suggesting a potential hepatoprotective effect of HM extract. This result is consistent with the findings from Owumi *et al. (*2019) who reported that *Cissampelos capensis* and *Pleiocarpa pycantha* plant extracts offer hepatoprotective effect. Similarly, the negative control group demonstrated an increase in total bilirubin and a decrease in total protein, further indicating liver dysfunction, and the treatment groups displayed trends toward normalization, supporting the potential hepatoprotective role of the extract. The study provides substantial evidence suggesting that DDVP induces hepatotoxic and potentially reproductive toxic effects in Wistar albino rats; also, it shows that the HM extract demonstrated promising hepatoprotective effects, as evidenced by improvements in organ weights and serum biomarkers. These findings conform with the findings of Adeneye *et*

al. (2008) who affirmed that aqueous root extract of Harungana protects rats' livers against acute and repeated dose models of acetaminophen hepatotoxicity, demonstrated by the fact that the biochemical changes and histological lesions generated by acetaminophen hepatotoxicity were significantly improved by the *H. madagascariensis* extract when administered orally in graded doses. Furthermore, Nku-Ekpang *et al.* (2021) affirmed this in their findings where they claimed that the administration of *H. madagascariensis* may have a protective impact on cellular and hepatocellular membranes, since the levels of certain liver enzymes were shown to be reduced.

In conclusion, hepatocyte lines with the hepatic artery, bile duct, portal vein, and central veins, as indicated among the various groups in plate 1.1 showed high level of histological lesions in group 2 indicative of the toxic damage caused by DDVP exposure in the liver of the experimental rats compare to the control which showed normal tissue cells. Succinctly the result of the treatment groups 3,4,5 showed moderate tissue lesion when compared to the control, this may be attributed to the effect of the administered the HM extract which helped in mitigating the negative impact of DDVP in the rats. Therefore, the findings of this study supported the hypothesis that HM extract possesses hepatoprotective properties against sub-chronic DDVP-induced toxicity in Wistar albino rats. This study sheds light on potential therapeutic interventions to counteract the harmful effects of pesticide exposure on liver function and overall health. Further research, including molecular investigations and long-term studies, is warranted to elucidate the underlying mechanisms and optimize the therapeutic potential of HM in mitigating pesticide-induced toxicity. In relation to other studies, this study stands out amongst its contemporaries given that while other studies in this field focus on the effects of HM along with other antioxidant compounds, this study seeks to exonerate HM of such associations in a bid to examine the hepatoprotective effect of *H. madagascariensis* alone. As, such, the results obtained can be more confidently relied upon because the study was well-designed and tightly controlled.

5.0 Conflict of Interest

The authors report no conflicts of interest in the study.

6.0 acknowledgment

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