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Gross Morphological Effect of *Benincasa hispida* on Paracetamol Induced Hepatotoxicity in Treated Wister Albino Rats

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ABSTRACT

Background: Paracetamol is an over-the-counter medication commonly used for managing low grade pain and fever. However, despite its clinical effectiveness, it has been associated with liver disease when taken in overdose. From literatures, *Benincasa hispida* has been associated with the hepatoprotective effects on drug induced hepatotoxicity. However, there is paucity of literature showing the hepatoprotective effects of *Benincasa hispida* on paracetamol-induced hepatotoxicity. **Aim:** To evaluate the gross morphological effect of *Benincasa hispida* on paracetamol-induced hepatotoxicity in Wister albino rats. **Materials and Methods:** This was a posttest-only true experimental design in which 25 Wister Albino Rats were used. Systematic random sampling method was used in recruiting and assigning the animals into control and experimental groups. Wister Albino rats as the sample population because of their strong genomic correlation with human being. The 25 animals were separated into five groups, each group having five animals. All the animals were concurrently treated with a constant dose of 1500mg/kg/bwt of Paracetamol and Different doses of *Benincasa hispida* except the Control that only fed on water *adlibitum.* Paracetamol was only administered with the 1500mg/kg/bwt. High, medium, low dose groups were administered with concurrent administration of 1500mg/kg/bwt of Paracetamol and 300, 200, 100mg/kg/bwt of *Benincasa hispida* respectively. On the 20th day, the animals were humanely sacrificed. The animals were dissected and the gross morphometric measurements such as the volume, weight, thickness and width of the liver were taken. The data was then uploaded into the statistical package for social science. The one-way ANOVA and post hoc test were used to test the significance level at the confidence interval at 95% and the $p < 0.05$ was considered significant. **Results:** There was a significance ($p < 0.05$) decrease in the terminal body weight of the paracetamol group as differentiated with the control. There was significance ($p < 0.001$) reduction in the weight, length, width and thickness of the liver in the Paracetamol group relative to the control group. There was a statistical ($p < 0.001$) difference of the weight, length, width and thickness of the liver of the High dose group as compared to the Paracetamol group. **Conclusion:** The gross morphological parameters such as the terminal body weight, Volume, thickness, weight of the liver can be used in assessing the histopathological changes on the hepatoprotective effects of *Benincasa hispida* on paracetamol induced hepatotoxicity.

Keywords: Hepatoprotective; *Benincasa hispida*; Gross morphometry; Paracetamol; Hepatotoxicity; Treated Wister albino rats.

1. Introduction

Hepatoprotection is the potential of a given chemical substance to prevent damage to the liver cell following an exposure to hepatotoxic agent. Several traditional herbal medications have been studied to find out their association with hepatoprotective effect following administration of hepatotoxic argent such as *Azadiracta indica* has been associated with hepatoprotection following administration of cisplatin (Abdel Moneim et al., 2014). Liv-52 is associated with hepatoprotection following paracetamol-induced toxicity (Girish et al., 2009). *Curcuma longa* has been associated with hepatoprotection following CCl_4-induced acute hepatic stress (Karamalakova et al., 2019), and also *Michelia nilagrica* has been associated with hepatoprotection following paracetamol induced toxicity (Aminabee et al., 2015).

Paracetamol (PCM) also known as acetaminophen widely used as over the counter analgesic and antipyretic agent (Blough & Wu, 2011). The healing dose of paracetamol is more secure however its overdose is also considered because of its narrow healing index. Its overdose can result in hepatic and renal harm in both human beings and experimental animals (Varughese, 2013). Liver is the main target organ for drug metabolism. Paracetamol toxicity can occur among patients with advanced hepatic injury; however, Paracetamol hepatotoxicity after overdose may be evident in animals too (Prescott, 2000).

Benincasa hispida (Synonym; *Bencasa cerifera*), which is commonly called (winter melon, ash guard, winter guard, white pumpkin and wax guard, white gourd, tallow guard, guard melon and Chinese watermelon belongs to the Cucurbitaceae family (Liu & Corma, 2018). It is a popular vegetable crop, especially among Asian communities both for nutritional and medicinal purposes. The major constituents of Benincasa fruits are volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes and uronic acid (Doharey et al., 2021). Young fruit is fleshy succulent and hairy while the mature fruit has thick deposit hairs which are easily removable. In Ayurveda, *Benincasa hispida* is recommended for management of peptic ulcers, internal organ bleeding.

Gross morphology includes the volume, thickness, width, length, height and weight of a structure. This can be used in detecting abnormal changes in an organ during a pathological process. In daily life, some imaging modalities are used in diagnosing some diseases using the gross morphological measurement. Ultrasound is used in detecting an enlarged prostate indicating a pathological process (Walter & Tukachinsky, 2020). An X–ray is used in elucidating an enlarged heart during a diagnosis of a chronic heart disease (Edwin et al., 2019). Gross morphological changes can be used as a marker for histopathological changes that is occurring in a structure.

This study helps in showcasing a solution for liver diseases associated with paracetamol-induced hepatotoxicity that can be as result of an increase or over use of the drug uptake. The study will be of great benefit to the population using paracetamol since *Benincasa hispida* is locally available and it will reduce the number of liver disease associated with drug induced hepatotoxicity.

1.1. Study Objective

The main objective of the study was to evaluate the gross morphological effect of *Benincasa hispida* on paracetamol-induced hepatotoxicity in Wister albino rats.

2. Materials and Methods

2.1. Study area: The study was conducted at Maseno University situated in Kisumu County along Kisumu-Busia Road. Breeding, weighing, handling and administration of *Benincasa hispida* was done at the zoology department in the school of biological sciences due to the availability of modernized animal cages and people with expertise to handle Wister albino rats. Tissue harvesting and examination was at the histology lab in the Department of human anatomy and blood sample analysis was done in the University of Nairobi, faculty of Clinical services at the Department of Veterinary Medicine.

2.2. Study design: A controlled post-test group experimental study design method was used. Only true of Wister Albino rats was used.

2.3. Study sample: The Wister albino rats of the species of *Rattus norvegicus* from a pure breed were adopted in the study.

2.4. Sample size determination: Sample size was calculated using the modified resource equation (Arifin & Zahiruddin) as given below,

$$n = DF/K+1 \quad (1)$$

n = Animals per group; K = Number of groups; DF = Error of degree of freedom [10 to 20]; N = Total number of animals.

Calculation of number of animals per group = 20/5 is 4 +1 = 5

Total number of animals N = Kxn (2)

K = 5; n = 5N = 5x5 = 25 rats.

2.5. Sampling methods: Random sampling method was used to sample and in selecting 25 Wister albino rats (*Rattus norvegicus*).

2.6. Selection criteria (Inclusion criteria): Healthy Wister albino rats (*Rattus norvegicus*); and Animals with the right weight for the study.

2.7. Handling of animals: The Wister Albino rats were put into the cages and left for seven days for acclimatization. The control group was only fed on water *adlibitum*. All of the experimental groups were treated with a constant dose of 1500mg/kg/bwt of paracetamol. Paracetamol group was only given 1500mg/kg/bwt, water *adlibitum.* High doses group was administered with 1500mg/kg/bwt of paracetamol and 300mg/kg/bwt of *Benincasa hispida.* Medium doses group was administered with 1500mg/kg/bwt of paracetamol and 200mg/kg/bwt of *Benincasa hispida.* Low doses group was administered with 1500mg/kg/bwt of paracetamol and 100mg/kg/bwt of *Benincasa hispida.* Before the animals were sacrificed, their terminal body weights were taken. All the animals were humanely sacrificed on the twentieth day.

The procedure for anaesthetizing:

1) Cotton wool was irrigated with solution of chloroform or diethyl.
2) The soaked cotton was introduced into the bell jar.
3) The rats were introduced into the bell jar for 15-20 minutes to be anaesthetized.
4) The rats were then placed on flat board in a dorsal position and mounted with tapes.
5) The rats were dissected using a pair of scissors and the forceps on the ventral medial side from the pubic symphysis until the sternal angle of the thoracic cage.
6) A perfusion needle was used to collect blood from the right side of the heart.
7) The blood sample was withdrawn from the left ventricle using normal saline of 0.85mol/lit.
8) The liver was excised and put in a fresh fixture.

2.8. Measurements of the liver parameters

Immediately the animals were sacrificed, they were dissected and the livers were grossly assessed. The gross morphometric measurements such the volume was taken using the water displacement principal, thickness and width was taken using the vernier caliper, weight of the liver was taken using an electric weight machine and the data was recorded as per the group presentation.

2.9. Data analysis: Data was uploaded in the excel sheet and further transferred into the Statistical package for the social science (SPSS) application version 27. One way ANOVA and post hoc Bonferroni test was used to find the difference within the group and treatment groups. The p≤ 0.05 was found to be of statistical significance at the confidence interval of 95%. The results were presented using the tables and figures.

2.10. Ethical approval

The ethical approval for conducting this animal study was obtained from the East Africa, Baraton University (Reference number: UEAB/ISERC/07/01/2023). The license for conducting this study was obtained from the National Commission for Science, Technology and innovation (Reference number: NACOSTI/P/23/30501). The study was conducted as per the standards and procedures of guidelines and protocol for care and use of laboratory animals in biomedical research (Guidelines & Kenya 2016).

3. Results

3.1. Terminal body weight of the Wister Albino Rats

The terminal body weight, the weight, length, width, and thickness of the liver measurements were taken. These variables were correlated among the control with paracetamol group and the different doses of *Benincasa hispida*. The P value ≤ 0.05 was considered to be statistically significance at 95% confidence interval.

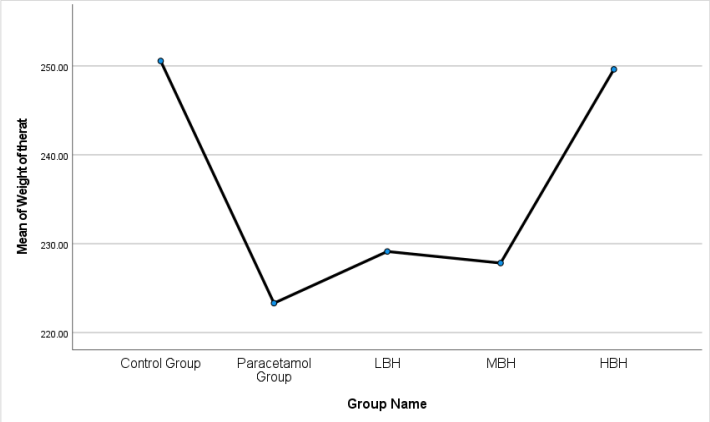

Figure 1. Mean terminal weight of the Wister albino rats

Table 1. The terminal body weight of the Wister Albino Rats

Group	Body weight (Mean ± SEM)	DF	F	Significance
Control group	250.56 ± 1.27	4	90.111	0.000
Paracetamol	223.31 ± 2.23	4	90.111	0.000
LBH	229.13 ± 0.82	4	90.111	0.690
MBH	227.23 ± 1.25	4	90.111	0.299
HBH	249.62 ± 0.66	4	90.111	0.000

Key: Paracetamol group was given 1500mg/kg of Paracetamol to induce hepatotoxicity, LBH, MBH, HBH group was given 100mg/kg, 200mg/kg, 300mg/kg of Benincasa hispida concurrently with 1500mg/kg of paracetamol respectively. SEM – Standard error of mean. Control group was only given water adlibitum. DF – Degree of freedom, F – Value of distribution.

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There was significance ($p < 0.05$) decrease in the terminal body weight of the paracetamol group as differentiated with the control. However, there was statistical ($p \leq 0.05$) significance of the body weight among the High dose of the *Benincasa hispida* group as compared to the paracetamol (Table 1).

3.2. Weight, volume, width and thickness of the liver compared among the control and treatment groups

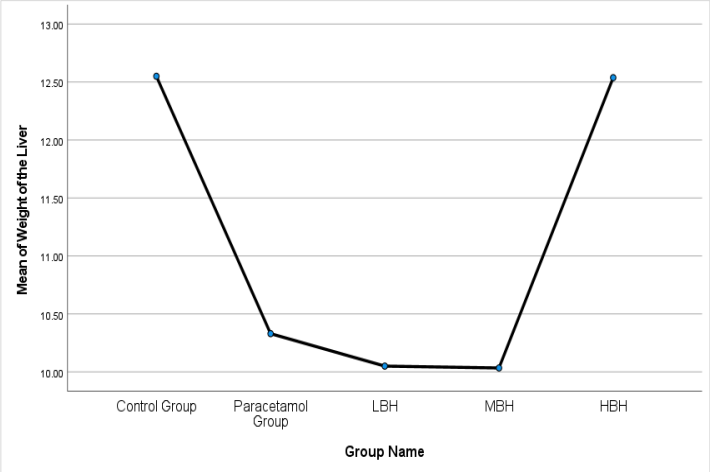

Figure 2. The mean weight of the weight of the Wister albino rats

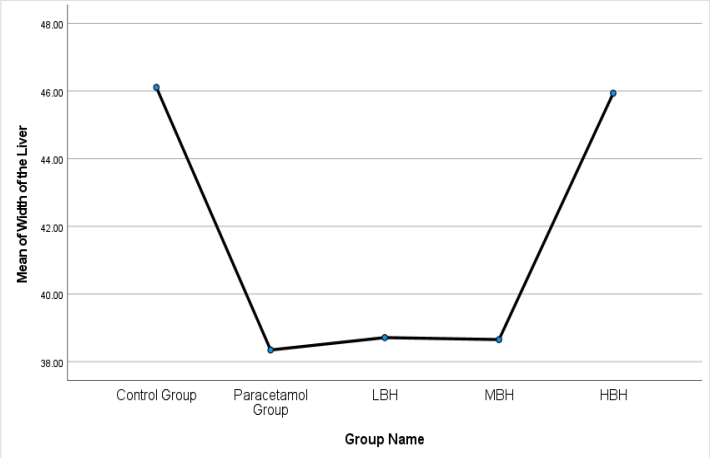

Figure 3. Mean Width of the Weight of the Wister albino rats

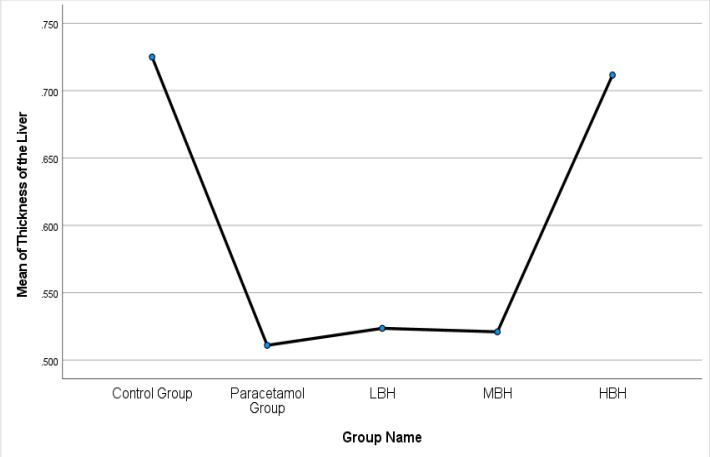

Figure 4. Mean thickness of the liver

Table 2. Mean weight, volume, width and thickness of the liver compared among the control and treatment groups

Groups	Weight of the liver	Volume of the liver	Width of the liver	Thickness of the liver	DF
Control group	12.55 ± 0.23	58.42 ±0.27	46.11 ± 0.16	0.73 ± 0.01	4
Paracetamol	10.33 ± 0.13	48.56 ± 0.34	38.35 ± 0.38	0.51 ± 0.01	4
LBH	10.05 ± 0.38	48.64 ± 0.41	38.71 ± 0.29	0.52 ± 0.12	4
MBH	10.05 ± 0.07	48.15 ± 0.29	38.65 ± 0.45	0.52 ± 0.01	4
HBH	12.54 ± 0.17	58.56 ± 0.34	45.94 ± 0.21	0.71 ± 0.01	4
F	83.44	276.59	169.150	108.31	

Key: Paracetamol group was given 1500mg/kg of Paracetamol to induce hepatotoxicity, LBH, MBH, HBH group was given 100mg/kg, 200mg/kg, 300mg/kg of Benincasa hispida concurrently with 1500mg/kg of paracetamol respectively. SEM – Standard error of mean. Control group was only given water adlibitum. DF – Degree of freedom, F – Value of distribution.

There was a significance ($p < 0.001$) reduction in the weight, length, width and thickness of the liver in the Paracetamol group as related with the control group. There was statistical ($p < 0.001$) difference of the weight, length, width and thickness of the liver of the HBH group as compared to the Paracetamol group (Table 2).

4. Discussion

Gross morphometrical parameters such as the Volume, Weight, Length, Width and the Height of the liver can be used in assessment of the physiological stress effects of the hepatotoxic activities (El Sayed et al., 2024). This study recorded significant change in the body weight of Wister albino rats of the control group as compared to the experimental groups these findings were in agreement with (Olajide et al., 2020) who also recorded significant changes in the weight of the Wister Albino rats in the control group as compared to the experimental groups. This is attributed to the physiological stress imposed on the experimental groups by the Paracetamol-induced hepatotoxicity.

The weights of the liver in the paracetamol, medium and low doses of *Benincasa hispida* experimental groups were significantly reduced as compared to the control group. These study findings were in line with (Khalid et al., 2022) who reported similar findings when melittin was attenuating Isoniazid and Rifampicin hepatotoxicity. The weight of the liver in the control group had no significant change as compared to the High dose group and these suggested hepatoprotection and these findings are in agreement with the (Buabeid et al., 2022) who also recorded the same results when *Solanum lycopersicum* was attenuating isoniazid and rifampicin-induced hepatotoxicity in Wistar albino rats.

The volumes of the liver in the paracetamol, medium and low doses of *Benincasa hispida* experimental groups were significantly reduced as compared to the control group. This study concurred with (Baran et al., 2022) who recorded the same results in hepatotoxicity induced by radiation and the protective effect of quercetin. This could be as a result of the shrinkage of the interstitial parts of the liver due to drug-induced hepatotoxicity. These findings differed with the (Ogunmoyole et al., 2021) who reported different findings where the Volume of the experimental

group increased significantly when compared to the control in *Mucuna pruriens* leaves extracts ameliorating carbon tetrachloride and rifampicin-induced hepatotoxicity. This may be attributed to the hypertrophy of the liver parenchyma cells hence increasing the volume. The Volume of the liver in the control group had no significant change as compared to the High dose group, and these suggested hepatoprotection, these findings are in agreement with (Libamila et al., 2023) who recorded no significant change in the liver volume when Liv-52 was protecting the liver from isoniazid and rifampicin induced hepatotoxicity.

The width and thickness of the liver in the paracetamol, medium and low doses of *Benincasa hispida* experimental groups were significantly reduced as compared to the control group. This study was in tandem with the findings of (Libamila et al., 2023) who recorded similar findings in rifampicin and isoniazid-induced hepatotoxicity. These might be a result of the atrophy of the liver cells that led to the decrease in length and width. The width and thickness of the liver in the control group had no significant change as compared to the High dose group and these suggested hepatoprotection and these findings are in agreement with the (Libamila et al., 2023) who recorded no significant change in the liver width and thickness when Liv-52 was protecting the liver from isoniazid and rifampicin-induced hepatotoxicity.

5. Conclusion

The gross morphological parameters such as the terminal body weight, Volume, thickness, and weight of the liver can be used in assessing the histopathological changes of the hepatoprotective effects of *Benincasa hispida* on paracetamol-induced hepatotoxicity. This will improve the quality of life in the population that is affected.

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis and manuscript writing equally.

Ethical approval

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