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The Protective Effects of Ethanolic Extract of *Vitis venifera* (Grape Leaf) on Spleen Induced with Paracetamol –In Adult Male Wistar Rats

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ABSTRACT

Aim: This study aimed to investigate the protective effects of ethanolic extract of *Vitis vinifera* on paracetamol-induced splenic toxicity in adult male Wistar rats.

Study Design: Twenty-five adult Wistar rats were randomly assigned into five groups (n=5). Group A served as the control group, Group B received 100 mg/kg of *Vitis vinifera* extract only, Group C received 100 mg/kg of paracetamol only, Group D received 100 mg/kg of paracetamol followed by treatment with 100 mg/kg of *Vitis vinifera* extract, while Group E received 100 mg/kg of paracetamol together with 200 mg/kg of *Vitis vinifera* extract.

Place and Duration of Study: This study was carried out at the Department of Human Anatomy, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria, over a period of four weeks.

Methodology: Twenty-five adult Wistar rats weighing between 150–190 g were acclimatized and randomly distributed into five experimental groups. Ethanolic extract of *Vitis vinifera* was administered orally for four weeks. At the end of the treatment period, the animals were anaesthetized using chloroform and sacrificed. The spleen tissues were harvested, fixed in 10% formalin, and processed for histological examination using Haematoxylin and Eosin staining technique. Morphometric analysis of body weight was also carried out, and data obtained were analyzed statistically with significance considered at $p<0.05$.

Results: Morphometric analysis revealed a significant ($p<0.05$) decrease in body weight in the treatment groups following paracetamol administration when compared with the control group. Histological examination of the spleen in Group C revealed severe focal aggregation of inflammatory cells and severe necrosis following administration of paracetamol. However, treatment with ethanolic extract of *Vitis vinifera* in Groups D and E demonstrated varying degrees of regeneration and restoration of splenic tissue architecture. The regenerative effect was more pronounced in Group E treated with the higher dose (200 mg/kg) of the extract, indicating a dose-dependent protective effect of the extract against paracetamol-induced splenic toxicity.

Conclusion: The findings of this study suggest that ethanolic extract of *Vitis vinifera* possesses protective and restorative effects against paracetamol-induced splenic toxicity in Wistar rats. The extract demonstrated dose-dependent ameliorative properties by reducing inflammatory changes and enhancing regeneration of splenic tissues following toxic injury.

Keywords: *Vitis venifera,* paracetamol, inflammation, histology, wistar rats

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INTRODUCTION

The spleen is an important secondary lymphoid organ responsible for immunological surveillance, blood filtration, erythrocyte destruction, and hematopoietic regulation. Due to its extensive vascularization and immunological activities, the spleen is highly susceptible to toxic insults caused by xenobiotics, pharmaceutical agents, environmental contaminants, and oxidative stress-inducing compounds. Drug-induced splenic injury has increasingly become a significant concern in toxicological and biomedical research because of the widespread and often uncontrolled use of analgesic medications such as paracetamol (acetaminophen). Although paracetamol remains one of the most frequently prescribed and self-administered analgesic and antipyretic drugs worldwide, overdose and prolonged administration have been associated with severe cellular degeneration and tissue necrosis in several organs including the liver, kidneys, brain, and spleen (James et al., 2003).

Paracetamol toxicity is largely mediated through the excessive formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes depletion of intracellular glutathione and enhances oxidative stress. Increased production of reactive oxygen species (ROS) subsequently leads to lipid peroxidation, inflammatory reactions, mitochondrial dysfunction, and cellular apoptosis. Histopathological manifestations of paracetamol-induced toxicity commonly include inflammatory cell infiltration, tissue necrosis, vascular congestion, and distortion of normal tissue architecture.

In recent years, there has been growing scientific interest in medicinal plants because of their therapeutic phytochemicals and antioxidant properties. Bioactive compounds such as flavonoids, phenols, tannins, alkaloids, anthocyanins, and saponins possess potent free radical scavenging abilities capable of ameliorating oxidative tissue damage. Consequently, plant-derived therapies are increasingly being explored as safer and cost-effective alternatives for the prevention and management of drug-induced organ toxicity.

Vitis vinifera (grape plant), belonging to the family Vitaceae, is widely recognized for its nutritional and medicinal importance. The leaves, fruits, and seeds of the plant contain abundant polyphenols including resveratrol, catechins, quercetin, proanthocyanidins, and anthocyanins, which possess anti-inflammatory, antioxidant, anti-apoptotic, and cytoprotective activities. Studies have shown that grape-derived phytochemicals exert protective effects against oxidative stress by stabilizing cellular membranes, reducing inflammatory mediators, and improving endogenous antioxidant defense systems (Xia et al., 2010).

Several experimental studies have demonstrated the protective effects of medicinal plants against chemically induced organ damage. Nweke et al. (2019) reported that ethanolic extract of *Annona muricata* exhibited significant hepatoprotective activities by improving liver architecture and reducing oxidative stress-mediated hepatic degeneration in experimental animals. Similarly, Nweke et al. (2025) evaluated the effect of ethanolic extract of *Solanum torvum* (eggplant) on renal health using Wistar rats and observed marked improvement in renal histology and biochemical parameters following treatment with the extract. Their findings further support the therapeutic potential of phytochemical-rich plant extracts in ameliorating toxicological damage.

In another related study, Nweke et al. (2019) investigated the protective effect of *Anacardium occidentale* on liver enzymes in paracetamol-induced toxicity in Wistar rats and reported significant restoration of altered liver enzyme activities following administration of the plant extract. The study attributed the observed hepatoprotective effects to the antioxidant constituents of the plant capable of scavenging reactive oxygen species generated during paracetamol metabolism.

Furthermore, recent neurotoxicological investigations by Anyiam et al. (2025) demonstrated the neuroprotective effects of ethanolic extract of *Aloe vera* on mercury-induced damage in the rat amygdala and hippocampus using histological and molecular docking approaches. The study revealed that the extract significantly reduced neuronal degeneration, oxidative stress, and tissue distortion caused by mercury toxicity. Their findings emphasized the growing importance of medicinal plants in mitigating oxidative stress-related tissue injuries through antioxidant and anti-inflammatory mechanisms.

Additional studies have also documented the efficacy of medicinal plants in ameliorating chemically induced organ damage. Uzozie, et al., (2019) reported that ethanolic extract of *Cucumis sativus* pulp significantly restored renal histology and kidney function in alloxan-induced nephrotoxicity in Wistar rats in a dose-dependent manner.

Despite numerous reports on paracetamol-induced hepatotoxicity and nephrotoxicity, there is limited information regarding the histological effects of paracetamol on splenic tissues and the potential protective role of *Vitis vinifera* leaf extract. Therefore, this study was designed to investigate the protective effects of ethanolic extract of *Vitis vinifera* (grape leaf) on paracetamol-induced splenic toxicity in adult male Wistar rats using morphometric and histological assessments. Findings from this study may further establish the medicinal relevance of *Vitis vinifera* and contribute to the development of plant-based therapeutic interventions against drug-induced splenic injury.

MATERIALS AND METHODS/ EXPERIMENTAL DETAILS / METHODOLOGY

ANIMALS USED

A total of 25 female wistar rats weighing between (150-250g) were used in this study. The experimental animals were obtained from Tochukwu Farm Nnewi, Anambra state and maintained in the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences Chukwuemeka Odumegwu OjukwuUniversity Uli. According to the principles of laboratory animal care, (National Institutes of Health Publication, 1985). The animals were weighed and randomly assigned to five (5) different cages and left to acclimatize for two (2) weeks. They were fed with normal rat chow bought from vital feed (Growers) made by Grand cereals limited a subsidiary of UAC Nigeria PLC KM 17, Zawan roundabout, Jos, Plateau State.

MATERIALS USED

- Cage
- Syringes and needles
- Normal rat chow
- Feeding tube
- Fresh vitis venifera leaves
- Paracetamol
- EDTA bottle
- Hand gloves
- Capillary tube
- Ketamine
- Saw dust (bedding material)
- Animal feed (Growers mesh)
- Laboratory coat and gloves
- Dissecting set and dissecting board
- Electronic weighing balance (NAPOO precision instrument JA-410)
- Animal weighing balance (ARMY)IILBX102
- Thermostat Oven DHG-9023A PEC MEDICAL USA.
- Measuring cylinder and plastic specimen bottle
- Water bath
- Desiccator
- Sample bottles
- 10% formal saline
- Cotton wool and anesthesia (diethyl ether)
- Graded alcohol (10%, 50%, 90% and absolute alcohol)
- Glass slides
- Hot plate
- Xylene

- Paraffin wax (molten)
- Embedding mould and pot
- Derpex (DPX) mountant
- Haematoxylin and eosin stain
- Light microscope
- Slide rack
- Mechanical shaker(Uniscope sm101)
- Diamond pencil
- Paracetamol
- Rotary Evaporator (Digital)TT-52 Techmel & Techmel USA.

ACUTE TOXICITY STUDY OF PARACETAMOL

The median lethal dose (LD50) of paracetamol was carried out in the department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Okofia campus. This was determined using the method of Dietrich Lorke (1983). In this study, a total of 13 rats were used. They received the extract via oral route and it was carried out in two phases.

PHASE I

Nine (9) rats were used and they were grouped into three groups of three rats each.

- Group 1 received 10mg/kg
- Group 2 received 100mg/kg
- Group 3 received 1000mg/kg

The animals were observed over a period of 24 hours for mortality. From the result of phase I, the second phase was carried out. In this phase, four (4) rats were used and they were grouped into four groups of one animal per group.

PHASE II

- Group 1 received 1200mg/kg
- Group 2 received 1600mg/kg
- Group 3 received 2900mg/kg
- Group 4 received 5000mg/kg

The animals were monitored over a period of another 24hours for mortality.

PHASE	DOSE	DEATH	OBSERVATION
1	10mg/kg	0/3	……………
	100mg/kg	0/3	……………
	1000mg/kg	0/3	……………
2	1200mg/kg	0/1	……………
	1600mg/kg	0/1	The animal was weak, died within 24hrs.
	2900mg/kg	1/1	Agitated and died within 12hrs.
	5000mg/kg	1/1	The animal died within 12 hours.

$LD_{50} = \sqrt{axb}$

A= maximum dosed with 0% mortality is 1600mg/kg

B= minimum dosed with 100% mortality is 2900mg/kg

$LD_{50} = \sqrt{axb}$

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$\sqrt{1600x\ 2900} = 46400000$

LD_{50}= 2154.06mg/kg

LD_{50} of acetaminophen is 2154.06mg/kg.

DETERMINATION OF DOSAGE

Paracetamol was obtained from the pharmaceutical unit at Chukwuemeka Odumegwu Ojukwu University, Uli.

1tablet of paracetamol contains 500mg

2tablets = 2x500 = 1000mg

Each group received 1000mg/kg

Dissolve 2 tablets in 100ml distilled water

Average body weight of rats-150g

150g to kg= 150/1000=0.15

0+.15x1000/100= 1.5ml

ACUTE TOXICITY STUDY OF ETHANOLIC LEAF EXTRACT OF *VITIS VENIFERA LEAF*

The median lethal dose (LD_{50}) of ethanolic leaf extract of *Vitis venifera leaf* was carried out in the department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Okofia Campus. This was determined using a method of Dietrich Lorke (1983). In this study, 13 rats were used. They received the extract via oral route and it was carried out in two phases.

PHASE I

A total of nine (9) rats were employed for the study and three rats were allocated for each group.

Group 1 received 10mg/kg per rat

Group 2 received 100mg/kg per rat

Group 3 received 1000mg/kg per rat

The animals were monitored for 24hrs for morbidity and mortality. The rats remained normal after 24 hours of observation in phase 1. Then, the study proceeded to the second phase were four rats were employed for the study comprising one rat per group.

PHASE II

Group 1 received 1200mg/kg per rat

Group 2 received 1600mg/kg per rat

Group 3 received 2900mg/kg per rat

Group 4 received 5000mg/kg per rat.

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The animal were monitored for another 24hrs for morbidity and mortality

PHASE	DOSE	DEATH	OBSERVATION
1	10mg/kg	0 / 3	The rats remained normal
	100mg/kg	0 / 3	The rats remained normal
	1000mg/kg	0 / 3	The rats remained normal
2	1200mg/kg	0 / 1	The rat remained normal
	1600mg/kg	0 / 1	The rat remained normal
	2900mg/kg	0 / 1	The rat remained normal
	5000mg/kg	0 / 1	No death occurred

$LD_{50} = \sqrt{a \times b}$

A= maximum dose with 0% mortality

B= minimum dosed with 100% mortality

$LD_{50} = \sqrt{a X b}$

LD_{50} of ethanolic leaf extract of *Vitis venifera* is above 5000mg/kg.

PREPARATION OF ETHANOLIC EXTRACT OF *VITIS VENIFERA*

Fresh leaves of *Vitis veinifera* were collected from Uli, Anambra state and washed of debris. The leaves were dried under ambient room temperature after which it was grounded using laboratory mill into coarse form 50g of the powdered leaves was macerated into 250mls of ethanol and was allowed to stay for 48hrs inside Mechanical shaker (Uniscope sm101), after which it was sieved using porcelain cloth into a clean bottle and was further filtered using filter paper. The filtrate was concentrated using Rotary Evaporator (Digital)TT-52 Techmel & Techmel USA. The mixture was further dried using Thermostat oven DHG-9023A PEC MEDICAL USA. The extract was stored in the refrigerator (Nexus) for future use.

DRUG AND EXTRACT ADMINISTRATION

GROUP A - These animals were the positive controls, they were fed with only the normal rat chow and water for the duration of the experiment.

GROUP B - The animals in this group received *Vitis veinifera* extract (100mg/kg) only for 21 days with no treatment. They were also fed with rat chow and water.

GROUP C - These animals received paracetamol (100mg/kg) only for 21 days and then They were also fed with rat chow and water.

GROUP D - The animals here, received paracetamol (100mg/kg) for three days and then treated with low dose of *Vitis veinifera* extract (200mg/kg). They were fed with rat chow and water.

GROUP E - The animals of this group were given paracetamol (100mg/kg) with high 200mg/kg ofV*vitis veinifera* extract. They also were fed with rat chow and water.

The extract was administered once daily for a duration of 4 weeks through the orogastric tube while the control group received equal volume of distilled water and feed for the same period.

At the end of the fourth week, chloroform was used to anesthetize the rats and the abdominal cavity was opened through a transverse abdominal incision. The liver was carefully removed, washed in normal saline solution.

HISTOLOGICAL STUDIES

The spleen collected was rinsed in normal saline solution and then the following tissue procession took place.

FIXATION

The spleen was fixed in 10% formal saline, in a container with light fitting lids for three (3) days to prevent autolysis, improve staining quality and also to aid optical differentiation of cells.

DEHYDRATION

The tissue was dehydrated to remove water that is immiscible with xylene and wax. This was done using different grades of alcohol ranging from 50% - absolute alcohol for 30 minutes each.

CLEARING/ DEALCHOLIZATION

The dehydrated tissue was cleared by removing the alcohol from the tissue. It was done by immersing the tissue through 3 changes of xylene for 30 minutes each.

WAX IMPREGNATION/ INFILTRATION

The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in a hot oven temperature of 60 degrees Celsius by passing it through three (3) changes of molten paraffin wax in a hot air oven for 30 minutes each.

EMBEDDING

The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify.

MOUNTING ON WOODEN BLOCK

The paraffin block of tissue was attached to a wooden block with the aid of a hot spatula held in between wood block and paraffin wax, the spatula melted the wax which solidified when spatula was removed.

MICROTOMY

The block of tissues was sectioned using Berg's rotary microtome machine. It was trimmed to obtain the cutting surface of the tissue at 15 microns and was sectioned at 5 microns. It was later dried in a hot plate for staining.

HAEMATOXYLIN/EOSIN STAINING PROCEDURE

PROCEDURE

- 3g of eosin powder was measured out and dissolved in 100mls of water.
- The slides were dewaxed in xylene for 30 minutes.
- The slides were placed in descending grades of alcohol ranging from absolute alcohol, 90%, 70%, 50% for 2 seconds each to remove xylene.
- Then rinsed in 2 changes of water.
- The tissue is then stained in haematoxylin for 20 minutes.
- Then rinsed in water again to remove excess stain.
- The tissue was differentiated in 1% acid alcohol for 1 minute. This is called bluing; it gives the tissue its characteristic background.
- Then the tissue was counter stained in eosin for 5 minutes depending on the strength of the eosin.
- The tissue is rinsed in water to remove excess stain.
- It is then mounted in D.X.P (Distrene Plasticider and Xylene) and dried for micrograph and interpretation.

Declaration of Interest

None

Funding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Statistical Analysis

Research objectives and hypothesis of the study was considered before analyzing data. The results were statistically analyzed using the SPSS software All Statistical data were analyzed using One-way Anova, followed by multiple comparism using Bonferroni, and data were considered significant at P<0.05. *P<0.05 shows that it was significant, and **P<0.05 shows that it was more significant. The results were expressed as mean± S.E.M.

RESULTS

Table 1: Effect of ethanolic extract *Vitis vinifera* on body weight following Paracetamol toxicity

	Initial body weight (g)	Final body weight (g)	p-value	t-value
	MEAN±SEM	MEAN±SEM		
Group A (control)	60.60±3.96	87.90±4.34	0.007*	-5.183
Group B (200 mg/kg of EVV)	90.00±3.00	78.00±0.00	0.057#	4.000
Group C (100 mg/kg PCM)	91.00±3.78	87.50±8.94	0.572#	0.670
Group D (100 mg/kg PCM + 100 mg/kg EVV)	88.66±4.66	95.50±0.28	0.289#	-1.429
Group E (200 mg/kg PCM + 200 mg/kg EVV)	84.66±5.89	81.00±5.19	0.235#	1.677

SEM: standard error of mean, PCM: paracetamol, EBV: ethanolic extract ***Vitis vinifera*** *: significant, #: not significant

Table 1 result showed a significant increase in the body weight when the initial weight was compared to the final weight in group A. However, groups B, C, D, and E had no significant difference in the body weight comparison but revealed a groups B, C, and E; group D had an increase.

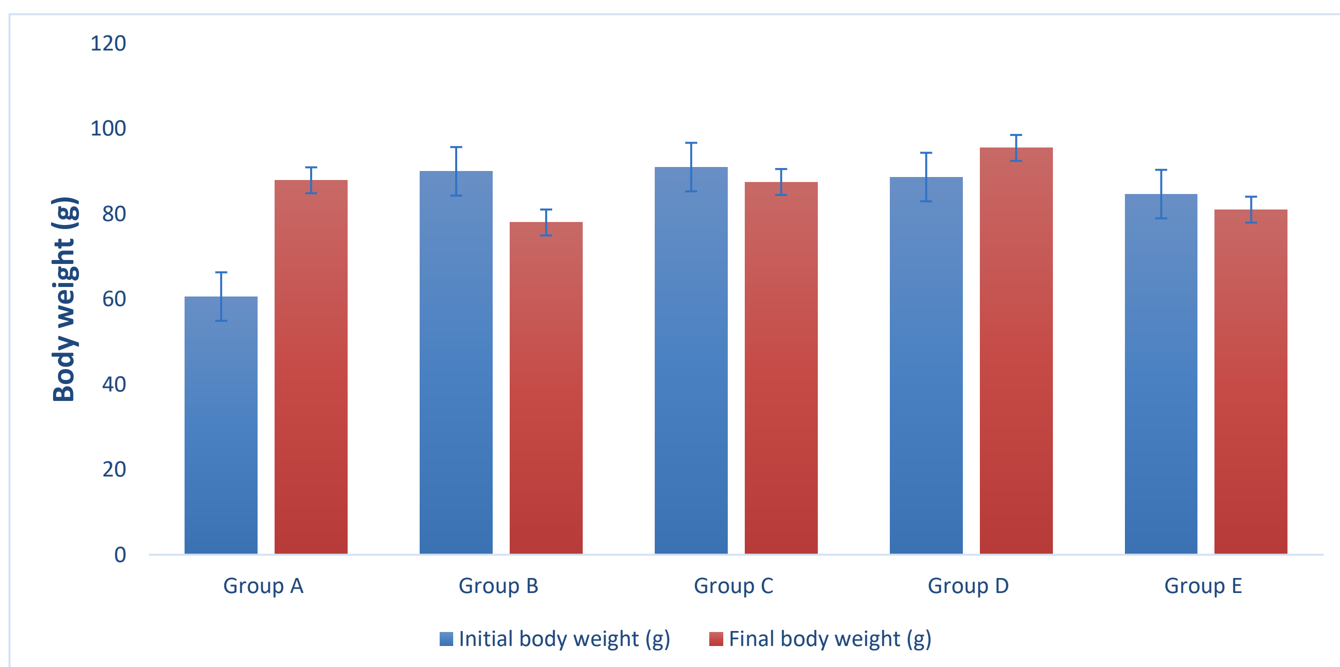


Fig 1: **effect of ethanolic extract *Vitis vinifera* on body weight following Paracetamol toxicity**

Table 2: effect of ethanolic extract of ***Vitis vinifera*** dried fruit on relative spleen weight following **Paracetamol** -induced toxicity

Groups (n=5)	**Relative spleen weight (g)**
	Mean±SEM
Group A (control)	0.39±0.03
Group B (200 mg/kg of EVV)	0.25±0.06#$
Group C (100 mg/kg PCM)	0.34±0.02#b
Group D (100 mg/kg PCM + 100 mg/kg EVV)	0.40±0.10#b$
Group E (200 mg/kg PCM + 200 mg/kg EVV)	0.31±0.02#b$
P-value	0.431
F-ratio	1.047

SEM: Standard error of mean, EVV: ethanolic extract of ***Vitis vinifera*** leaves; *: significant, #: not significant compared to group A, @: significant, $: not significant when compared to group C. a: significant, b: not significant compared to group B

Table 2 result revealed a non-significant decrease in in the relative spleen weight in groups B, C, and E (*p=0.136, p=0.569, p=0.369*), group D (*p=0.875*) had a non-significant increase compared to group A. Also, groups C, D, and E (*p=0.327, p=0.15, p=0.513*) had a non-significant increase compared to group B. Further, groups B and E (*p=0.327, p=0.732*) had a non-significant decrease and group D (*p=0.471*) had non-significant increase compared to group C.

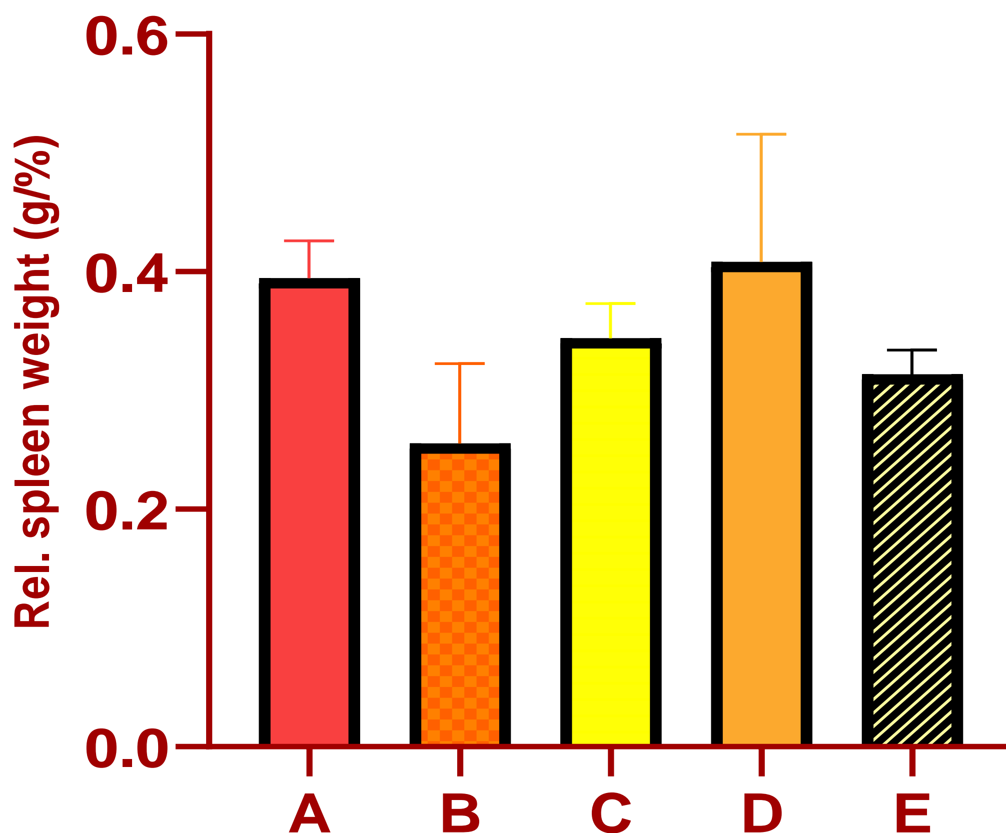
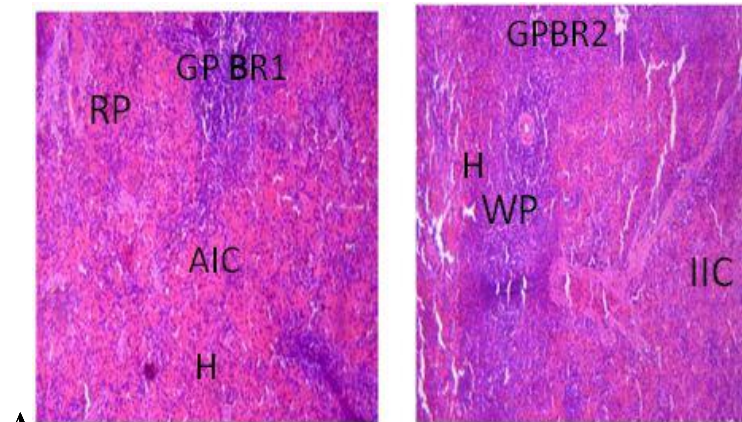
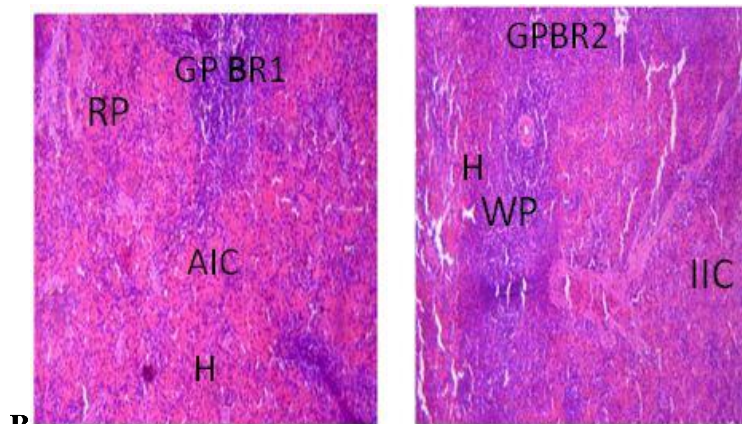


Fig 2: effect of ethanolic extract of ***Vitis vinifera leaves*** on relative spleen weight following paracetamol-induced toxicity

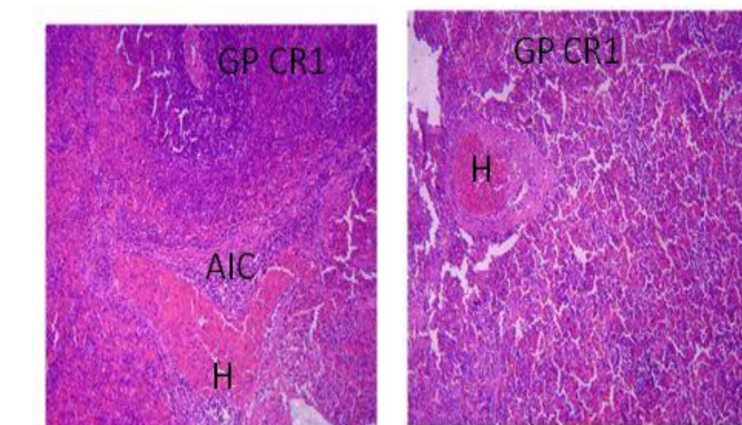
HISTOLOGICAL ANALYSIS



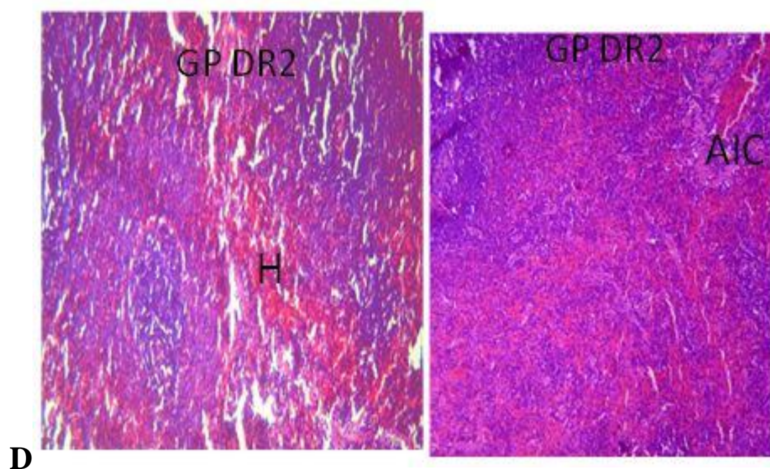
A



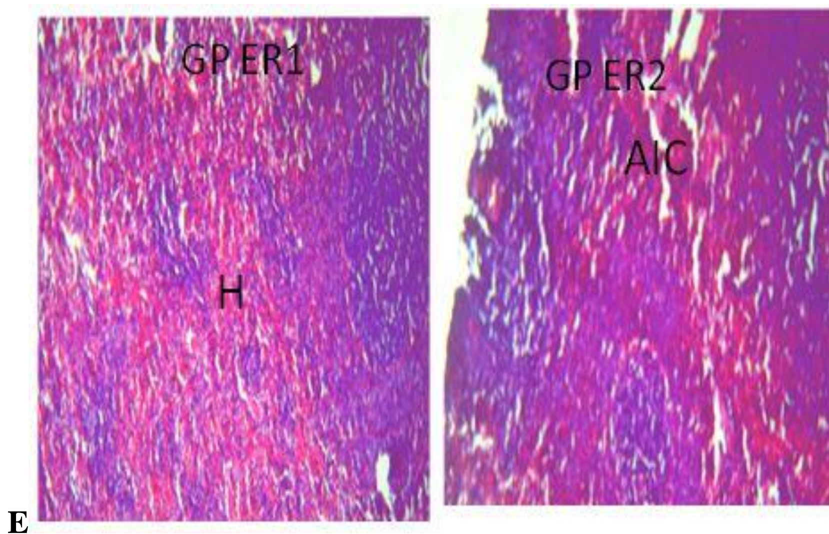
B



C



D



E

Fig. 3: Photomicrograph of spleen; (a)AR1R2 section of the spleen on (X150)(H/E) shows normal splenic tissue with well outlined white and red pulp, (b)Group BR1R2 section of the spleen administered with 100mg/kg of extract of Vitis venifera (X150)(H/E) shows active splenic tissues with well outlined whilte pulp(WP) and red pulp(RP),(c)Group CR1R2 section of spleen induced with 100mg/kg paracetamol (X150)(H/E) shows severe effect on the splenic tissue with severe focal area of necrosis(N), Severe aggregation of inflammatory cells(AIC) around the hemorrhage(H) area, (d)Group DR1R2 section of spleen induced with 100mg/kg paracetamol treated with Vitis venifera 100mg/kg low dose of extract (X150)(H/E) shows mild healing with moderate aggregate of inflammatory cells (AIC) and focal area of hemorrhage(H) However, the white and red pulp area diffusely infiltrated in R1,(e)Group ER1R2 section of spleen induced with 200mg/kg paracetamol treated with Vitis venifera 200mg/kg (X150)(H/E) shows mild healing with moderate aggregate of inflammatory cells (AIC) and focal area of hemorrhage(H) However, the white and red pulp area diffusely infiltrated in R1

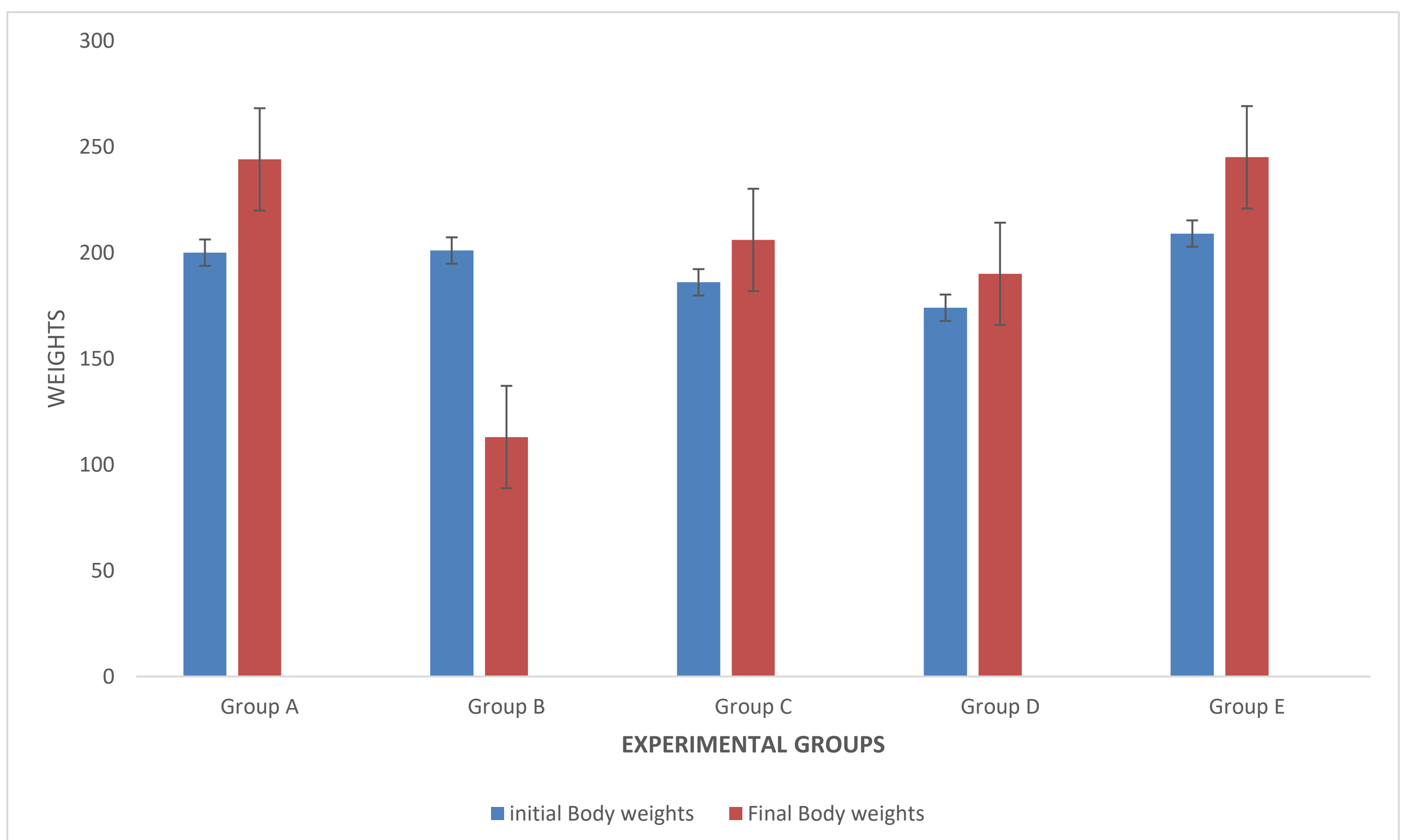


Fig. 4. Body weights of animals

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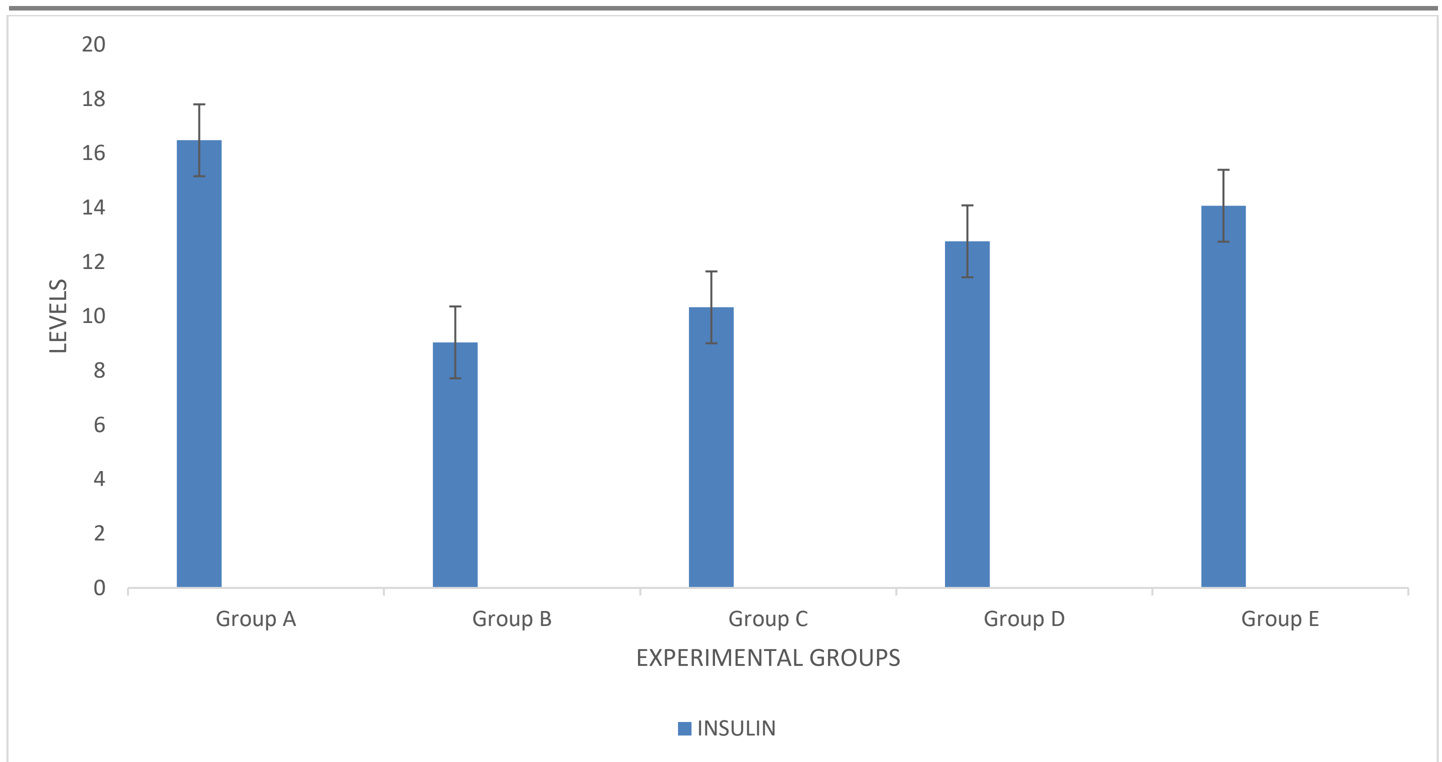

Fig. 5. Insulin levels of the animals

DISCUSSION

Medicinal plants continue to play significant roles in both traditional and modern healthcare systems because of their diverse pharmacological properties and relatively low toxicity The therapeutic properties of many medicinal plants have been linked to the presence of biologically active phytochemicals such as flavonoids, alkaloids, tannins, saponins, phenols, and polyphenolic compounds, which possess potent antioxidant, anti-inflammatory, and cytoprotective activities capable of mitigating oxidative stress-mediated tissue damage (Zhang and Tsao, 2016).In recent years, increasing scientific attention has been directed toward the use of plant-derived compounds in the management of oxidative stress-mediated tissue injuries induced by drugs and environmental toxicants.

The present study investigated the protective effects of ethanolic extract of *Vitis vinifera* leaf on paracetamol-induced splenic toxicity in adult male Wistar rats. Histological examination of the spleen from the control group revealed normal splenic architecture characterized by well-defined white pulp and red pulp regions. Similarly, animals treated with *Vitis vinifera* extract alone showed preserved splenic histology with intact tissue organization, suggesting that the extract did not exert toxic effects on splenic tissues at the administered dose.

However, administration of paracetamol alone resulted in severe histopathological alterations including focal necrosis, hemorrhage, and aggregation of inflammatory cells within the splenic tissue. These findings indicate that paracetamol overdose induces significant splenic injury, likely mediated through oxidative stress, inflammatory responses, and cellular degeneration. Similar observations have been reported in studies evaluating toxicant-induced organ damage in experimental animals. Previous studies have shown that excessive metabolism of paracetamol leads to the formation of the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which induces glutathione depletion, oxidative stress, lipid peroxidation, mitochondrial damage, and eventual cellular necrosis (McGill & Jaeschke, 2013).Additionally, oxidative stress and mitochondrial impairment have been identified as major mechanisms involved in acetaminophen-induced cellular and tissue injury, particularly following excessive production of reactive metabolites and depletion of endogenous antioxidant defenses (Jaeschke et al., 2012).

The severe aggregation of inflammatory cells and necrotic changes observed in the untreated paracetamol group in this study may therefore be linked to oxidative tissue damage induced by reactive oxygen species

generated during paracetamol metabolism. Similar inflammatory and degenerative alterations were also reported by Onyejike et al. (2024) in their study on the nephrotoxic effects of Odogwu bitters and Goko cleanser on Wistar rat kidneys, where exposure to toxic substances caused severe distortion of tissue architecture and inflammatory infiltration.

Treatment with ethanolic extract of *Vitis vinifera* following paracetamol administration resulted in varying degrees of tissue regeneration and histological improvement. Animals treated with higher doses of the extract demonstrated better restoration of splenic architecture with reduced inflammatory cell infiltration and diminished necrotic lesions compared to untreated groups. This suggests that the protective effects of *Vitis vinifera* are dose-dependent and may be associated with its antioxidant phytoconstituents such as resveratrol, anthocyanins, flavonoids, and proanthocyanidins.

The observed regenerative effects in this study are consistent with previous findings on the protective activities of medicinal plants against toxicant-induced organ injury. Uzozie et al. (2019) reported that ethanolic extract of *Cucumis sativus* significantly ameliorated alloxan-induced renal toxicity in Wistar rats by restoring tissue architecture and improving histological integrity in a dose-dependent manner. Similarly, Nweke et al. (2024) demonstrated that methanol seed extract of unripe *Carica papaya* exerted hepato- and reno-protective effects against carbon tetrachloride-induced toxicity through restoration of normal tissue morphology and reduction of oxidative stress.

Furthermore, recent studies have continued to establish the therapeutic relevance of medicinal plants in toxicological research. Anyiam et al. (2025) demonstrated that ethanolic extract of *Aloe vera* significantly protected the amygdala and hippocampus against mercury-induced neurotoxicity by reducing neuronal degeneration and improving tissue histology. Likewise, Onyejike et al. (2025) reported that *Tetracarpidium conophorum* exhibited substantial hepatoprotective activity in mercury-exposed Wistar rats through attenuation of oxidative stress and restoration of hepatic architecture.

The protective effects observed in the present study may therefore be attributed to the antioxidant and anti-inflammatory properties of *Vitis vinifera*. Grape-derived polyphenols have been reported to scavenge free radicals, inhibit lipid peroxidation, stabilize cellular membranes, and enhance endogenous antioxidant defense systems (Xia et al., 2010). These biological activities likely contributed to the reduced inflammatory reactions and enhanced tissue regeneration observed in the treatment groups.

CONCLUSION

Findings from this study demonstrated that ethanolic extract of *Vitis vinifera* leaf possesses protective effects against paracetamol-induced splenic toxicity in adult male Wistar rats. Histological observations revealed that treatment with the extract ameliorated necrosis, inflammatory cell aggregation, and hemorrhagic alterations induced by paracetamol administration. The protective activity of the extract appeared to be dose-dependent, with higher doses producing greater regenerative effects on splenic tissues. The study therefore suggests that *Vitis vinifera* leaf extract may serve as a potential therapeutic agent against oxidative stress-mediated splenic injury due to its antioxidant and cytoprotective properties.

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COMPETING INTERESTS

The authors declare that they have no known financial interests that influenced the work reported in this paper. The research was conducted independently, without any financial support, commercial sponsorship, or affiliations that might present a conflict of interest.

AUTHORS' CONTRIBUTIONS

'Nweke, Elizabeth Obioma' designed the study and managed the literature searches, 'Nweke, Miracle Tochukwu' wrote the protocol and performed the statistical analysis, and 'Anyiam, Kennedy Ekenedirichukwu' wrote the first draft of the manuscript…All authors read and approved the final manuscript.

ETHICAL APPROVAL

This was obtained from the ethical committee, faculty of basic medical sciences Chukwuemeka Odumegwu Ojukwu University, Uli campus in compliance with the relevant laws and institution's guidelines.

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee**.**

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