

Evaluation Of Hepatoprotective Potential Of Hydroalcoholic Leaf Extract Of Calotropis Gigantea Against Thioacetamide-Induced Hepatic Injury In Wistar Albino Rats

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ABSTRACT

Hepatotoxicity stands as a formidable challenge within the realm of medical science, demanding an in-depth exploration of the intricate interplay between various substances and the liver's vulnerability. The liver, a central metabolic powerhouse, intricately processes an extensive array of compounds, ranging from pharmaceutical drugs to herbal supplements and environmental toxins. Hepatotoxicity unfolds as a complex cascade of events, often involving the formation of reactive metabolites, oxidative stress, and inflammation. The hydroalcoholic extract of *Calotropis gigantea* demonstrates promising hepatoprotective potential in this experimental model of TAA-induced liver damage. The significant reduction in liver injury markers and the concurrent enhancement of antioxidant defenses suggest a multifaceted protective mechanism. Further research and exploration are warranted to elucidate the specific bioactive compounds responsible for these effects and to determine the translational potential of HCEG in mitigating liver damage in clinical contexts. These findings contribute to the understanding of herbal interventions for liver health and may pave the way for the development of novel therapeutic strategies for liver disorders.

Keywords: *Calotropis gigantea*, in vitro assays, medicinal plant, Hepato productive.

INTRODUCTION

Herbal medicine is the oldest form of health care system known to mankind and all living being including man has been troubling by many ailments from past so many centuries and most often nature has provided the cure. Herbs had been used by all cultures throughout history and are the important members of nature. Herbs has played the great role from years in combating the disease of human race.

The liver plays a critical role in human metabolism, contributing to various essential physiological functions. It is responsible for detoxifying harmful substances, synthesizing proteins, and producing vital biochemicals required for digestion. Some of its key roles include:

1. **Detoxification:** The liver filters toxins and waste products from the blood, neutralizing and eliminating harmful substances such as drugs, alcohol, and metabolic waste.
2. **Protein Synthesis:** It synthesizes several important proteins, including albumin (which

helps maintain blood volume and pressure) and clotting factors (which are essential for blood coagulation).

3. **Metabolism of Nutrients:** The liver regulates glucose, fat, and protein metabolism. It stores excess glucose as glycogen and releases it when needed to maintain blood sugar levels. It also converts excess nutrients into forms that can be stored or used by the body.
4. **Bile Production and Secretion:** The liver produces bile, a digestive fluid that is stored in the gallbladder and released into the small intestine when needed. Bile is essential for emulsifying fats, allowing them to be broken down and absorbed during digestion.
5. **Cholesterol and Lipid Regulation:** The liver is responsible for regulating cholesterol levels in the body by synthesizing and breaking down lipids. It also produces lipoproteins, which are vital for transporting fats through the bloodstream.

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While the liver is crucial for survival and no current technology can fully compensate for the absence of liver function long-term, it has an extraordinary ability to regenerate. If a portion of the liver is damaged or removed, healthy liver cells can grow back, allowing it to restore its function. This regenerative ability is one of the liver's most remarkable features. However, sustained damage (e.g., from chronic alcohol abuse, viral infections like hepatitis, or liver diseases such as cirrhosis) can overwhelm its capacity to regenerate, leading to irreversible damage and potentially liver failure.

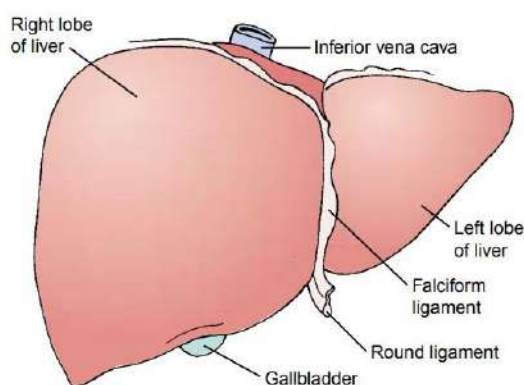


Figure 1. Anatomy Of Liver

Toxic liver injury, or **hepatotoxicity**, can be caused by a wide range of substances, including drugs, chemicals, and environmental or occupational exposures. These toxins can mimic natural liver diseases, making it difficult to distinguish them from conditions like hepatitis or cirrhosis. The severity of liver damage is often worsened if the harmful substance is not discontinued once symptoms begin to appear.

A) Inorganic Compounds:

Certain inorganic compounds are known to cause hepatotoxicity, including:

- **Arsenic:** A well-known toxin that can lead to chronic liver damage and has been linked to cancer in the liver and other organs.
- **Copper:** Excess copper in the body (e.g., in conditions like Wilson's disease) can accumulate in the liver, leading to hepatotoxicity and liver failure.
- **Phosphorus:** Exposure to phosphorus, especially in industrial settings, can cause severe liver damage, often manifesting as fatty degeneration and necrosis.
- **Iron:** Excessive iron, typically seen in conditions like hemochromatosis, can accumulate in liver cells, leading to oxidative stress and liver injury.

B) Organic Agents:

Organic compounds, including naturally occurring toxins from plants and fungi, also pose significant risks to liver health. Some of these agents include:

- **Pyrrolizidine Alkaloids:** Found in some plants, these compounds can cause severe liver damage, including fibrosis and cirrhosis, especially after prolonged exposure.
- **Mycotoxins:** Produced by certain fungi, these toxins can contaminate food and, when consumed, cause liver injury, often leading to acute hepatitis or chronic liver disease.
- **Alkaloids:** Some plant alkaloids, such as those found in Aconitum species (aconitine), can be hepatotoxic, leading to liver failure in severe cases.
- **Bacterial Toxins:** Some bacteria produce toxins that can lead to liver injury, such as those produced by Clostridium perfringens, which can cause liver necrosis.

C) Environmental, Occupational, and Domestic Exposure:

Exposure to hepatotoxic compounds can occur in various settings:

- **Environmental:** People may be exposed to liver-damaging chemicals through polluted air, water, or food.
- **Occupational:** Workers in industries dealing with chemicals such as solvents, pesticides, or heavy metals may be at higher risk of developing hepatotoxicity.
- **Domestic:** Accidental ingestion of toxic substances like household cleaning agents or pesticides can result in liver damage.
- **Intentional Ingestion:** In rare cases, toxic liver injury may be due to **homicidal or suicidal** ingestion of chemicals or drugs. This can include the intentional consumption of substances like toxic mushrooms, industrial chemicals, or poisons.

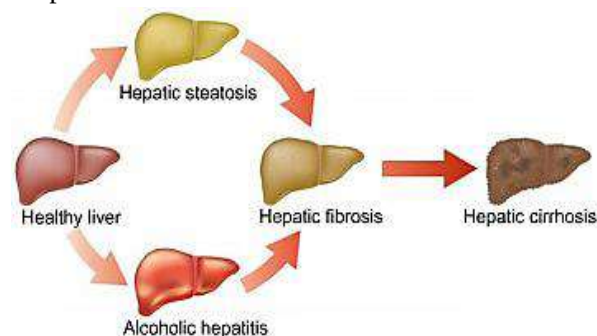


Figure 2. Liver Disease

1. PLANT PROFILE:

Medicinal plant is used from the ancient times as the major sources of drugs. The main fact is that, we can obtain various life-saving drugs are present, either directly in the extract form or in the modified synthetic form. *Calotropis gigantea* is a large shrub, gregarious, much branched and young branches covered with white, cottony hairs, contains milky latex.

2.1 Geographical Distribution:

It is widely distributed in almost all over the world. In native of India, China, Malaysia and found chiefly in lower Bengal, Himalaya, Punjab, Assam, Madras and South India. Common in waste land, railway embankments, road sides ascending to about 1000 m in the Himalayas from Punjab to Assam.



Figure 3. *Calotropis Gigantea*

2.2 Taxonomical Classification:

Subkingdom : Tracheobionta

- Class : Dicotyledones
 - Sub class : Asteridae
 - Order : Gentianales
 - Family : Apocynaceae
 - Subfamily : Asclepidiaceae
 - Genus : *Calotropis*
 - Species : *Calotropis gigantea*
- Subkingdom : Tracheobionta
- Class : Dicotyledones
 - Sub class : Asteridae
 - Order : Gentianales
 - Family : Apocynaceae
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 - Genus : *Calotropis*
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Table. 1 Taxonomical Classification

KINGDOM	PLANATE
Order	Gentianales
Family	Apocynaceae
Subfamily	Asclepiadaceae
Genus	<i>Calotropis</i>
Species	<i>C. gigantea</i>

2.3 Chemical Constituent:

Studies on *Calotropis*' phytochemistry have revealed a variety of different chemicals, including Cardenolide, triterpinoids, alkaloids, resins, anthocyanins, and proteolytic enzymes in the latex. Multiflorenol, cyclisadol, and -terpenes are found in flowers. Amyrin, amyirin acetate, β -sitosterol, urosolic acid, cardenolides, calotropin, and calotropagenin are the primary compounds found in the leaves.

1.4 Pharmacological Activity:

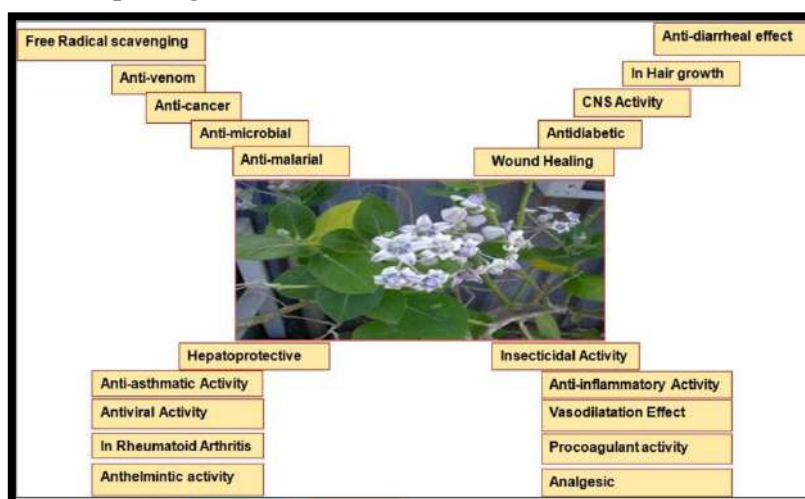


Figure 4. Pharmacological Activity

MATERIALS AND METHODS:

3.1 Pharmacognostical Studies:

- Ash Values
- Extractive Value
- Loss On Drying

3.2 Extraction Of Leaf Of Calotropis Gigantea L:

About 200 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days to remove fatty substances. At the end of 2nd day the powder was taken out and it was dried. After drying it was again packed and extracted by using Hydroalcoholic (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness.

3.3 Chemical Tests:

- Test For Carbohydrates
- Test For Alkaloids
- Test For Steroids And Sterols
- Test For Glycosides
- Test For Saponins
- Test For Flavonoids
- Test For Tri-Terpenoids
- Tests For Tannins and Phenolic Compounds
- Test For Fixed Oils and Fatty Acids
- Test For Gums and Mucilage
- Test For Proteins and Amino Acids

3.4 Animals:

Healthy Wistar albino rats of 2 to 3 months of age and approximately weighing between 150-250g were used in the present study. Rats were housed in a polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature 25±2°C.

All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethics committee, Aadhibhagawan College of Pharmacy, Rantham.

3.5 Acute Toxicity Studies:

3.5.1 Experimental Animals:

Wistar albino rats (120-125gm) of male rats were purchased from, Chennai, India. All animals were maintained in an air-conditioned room at 25°C±2°C, with a relative humidity of 75%±5%, and a 12-h

light/dark cycle. A basal diet and tap water were provided ad libitum. Male and female rats were assigned to each dose group by stratified random sampling based on body weight. The animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments.

3.5.2 Experimental procedure:

Wistar albino rats (120-125gm) were used for the study. The starting dose level of HECG . Baker was 5, 50, 300, and 2000 mg/kg body weight p.o. Dose was administered to overnight fasted mice's. Food was withheld for a further 3-4 hours after administration of HECG and observed for signs for toxicity. The body weight of the Wistar albino rats before and after administration were noted that changes in eyes and mucous membranes, skin and fur, respiratory, circulatory, autonomic, and central nervous systems, and also motor activity and behavior pattern. Special attention was directed to observations of convulsions, tremors, diarrhea, salivation, lethargy, sleep, and coma were noted. The onset of toxicity and signs of toxicity of LD50 values are noted.

3.5.3 Thioacetamide Induced Hepatotoxicity In Rat (Wistar Albino Rat) Model:

Thioacetamide, known for its hepatotoxic effects, was thus employed as a tool to simulate conditions akin to liver cirrhosis in these experimental subjects. By administering the injections at regular intervals over the specified duration, the study sought to elucidate the extent to which TAA could replicate the complex manifestations of liver cirrhosis, contributing valuable insights into the pathophysiological aspects of this hepatic condition. The careful preparation and administration of the TAA solution underscore the precision and methodological rigor employed in this experimental design, paving the way for a comprehensive understanding of the biochemical and morphological variations induced by TAA in the rat model.

A 0.03% w/v stock solution of Thioacetamide (TAA) was meticulously prepared by dissolving 30 mg of solid crystals in 100 ml of sterile distilled water. This solution, carefully concocted, served as the basis for an intriguing experiment in which rats were subjected to intraperitoneal injections three times a week for a span of two months. The concentration of TAA in these injections was 200 mg/kg (w/w). The choice of this concentration was deliberate, as it aimed to

induce morphological and biochemical alterations in the rats, mirroring the characteristics observed in human liver cirrhosis.

3.6 Grouping:

Animals are divided into 5 groups; each group consists of 5 rats (n) as follows; and placed into respective cages.

Group I: Control animals treated with Tween 20 (10% w/v) for about 28 days

Group II: Thioacetamide (200 mg/kg/ b.w.) administered intraperitoneally three times a week for about two months. (inducing agent) – Negative Control

Group III: Thioacetamide (200 mg/kg/ b.w.) I.P. for 3 times a week for 2 months + silymarin (50 mg/kg/b.w.) p.o. for 28 days after induction period.

Group IV: Thioacetamide (200 mg/kg/ b.w.) I.P. for 3 times a week for 2 months + HECG (200 mg/kg/b.w.) p.o. for 28 days after induction period.

Group V: Thioacetamide (200 mg/kg/ b.w.) I.P. for 3 times a week for 2 months + HECG (400 mg/kg/b.w.) p.o. for 28 days after induction period.

3.7 Parameters:

- Estimation of aspartate transaminase (SGPT)
- Estimation of (SGOT)
- Estimation of serum alkaline phosphatase (ALP)
- Estimation of serum bilirubin
- Estimation of SOD
- Estimation of glutathione- S-transferase (GST)
- Estimation of catalase (CAT)
- Estimation of Reduced Glutathione (GR)
- Estimation of Glutathione Peroxidase (GPX)
- Estimation of MAD
- Estimation of GSH

RESULTS AND DISCUSSION:

4.1 Extraction Of Leaf Of Calotropis Gigantea L:

Table. 2 Nature and colour of Hydroalcoholic extract of Calotropis Gigantea L

S.NO.	NAME OF EXTRACT	COLOUR	CONSISTENCY	YIELD% W/W
1	Hydro alcoholic extract	Dark greenish	Sticky mass	14.6

4.2 Ash Values:

Table. 3 Ash Value

S.No	PARAMETER	%w/w
	ASH VALUES	
1.	Total Ash	8.1
2.	Water Soluble Ash	2.8
3.	Acid Insoluble Ash	3.1
4.	Sulphated Ash	4.8

4.3 Extractive Values And Loss On Drying:

Table. 4 Data For Extractive Values And Loss On Drying

Analytical parameter	Percentage (w/w)
Water soluble extractive	3.5 %
Alcohol soluble extractive	4.4 %
Loss on drying	4.7 %

1.5 Preliminary Phytochemical Studies:

Table. 5 Results Of Phytochemical Analysis Of Calotropis Gigantea L

PHYTOCONS TITUENTS	HYDROETHANOLIC EXTRACT
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Alkaloids	+
Saponins	-
Glycosides	+
Carbohydrates	+
Tannins	-
Flavanoids	+
Terpenoids	+
Steroids	+
Phenolic compounds	+
Proteins and amino acids	+
Fixed oils and fatty acids	+
Gums and mucilage	+

4.5 Pharmacological Activity:

4.5.1 Effect Of HECG On SGOT, SGPT, ALP & GGT:

Table. 6 Effect Of HECG On SGOT, SGPT, ALP & GGT

S.NO	GROUPS	SGOT VALUES (IU/L)	SGPT VALUES (IU/L)	ALP VALUES (IU/L)	GGT VALUES (IU/L)
1	Control	197.6± 0.8048	84.60 ±2.731	244.9±4.095	8.688 ± 0.19
2	Negative Control	390.2 ± 1.889 a****	380.2±6.741 a****	462.7±8.462 a****b****	44.78 ± 0.73 a****
3	Positive Control	210.3 ± 1.650 a****b****	90.84±1.822 a ^{ns} b****	250.6±7.149 a ^{ns} b****	13.44 ± 0.40 a****b****
4	HECG (200 mg/kg) (Low dose)	282.2 ±1.393 a****b****c****	144.5±2.001 a****b****c****	333.4±9.519 a****b****c****	28.21 ± 0.30 a****b****c****
5	HECG (400 mg/kg) (High dose)	221.9 ±1.024 a****b****c****	101.4±3.055 a* b****c ^{ns}	279.0±3.081 a* b****c ^{ns}	18.64 ± 0.41 a****b****c****

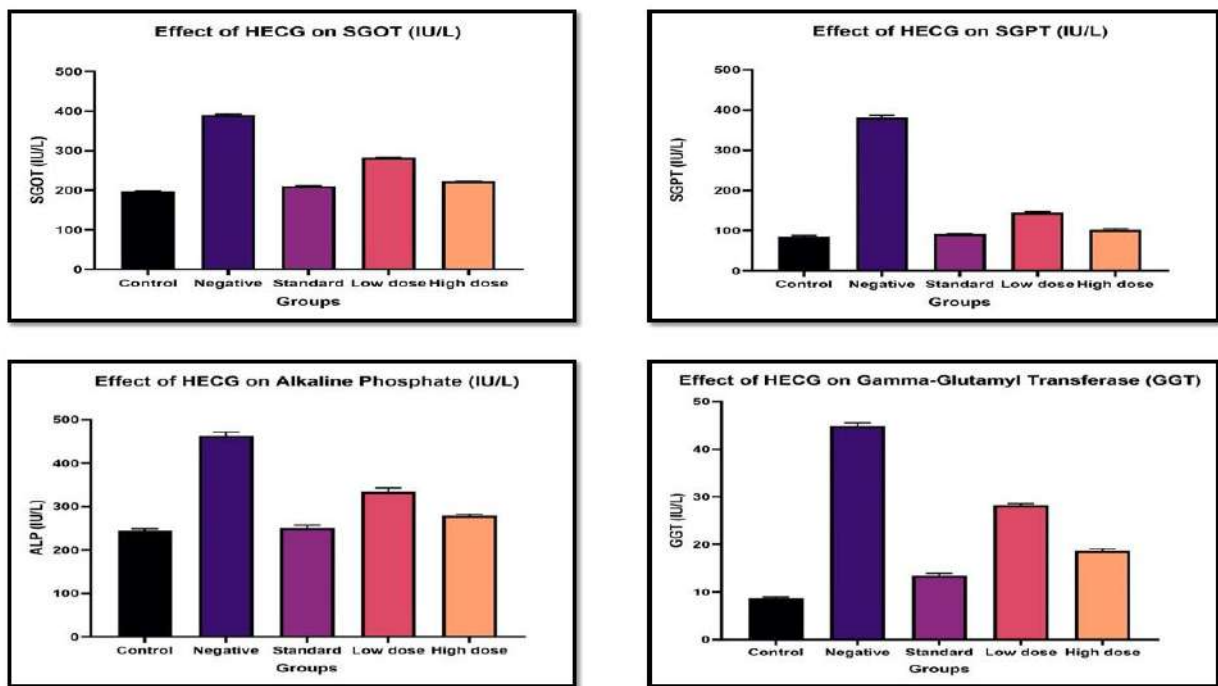


Figure 5. Effect Of HECG On SGOT, SGPT, ALP & GGT

4.5.2 Effect Of HECG On TB, TP, SOD & CAT:

Table. 7 Effect Of HECG On TB, TP, SOD & CAT

S.NO	GROUPS	TOTAL BILIRUBIN VALUES (µM)	TOTAL PROTEIN (mg/dl)	SOD VALUES (µmol/mg Protein)	CAT VALUES (nmol/min/ml)
1	Control	2.650±0.068	10.28±0.083	6.964 ±0.047	3.431±0.008
2	Negative Control	9.490 ± 0.05 a****	6.162±0.037 a****	2.294±0.179 a****	1.523±0.047 a****
3	Positive Control	4.378 ± 0.05 a**** b****	9.262±0.069 a**** b****	5.772 ±0.093 a**** b****	3.372±0.028 a ^{ns} b****
4	HECG (200 mg/kg) (Low dose)	7.180 ±0.050 a**** b**** c****	7.150±0.025 a**** b**** c****	4.436 ±0.162 a**** b**** c****	2.746± 0.032 a**** b**** c****

5	HECG (400 mg/kg) (High dose)	6.378 ±0.057 a**** b**** c****	8.718±0.05962 a**** b**** c****	5.052 ±0.067 a**** b**** c**	3.105±0.002 a**** b**** c****
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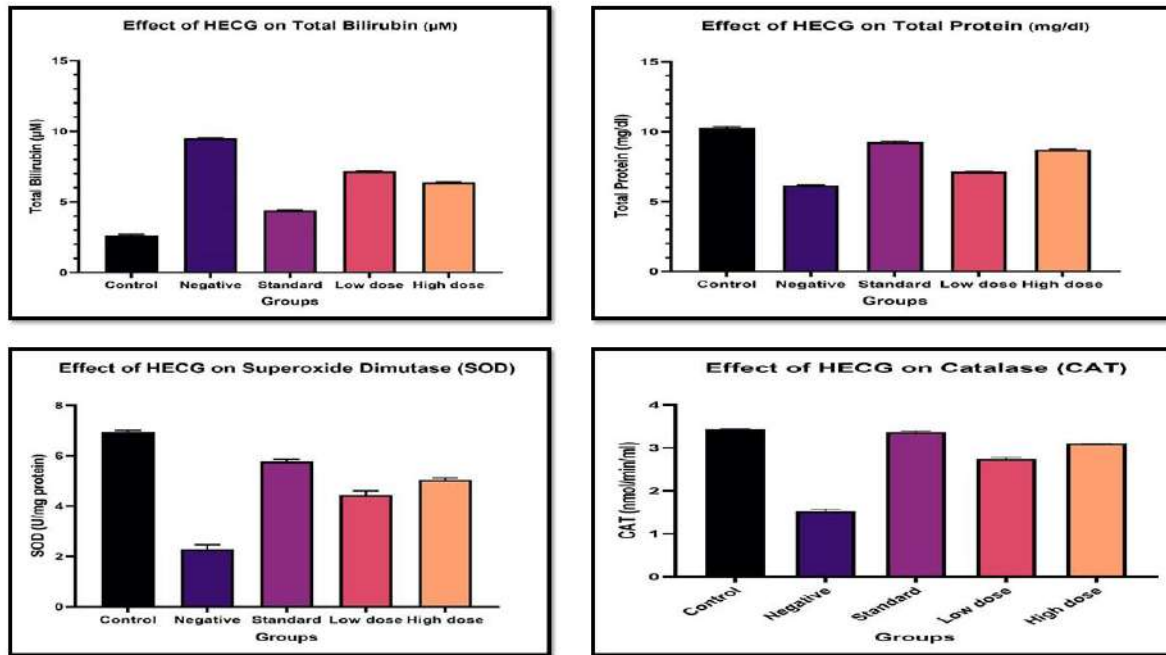


Figure 6. Effect Of HECG On TB, TP, SOD & C

4.5.3 Effect Of HECG On MDA, GSH, GR & GPx:

Table. 8 Effect Of HECG On MDA, GSH, GR & GPx

S.NO	GROUPS	MDA (µM)	GSH (n mol/g Tissue)	GR (n mol NADPH Consumed/min/mg Protein)	GPx (n mol NADPH Consumed/min/mg Protein)
1	Control	11.39±0.059	4.592±0.05024	18.84 ± 0.1873	12.70 ± 0.163
2	Negative Control	35.60±0.10 a****	1.094 ± 0.04445 a****	8.516 ± 0.1731 a****	4.89 ± 0.393 a****
3	Positive Control	13.33±0.051 a** b****	3.570 ± 0.1225 a**** b****	15.53 ± 0.1792 a**** b****	10.50 ± 0.210 a** b****
4	HECG (200 mg/kg) (Low dose)	19.04±0.31 a**** b**** c****	2.760 ± 0.05941 a**** b**** c****	10.87 ± 0.1204 a**** b**** c****	7.50 ± 0.316 a**** b**** c****
5	HECG (400 mg/kg) (High dose)	14.72±0.57 a**** b**** c*	4.064 ± 0.04320 a** b**** c**	13.44 ± 0.1237 a**** b**** c****	10 ± 0.316 a**** b**** c ^{ns}

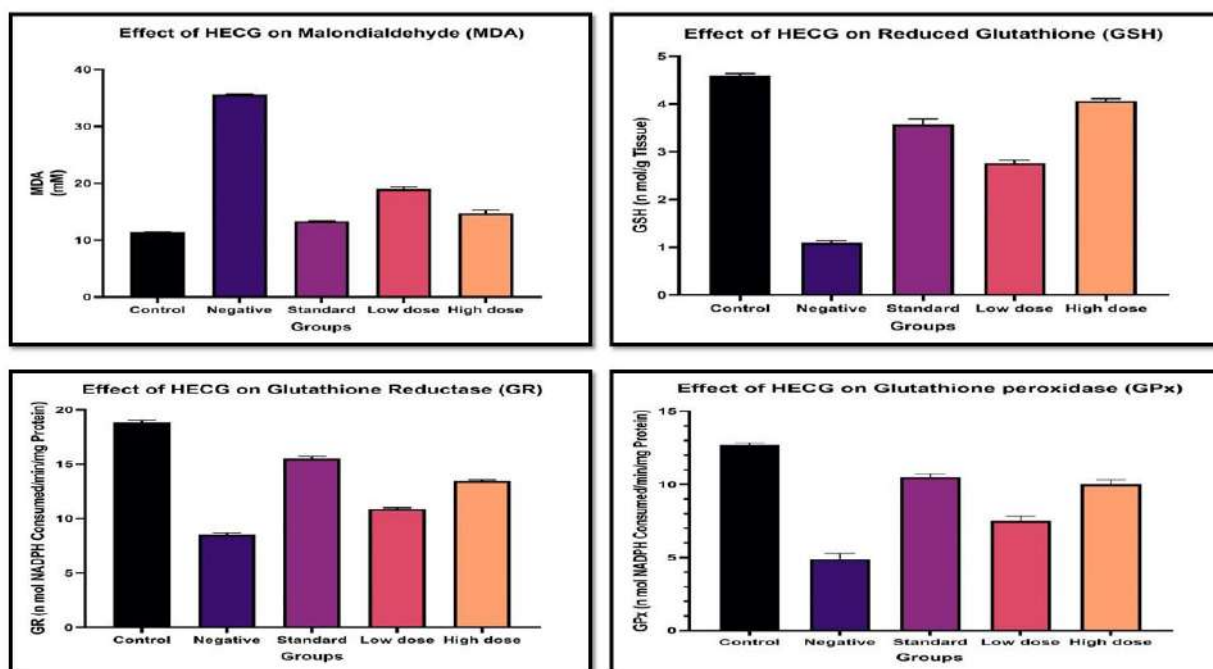


Figure 7. Effect Of HECG On MDA, GSH, GR & GPx

4.6 Histopathology Analysis:

Group I: The liver tissue from control rats exhibits a histologically normal state, characterized by an intact central vein (CV) and healthy hepatic parenchymal cells. The central vein displays no signs of damage or distortion, and the parenchymal cells show regular morphology, indicating a well-preserved and functional liver. Overall, the histological features suggest the absence of pathological changes in the liver of control rats.

Group II: In rats subjected to TAA administration, the liver displays disarray and degeneration of normal hepatic cells, characterized by intense centrilobular necrosis, sinusoidal hemorrhage, and dilatation. Notably, chronic inflammatory cell infiltrate is evident in the portal tract, indicating a sustained inflammatory response. These histopathological changes collectively signify severe hepatocellular damage and disruption of normal liver architecture due to TAA-induced toxicity.

Group III: In this group III treated with the stranded drug exhibit moderate fibrous proliferation in portal areas, incomplete septa extension, mild inflammatory cell infiltration, and proliferation of bile duct epithelial cells. Notably, histopathological analysis suggests ongoing healing processes in the damaged liver tissue.

Group IV: Treatment with HECG (200 mg/kg) in rats brings about partial improvement in hepatocyte

degeneration. However, liver sections still exhibit signs of cloudy swelling and mild fatty changes, suggesting a lingering impact on hepatic morphology.

Group V: Treatment with HECG (400 mg/kg) in rats shows nearly normal lobular patterns without degenerative alterations, the cytoplasm was preserved, featuring a prominent nucleus devoid of intracellular lipid accumulation.

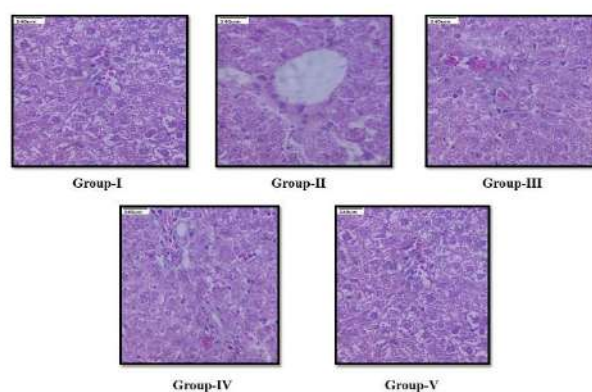


Figure 8. Histopathology Report

CONCLUSION:

In conclusion, the hydroalcoholic extract of *Calotropis gigantea* demonstrates promising hepatoprotective potential in this experimental model of TAA-induced liver damage. The significant reduction in liver injury markers and the concurrent enhancement of antioxidant defenses suggest a multifaceted protective mechanism. Further research and exploration are warranted to elucidate the specific bioactive compounds responsible for these effects and

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