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Hepatoprotective Effect of *Padina gymnospora* in Albino Wistar Rat; A Biochemical and Histopathological Studies

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The methanolic extract of brown algae, *Padina gymnospora* (PG) was tested for hepatoprotective properties against paracetamol (acetaminophen, APAP)-induced liver damage in male albino Wistar rat through in-vitro model. Liver is damaged by the variation of serum enzymatic and biochemical markers, such as serum glutamate oxaloacetate transaminase (SGOT or AST), serum glutamate pyruvate transaminase (SGPT or ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), bilirubin, albumin, and lipid

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peroxidation and antioxidant status were measured by SOD and SDH. The study found that PG caused dose-dependent histopathological changes in the liver and kidney of the rats. While low doses caused minimal alterations, higher doses resulted in significant tissue damage. This suggests that, despite its therapeutic potential, careful consideration is needed regarding dosage to ensure safety. Acute toxicity studies revealed that the LD_{50} value exceeded 3 g/kg body weight. These results indicate that the methanolic extract of PG contains active compounds with antioxidant, anti-inflammatory, detoxification support, liver regeneration, cholesterol regulation, prevention of liver fibrosis and hepatoprotective effects against high doses of paracetamol-induced hepatotoxicity [Fig. 1].

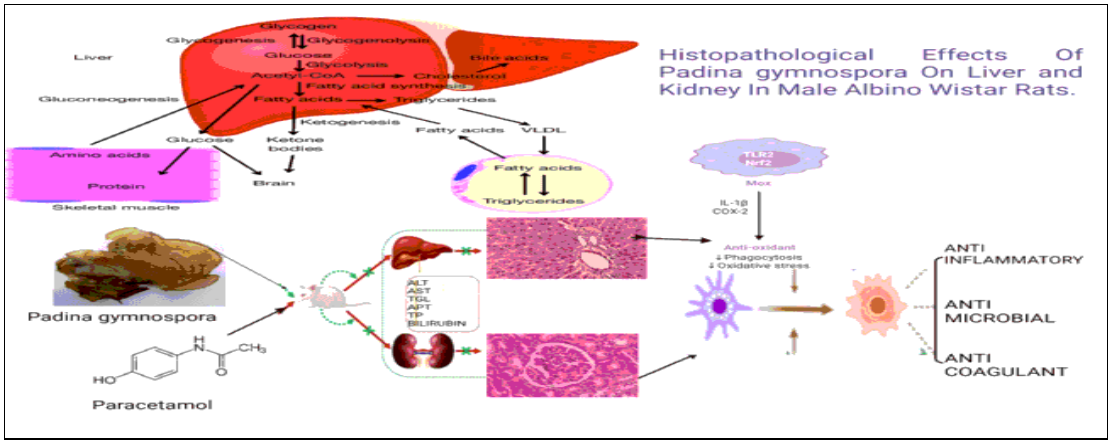


Fig. 1. Graphical abstract of the histological effects of Padina gymnosporaon liver and kidney

(created by BioRender.com)

Keywords: Histopathological; Padina gymnospora; antioxidant; hepatoprotective; biochemical parameter; and methanolic extract.

1. INTRODUCTION

Food habit, changing lifestyle due to work pressure, excessive chronic alcohol consumption, and depression all are helping to unbalance of enzyme and hormone secretion. Dysfunction or injury of liver is the common and serious health issue in throughout the world irrespective of age, sex, region or race. Large internal body organ liver plays crucial role in much essential physiological process such as a wide variety of toxic, microbial, metabolic, circulatory and neoplastic insults. PG was collected from Gulf of Mannar or Mandapam area, Tuticorin located (8.76°42″ N; 78.13° 48″ E) at Rameswaram, district Ramanathapuram in Tamil Nadu. Phaeophyta (brown algae) are more abundant in a shallow rocky, low tide area. The plant was identified and authenticated at the herbarium (AU Bota#609) of Botany, Professor Dr.L.Mullainathan , Academic affairs Director, Faculty of Science, Annamalai University, Tamilnadu, India [Fig. 2].

Paracetamol, administered orally at a dose of 3 g/kg body weight for one day, caused liver damage, evident by a significant increase in serum levels of AST, ALT, ALP, GGT, LDH, bilirubin, and cholesterol, along with a decrease in protein levels compared to the control group. Treatment with Indigofera tinctoria (250 and 500 mg/kg body weight) and silymarin (25 mg/kg body weight) once daily for 28 days in paracetamol-treated rats significantly reduced the elevated serum biochemical parameters and increased protein levels (Muthulingam et al.,2010). The liver is unique among organs in the body because it has the remarkable ability to replace damaged tissue with new cells instead of scar tissue. For instance, an overdose of acetaminophen (Tylenol) can destroy liver cells in less than a week (McClain et al.,1999). However, barring complications, the liver can fully regenerate, and within a month, the patient will exhibit no signs of damage (Koniaris et al.,2003) At times, the liver may become overwhelmed and unable to fully repair itself,

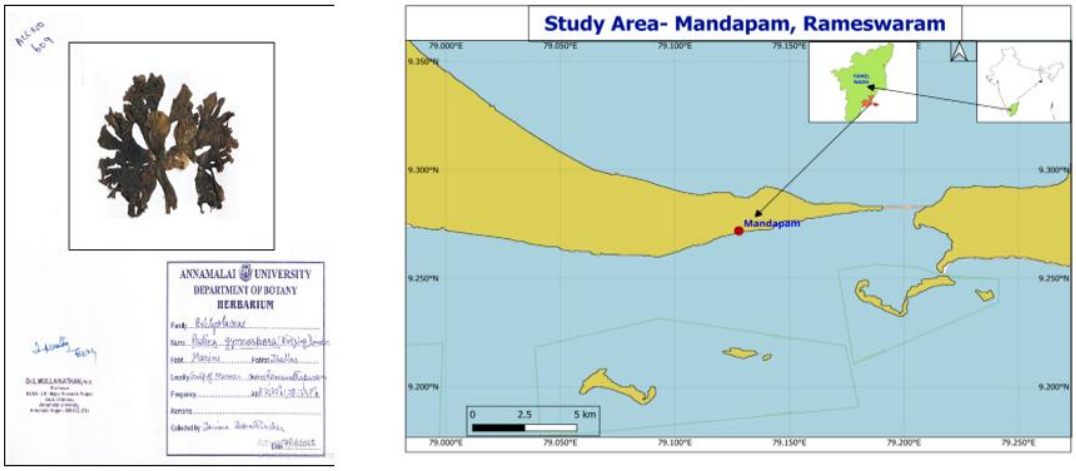


Fig. 2. Herbarium sheet of PG; Gulf of Mannar or Mandpam, Rameswarm, Tamilnadu, India

particularly if it continues to be affected by a virus, drug, or alcohol. This can result in the development of scar tissue, which is challenging to reverse and can ultimately lead to cirrhosis (Rappaport et al.,1983).

2. MATERIALS AND METHODS

2.1 *Padina gymnospora* Extracts Both Soxhlet and Filtration Process

The choice of solvent for soxhlet extraction is crucial and typically based on polarity and the phytoconstituent isolation process. Methanol, being a semi-polar solvent, is often preferred as it can effectively extract PG phytoconstituents.

2.2 Extraction by Filtration Processes

500 g of powdered sample is extracted in 500ml of methanol in a room temperature (summer season, temperature 40^0 C) for 3 days.The solution is filtered through sterile cloth like as a strainer. Keep the filtrate solution aside which is covered by hole aluminium foil for evaporation of methanol. The residue is mixed again with 500ml of methanol and keeps it for another 3 days. Repeat this process for 3 times.

2.3 Drying Desiccators

Both dried powders are kept in a desiccator, which is an air-tight glass vessel containing a suitable drying agent, anhydrous calcium chloride, placed at the bottom. Measured every 12 hours until it's' weight stabilizes.

2.4 About Experimental Animals

The scientific classification for outbred strin of albino wistar rat is *Rattus norvegicus*.[Berkenhout,1769] (Hedrich, 2020). The wistar rat is known as the Rcc Han: WIST, the Crl: WI(Han), and Wistar Hannover GALAS strain. Total 42 male wistar rats, have aged 3-4 months, each weighing between 130 and 170 grams, were used 28 days for this study. Male rats were selected due to their consistent hormonal levels compared to females, facilitating easier observation and measurement of physiological changes or reactions (Kajantie and David, 2006). The rats were sourced from Biogen Laboratory Animal Facility, India. Under Estimate No. BLAF/QN/128, Date 02.03. 2023. Rats are housed, from the inbred colonies of the department of experimental medicine, the central Animal House, Raja Muthiah Medical College, Annamalai University, Annamalai Nagar, Tamilnadu, India were subject to investigation. Under standard laboratory conditions with controlled temperature $27^0 \pm 2^0$ C, humidity 40-70%, and a 12-hour (6:00-18:00) light-dark cycle. Access to food and water is provided ad libitum. The animals were euthanized by cervical dislocation 24 hours after the final dose, marking the end of the tentative test. Blood specimens were obtained without the use of an anticoagulant to separate serum and measure serum marker enzymes such as ALT, AST, ALP, GGT, LDH, total protein, albumin, SDH, SOD and bilirubin levels. The liver and kidney tissue was instead of taken away, washed with cold physiological saline and preserved in 10% formalin for subsequent histological analyses.

2.5 Experiment Design

The rats were segregated into 7 groups, each consisting of six individuals:

Group 1: (Control)	Administered physiological saline solution at a dosage of 10ml/kg body weight.
Group 2:(Inducer)	Received a single dose of paracetamol at 3 g/kg weight of the body via oral gavage (Size 16 Gauge, Length 3 inches, Ball diameter 3mm).
Group 3:(With drug low dosage)	Given paracetamol along with PG at a dosage of 50 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.
Group 4:(With drug in medium dosage)	Given paracetamol along with PG at a dosage of 100 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.
Group 5:(With drug in high dosage)	Given paracetamol along with PG at a dosage of 200 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.
Group6:(With standard drug)	Given paracetamol along with silymarin at a dosage of 25 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.
Group7:(Drug alone)	Administered PG alone at a dosage of 200 mg/kg weight of the body daily one time for 28 days, using the same oral gavage method as Group 2.

2.6 Preparation of Tissue Homogenate

A specific quantity of hepatic tissue was homogenized in Tris-HCl buffer. The homogenate was then centrifuged at 12,000 rpm for 20 minutes at 4°C. The supernatant was collected and stored at -80°C for subsequent assays of marker enzymes and antioxidant studies.[Fig. 3:A),(B)]. Histopathological slides of liver and kidney prepared.

2.7 Statistical Analysis of the Data

The statistical analysis for each test has conducted on cases with complete data for all variables. For each dependent variable presented in the table, user-defined missing values for both the dependent and grouping variables are considered as missing data. The cases included in each table have no missing values for any independent variables, though some dependent variables may still have missing values. The means table for initial bodyweight and water intake by groups includes calculations of mean, count, and standard deviation. An ANOVA was also performed to assess differences among the groups.

User-defined missing values treated as missing in the analysis. Statistics for each analysis are based on cases with no missing data for any variable involved. A one-way ANOVA was conducted on initial bodyweight and water intake by groups, using polynomial contrasts. Descriptive statistics were calculated, mean plots were generated, and missing data were analyzed. For post-hoc comparisons, Duncan's test was applied with an alpha level of 0.05.

Fig. 3. (A) Removing organs;(B) After removing liver, kidney and pancreas

3. RESULTS

3.1 Qualitative and Quantitatively Using Biomarkers for Phytochemical Analysis Methyl Alcohol Extract of PG

The phytochemical analysis of the methyl alcohol extract of *Padina gymnospora* reveals the presence of various bioactive compounds. Tests indicate that the extract is positive for flavonoids, tannins, phenols, saponins, phytosteroids, coumarins, terpenoids, carbohydrates, and proteins, highlighting a diverse phytochemical profile (Thamizharasan, 2018, Nazarudin et al., 2022). Alkaloids were partially present, as only Wagner's test returned positive. Cardiac glycosides and quinones were absent. These findings suggest that PG may possess therapeutic potential due to its rich composition of bioactive molecules [Table 1].

3.2 Biochemical Studies

3.2.1 Acetaminophen (APAP) induced hepatotoxic Studies in Wister albino male rats

Hepatic damage from alcohol and drug abuse is a widespread concern globally. The liver plays a crucial role in nutrient digestion, metabolism, and storage. Recently, the increasing recognition of liver diseases, particularly those induced by pharmacological treatments, highlights the need for understanding liver injury mechanisms. This has led to the development of new therapeutic strategies (Pantev et al., 2006).

Acetaminophen (N-acetyl-para-aminophenol), a widely used over-the-counter medication for fever and pain relief, is a key ingredient in many cold and flu remedies. Although generally safe at recommended doses, acetaminophen can cause severe liver damage in cases of overdose or even with normal doses under certain conditions. Consequently, acetaminophen overdose is a leading cause of drug poisoning worldwide. Excessive use of acetaminophen can lead to damage in multiple organs, notably the liver and kidneys (Bertolini et al., 2006, Altuncu et al., 2007).

The excessive production of reactive oxygen free radicals can cause tissue injury through covalent binding and lipid peroxidation, contributing to fibrosis as evidenced by increased collagen synthesis (Geesin et al., 1990). Antioxidants that scavenge free radicals may help mitigate fibrosis and tissue damage (Thresiamma and Kuttan, 1996). Furthermore, free radicals may contribute to a progressive decline in immune system function (Daneshyar et al., 2011). Binding of drugs to blood serum proteins can influence both therapeutic efficacy and toxicity (Kratz et al., 2012). The body's defense against free radical damage relies on antioxidant nutrients and enzymes that work cooperatively to protect against oxidative stress.

Table 1. Qualitative and quantitatively using biomarkers for Phytochemical Analysis Methyl Alcohol Extract of PG

Phytochemical Test	Methyl Alcohol Extract of *Padina gymnospora*
1.Alkaloids:	
a). Mayer’s test	-
b). Wagner’s test	+
c).Dragendorff’s test	-
2.Flavonoids	+
3.Tannins	+
4.Phenols	+
5.Saponins	+
6.Phytosteroids	+
7.Cardiac glycosides	-
8. Coumarin	+
9. Quinones	-
10. Terpenoids	+
11. Carbohydrates	+
12. Proteins	+

**Abbreviation: (–)= Absent, (+) =Present.*

3.3 Heat Map-Colour Variance

A heat map (Fig. 4) visually represents the values of a primary variable across two axis variables using a grid of colored squares. The axis variables are segmented into ranges similar to those in a bar chart. Each colored cell in the grid corresponds to the value of the primary variable within that range, providing a clear, color-coded comprehensive overview of the effective data density which immediate visual insights, highlighting variability and user-friendly (Yau, 2014).

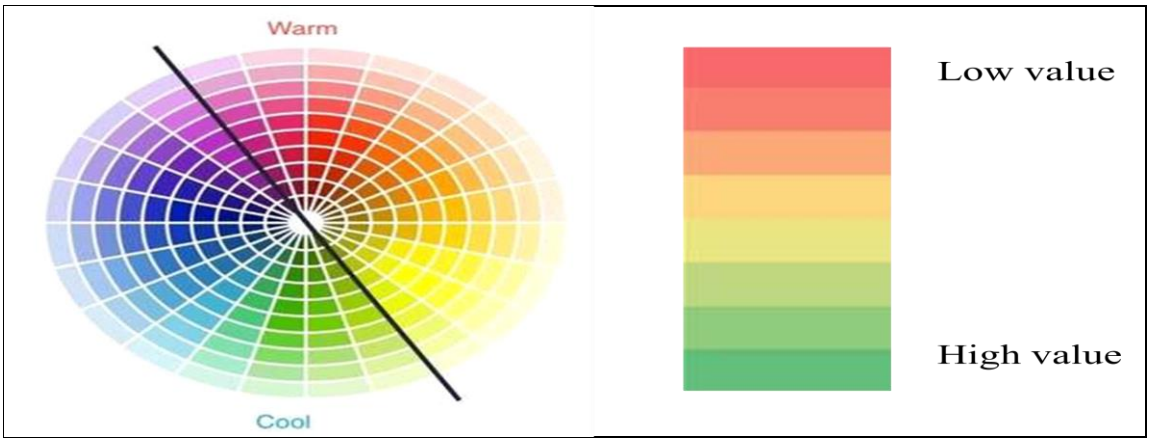
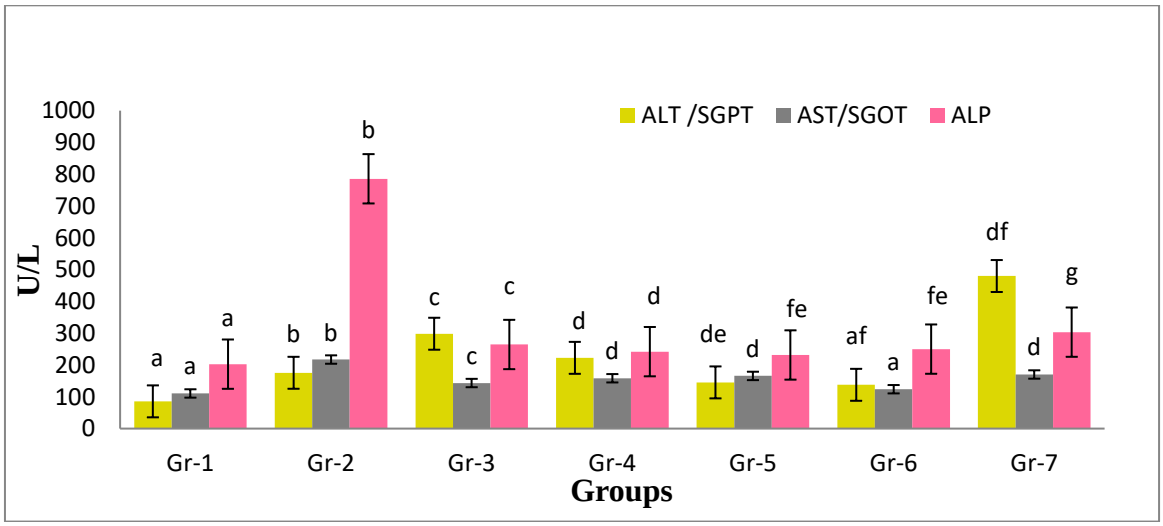


Fig. 4. Heat map-colour variance

Table 2. ALT or SGPT, AST or SGOT and ALP test results

GROUPS	ALT /SGPT	AST/SGOT	ALP
Gr-1	85.66	110.5	202.66
Gr-2	175.5	217.16	785.66
Gr-3	298.33	143.33	264.5
Gr-4	222.5	158.33	242
Gr-5	145.33	165.66	231.5
Gr-6	137.66	123.83	250
Gr-7	479.83	170.16	303.33

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p <0.05(Duncan Multiple Range Test)



Graph 1. Compare ALT, AST and ALP test of albino wistar rats

4. DISCUSSION

The heat map and graph, it is clear that ALT/SGPT, AST/SGOT and ALP increased more in Group 2 rats, which were treated with a single dose of 3 g/kg body weight of Paracetamol (APAP), compared to Group 6 rats that received both APAP and the standard drug Silymarin (Sly) at a dosage of 25 mg/kg body weight, with values of 175.5±3.59, 217.16± 4.88 and 785.66± 11.68 versus 137.66±2.47, 123.83±4.00 and 250±5.63 respectively. [Table 2 & Graph 1].

Additionally, the heat map and graph show that Group 7 rats, which were treated with PG alone at a dosage of 200 mg/kg body weight, exhibited a greater increase in ALT/SGPT, AST/SGOT and ALP compared to Group 1 control rats that received saline water at a dosage of 10 ml/kg body weight, with values of 479.83±5.42, 170.16± 6.94 and 303.33± 6.95 versus 85.66±3.06, 110.5± 2.38 and 202.66±3.76 respectively. [Table 2 & Graph 1]

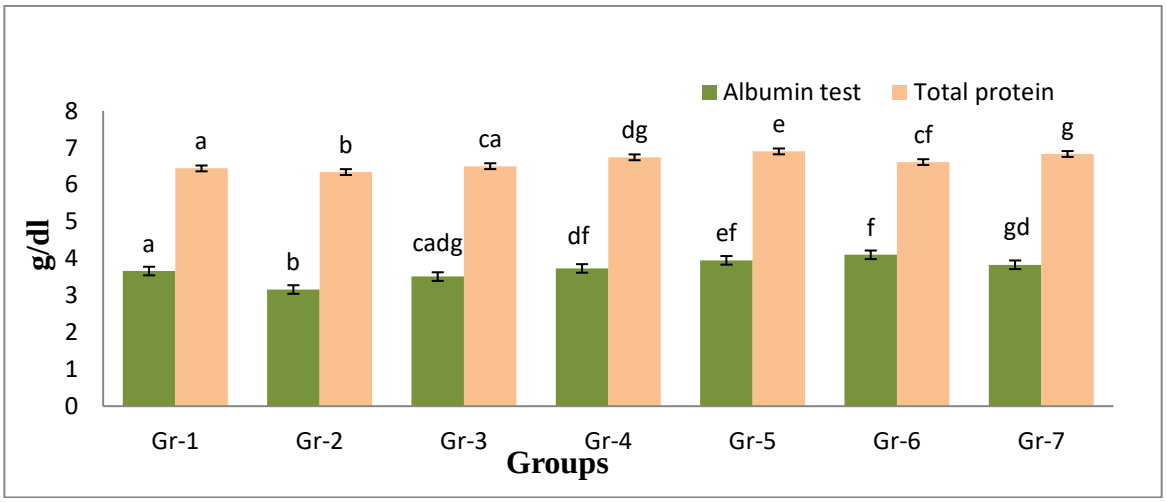
The data show that ALT/SGPT, AST/SGOT and ALP levels were significantly higher in Group 2 rats, which received a single dose of 3 g/kg body weight of APAP, compared to Groups 3, 4, and 5, which were administered APAP in combination with PG at doses of 50, 100, and 200 mg/kg body weight, respectively. The values were 175.5±3.59, 217.16± 4.88 and 785.66± 11.68 for Group 2, and ALT values 298.33±6.53, 222.5±6.37, and 145.33±2.64; AST values 143.33± 5.02, 158.33±3.54, and 165.66± 5.12; ALP values 264.5±5.23, 242±7.16, and 231.5±4.92f for Groups 3, 4, and 5, respectively. This indicates that increasing the dose of PG is associated with a reduction in ALT/SGPT, AST/SGOT and ALP levels. [Table 2 & Graph 1].

The heat map and graph clearly show that Albumin and Total Protein levels decreased more in Group 2 rats, which were administered a single dose of 3 g/kg body weight of Paracetamol (APAP), compared to Group 6 rats, which received both APAP and the standard drug Silymarin (Sly) at a dosage of 25 mg/kg body weight. Specifically, Albumin and Total protein levels were 3.16±0.03 and 6.34±0.15 in Group 2 versus 4.1±0.21 and 6.34±0.15 in Group 6 respectively. [Table 3 & Graph 2].

Table 3. Albumin and Total Protein test results

Groups	Albumin test	Total protein
Gr-1	3.66	6.44
Gr-2	3.16	6.34
Gr-3	3.51	6.5
Gr-4	3.73	6.74
Gr-5	3.95	6.9
Gr-6	4.1	6.61
Gr-7	3.83	6.83

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p <0.05(Duncan Multiple Range Test)

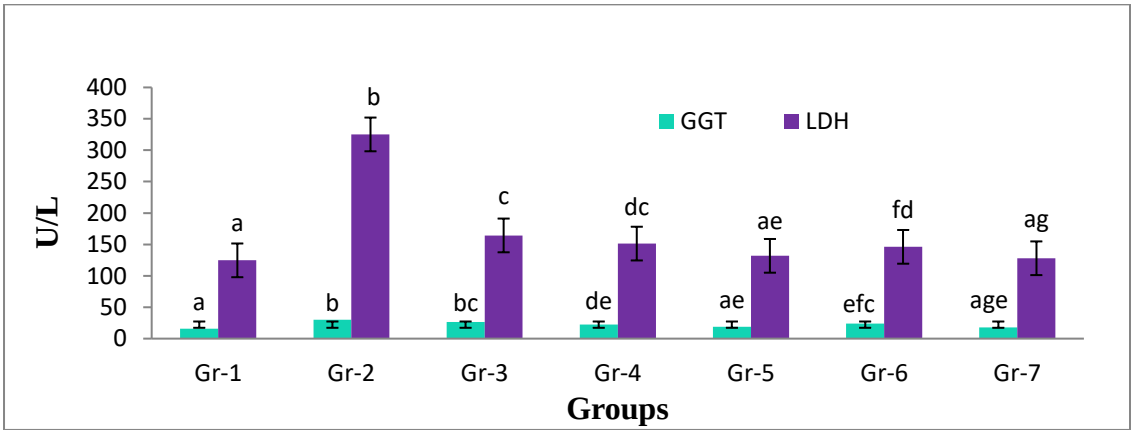


Graph 2. Compare Albumin and Total protein test of albino wistar rats

Table 4. GGT and LDH test results:

Groups	GGT	LDH
Gr-1	16	124.66
Gr-2	30	325.16
Gr-3	26.33	164.33
Gr-4	22.66	151.33
Gr-5	18.83	131.83
Gr-6	24.16	146.16
Gr-7	17.83	128

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p <0.05(Duncan Multiple Range Test)



Graph 3. Compare GGT and LDH test of albino wistar rats

Additionally, the heat map and graph reveal that Group 7 rats, which were treated with PG alone at a dosage of 200 mg/kg body weight, showed a greater increase in Albumin levels compared to Group 1 control rats that received saline water at a dosage of 10 ml/kg body weight. Specifically, Albumin and Total protein levels were 3.83±0.05 and 6.83±0.11 in Group 7 compared to 3.66±0.03 and 6.44±0.15 in Group1 respectively.[Table 3 & Graph 2].

The data indicate that Albumin and Total protein levels were notably higher in Group 2 rats, which received a single dose of 3 g/kg body weight of APAP, compared to Groups 3, 4, and 5, which received APAP in combination with PG at doses of 50, 100, and 200 mg/kg body weight, respectively. Specifically, Albumin and Total protein levels were 3.16±0.03and 6.34±0.15 in Group 2, while they were 3.51±0.09, 3.73±0.04, and 3.95±0.15; 6.5±0.14, 6.74±0.07, and 6.9±0.03 in Groups 3, 4, and 5 respectively. These results suggest that increasing the dose of PG correlates with a decrease in Albumin and Total protein levels. [Table 3 & Graph 2].

According to the heat map and graph, it is clear that GGT and LDH increased more in Group 2 rats, which were treated with a single dose of 3 g/kg body weight of Paracetamol (APAP), compared to Group 6 rats that received both APAP and the standard drug Silymarin (Sly) at a dosage of 25 mg/kg body weight, with values of 30± 2.21and 325.16±6.74 versus 24.16±1.23 and 146.16±2.72 respectively. [Table 4 & Graph 3].

Additionally, the heat map and graph show that Group 7 rats, which were treated with PG alone at a dosage of 200 mg/kg body weight, exhibited a greater increase in GGT and LDH compared to Group 1 control rats that received saline water at a dosage of 10 ml/kg body weight, with values of 17.83± 1.55 and 128±2.72 versus 16±1.24 and 124.66±2.05 respectively. [Table 4 & Graph 3].

Furthermore, the data show that GGT and LDH levels were significantly higher in Group 2 rats, which received a single dose of 3 g/kg body weight of APAP, compared to Groups 3, 4, and 5, which were administered APAP in combination with PG at doses of 50, 100, and 200 mg/kg body weight, respectively. The GGT and LDH values were 30±2.21 and 325.16±6.74 for Group 2, and GGT values 26.33±1.38, 22.66±1.19, and 18.83±1.65; LDH values 164.33±0.03,

151.33±7.75 and 131.83±5.01 for Groups 3, 4, and 5, respectively. This indicates that increasing the dose of PG is associated with a reduction in GGT and LDH levels. [Table 4 & Graph 3].

The heat map and graph indicate that SOD and SDH levels significantly increased in Group 2 rats, which were given a single dose of 3 g/kg body weight of paracetamol (APAP). In contrast, Group 6 rats, treated with both APAP and the standard drug silymarin (Sly) at a dosage of 25 mg/kg body weight, showed lower levels of SOD and SDH, with values of 8.62 ± 0.04 and 4.25± 0.14 for Group 2 compared to 6.02 ± 0.11and 3.25±0.10 for Group 6 respectively. [Table 5& Graph 4].

Furthermore, the heat map and graph reveal that Group 7 rats, which received Padina gymnospora (PG) alone at a dosage of 200 mg/kg body weight, showed a larger increase in SOD and SDH levels compared to Group 1 control rats that were given saline water at a dosage of 10 ml/kg body weight. The values were 5.69 ± 0.23 and 3.04± 0.12 for Group 7 and 5.32 ± 0.05 and 1.20±0.07 for Group 1 respectively. [Table 5 & Graph 4].

Moreover, the data indicate that SOD and SDH levels were significantly higher in Group 2 rats, which received a single dose of 3 g/kg body weight of APAP, compared to Groups 3, 4, and 5, which were given APAP in combination with PG at doses of 50, 100, and 200 mg/kg body weight, respectively. The SOD and SDH values were 8.62 ± 0.04 and 4.25± 0.14 for Group 2, while Groups 3, 4, and 5 showed values of SOD 4.17 ± 0.14, 4.88 ± 0.20, and 5.04 ± 0.12; SDH values 2.87 ± 0.09, 3.15 ± 0.17, and 3.35 ± 0.13, respectively. This suggests that increasing the dosage of PG is associated with a rise in SOD and SDH levels. [Table 5& Graph 4].

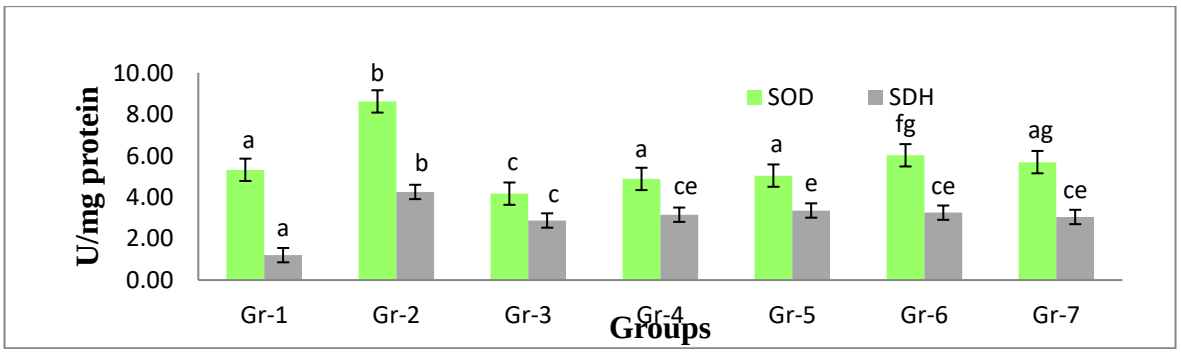
The heat map and graph clearly show that Bilirubin levels decreased more in Group 2 rats, which were administered a single dose of 3 g/kg body weight of Paracetamol (APAP), compared to Group 6 rats, which received both APAP and the standard drug Silymarin (Sly) at a dosage of 25 mg/kg body weight. Specifically, Bilirubin levels were 0.21±0.02 in Group 2 versus 0.38±0.03 in Group 6. [Table 6 & Graph 5].

Additionally, the heat map and graph reveal that Group 7 rats, which were treated with PG alone at a dosage of 200 mg/kg body weight, showed a greater increase in Bilirubin levels compared to Group 1 control rats that received saline water at a dosage of 10 ml/kg body weight. Specifically, Bilirubin levels were 0.4±0.03 in Group 7 compared to 0.24±0.63 in Group1.[Table 6& Graph 5].

Table 5. SOD and SDH test results

Goups	SOD	SDH
Gr-1	5.32	1.20
Gr-2	8.62	4.25
Gr-3	4.17	2.87
Gr-4	4.88	3.15
Gr-5	5.04	3.36
Gr-6	6.02	3.25
Gr-7	5.69	3.05

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p <0.05(Duncan Multiple Range Test)

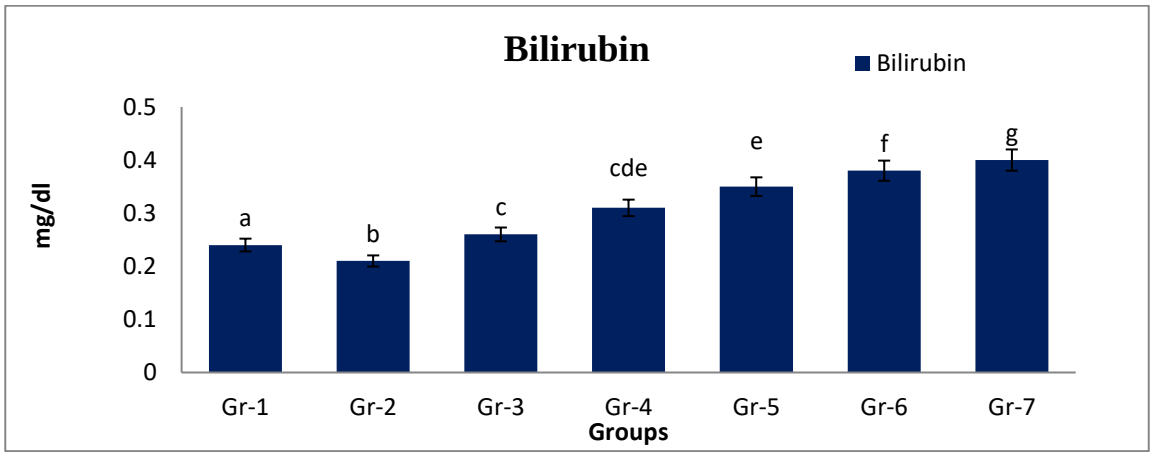


Graph 4. Compare SOD and SDH test of albino wistar rats

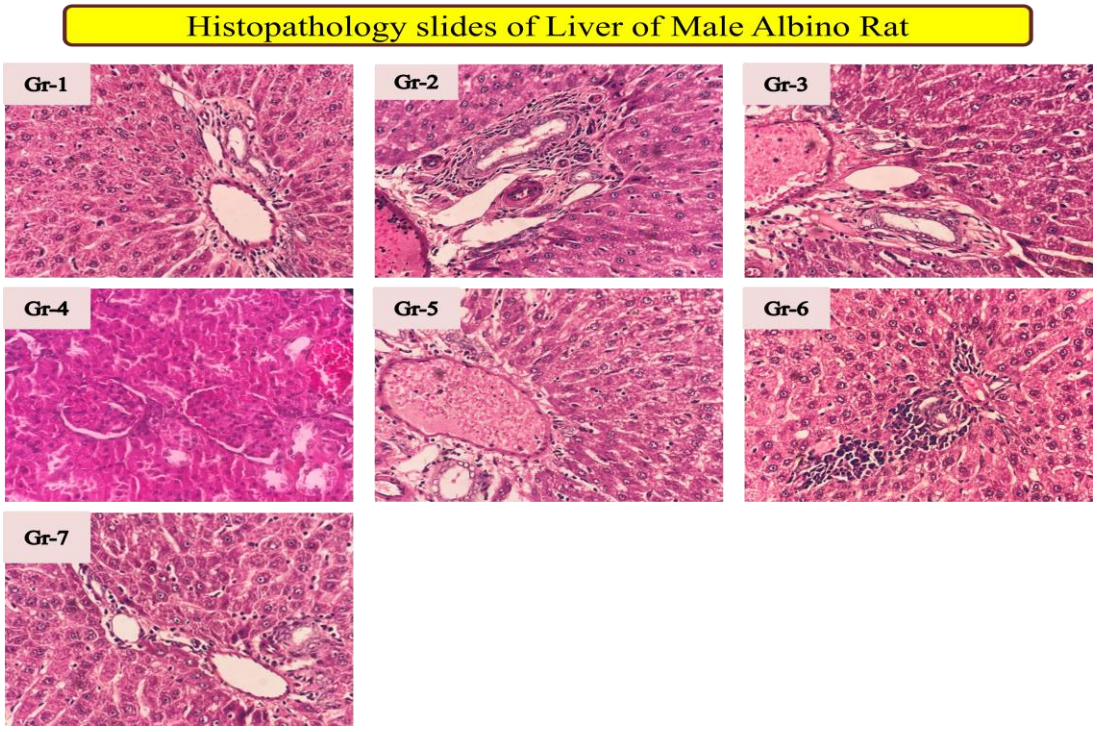
Table 6. Bilirubin test results

Bilirubin test						
Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
0.16	0.14	0.23	0.34	0.43	0.53	0.59
0.29	0.31	0.26	0.41	0.49	0.44	0.41
0.38	0.24	0.27	0.22	0.21	0.35	0.33
0.17	0.15	0.24	0.42	0.39	0.28	0.35
0.21	0.26	0.28	0.24	0.32	0.35	0.34
0.24	0.19	0.28	0.23	0.31	0.38	0.39

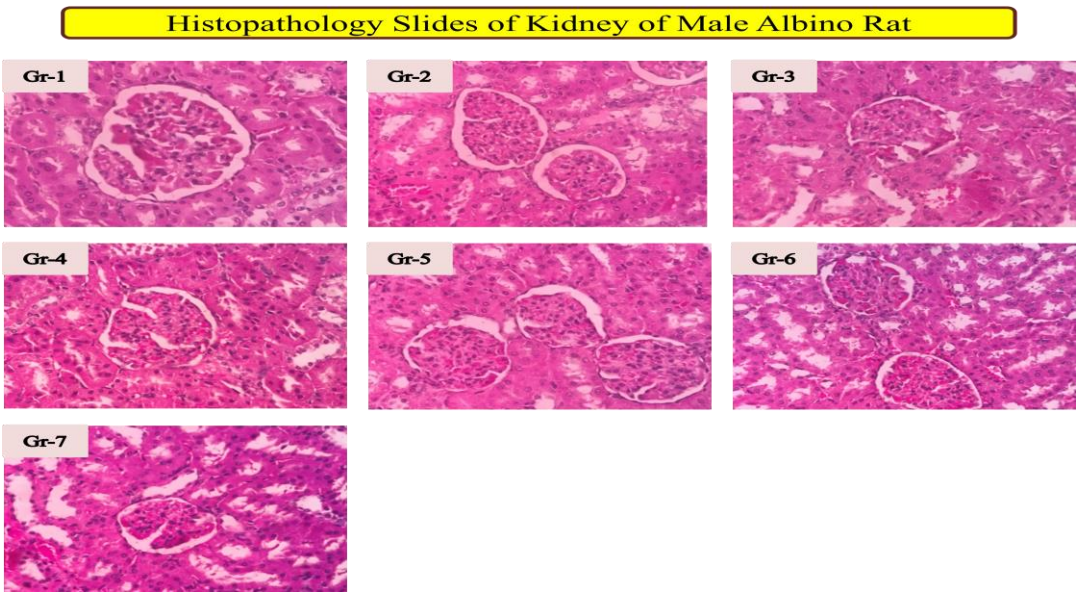
Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p <0.05(Duncan Multiple Range Test)



Graph 5. Bilirubin test of albino wistar rats



Slides 1. Microscopic observations of liver tissue pre-treated with various concentrations of PG followed by treatment against APAP. induced liver injury under 40x magnification



Slides 2. Microscopic observations of kidney tissue pretreated with various concentrations of PG followed by treatment against APAP induced liver injury under 40x magnification

The data indicate that Bilirubin levels were notably higher in Group 2 rats, which received a single dose of 3 g/kg body weight of APAP, compared to Groups 3, 4, and 5, which received APAP in combination with PG at doses of 50, 100, and 200 mg/kg body weight, respectively. Specifically, Bilirubin levels were 0.21±0.02in Group 2, while they were 0.26±0.007, 0.31±0.03, and 0.35±0.003 in Groups 3, 4, and 5. These results suggest that increasing the dose of PG correlates with a decrease in Bilirubin levels. [Table 6 & Graph 5].

4.1 Histopathology Slides Drescriptions by Paracetamol Inducer of PG Extract on Standard Drug of Silymarin

4.1.1 The histological changes in the liver of control and experimental rats

In Group 1, the hepatic slide shows no degenerative changes and the sinusoidal spaces remain intact without any obliteration. Group 2, which received an inducer (3 g/kg body weight), exhibits minute degenerative changes with increased congestion in the sinusoidal spaces. In Group 3, treated with both the inducer and a drug (50 mg/kg body weight), the hepatic slide reveals microvesicular steatosis, obituary changes, and congestion due to triglyceride washout. Group 4,with a higher drug dosage (100 mg/kg body weight), shows periportal inflammatory cell infiltration and hepatoprotective effects against degenerative necrosis. Group 5, given the highest drug dose (200 mg/kg body weight), presents extensive periportal inflammation and a high percentage of hepatocyte degeneration and necrosis. In Group 6, which received the inducer along with a standard drug (25 mg/kg body weight), the hepatic slide reveals periportal inflammatory cells, microvesicular steatosis, degenerative changes, and congested sinusoidal spaces. Lastly, Group 7, treated with the drug alone (200 mg/kg body weight), shows peripheral inflammation, hepatocyte necrosis, and degeneration, along with congested sinusoidal spaces and predominant eosinophil involvement.[Slides 1].

4.2 The Histological Changes in the Kidney of Control and Experimental Rats

In Group 1, the hepatic slide shows a normal structure of the glomerulus and tubules, with no visible abnormalities. Group 2, treated with an inducer (3 g/kg body weight), exhibits minute degenerative changes at the glomerulus and tubules. Group 3, which received the inducer and drug (50 mg/kg body weight), shows both the glomerulus and tubules as congested and slightly degenerative. In Group 4, treated with a higher drug dose (100 mg/kg body weight), both the glomerulus and tubular systems are congested, with signs of red blood cell (RBC) extravasation. Group 5, given an even higher dose (200 mg/kg body weight), reveals glomerulus extravasation and congestion, while the tubules show

degeneration, mild necrosis, and infiltration of inflammatory cells. In Group 6, which received the inducer with a standard drug (25 mg/kg body weight), the glomerulus appears congested with a narrow capsule space, and focal renal lesions show inflammatory cell infiltration, while the tubular system appears congested. Lastly, in Group 7, treated with the drug alone (200 mg/kg body weight), the glomerulus appears congested and reddish, with adequate capsule space. The tubules show edematous areas with occasional infiltration of inflammatory cells around them, though some regions appear normal [Slides 2].

4.3 Total Biochemical and Histopathological Discussion

4.3.1 Safety evaluation study discussion

Rats administered methanol extract of PG orally at doses up to 3000 mg/kg body weight showed no mortality or noticeable behavioral changes during the initial 72-hour observation period and throughout the 28-day follow-up. This is an indication that the extract has low acute toxicity when administered to albino rats.

4.4 Discussion of Acetaminophen (APAP) Induced Hepatotoxic Studies in Albino Rats

In the APAP-induced group (Group 2), levels of ALT, AST, and bilirubin were significantly elevated ($P<0.001$). ALP activity was also significantly increased ($P<0.001$) compared to normal rats. Bilirubin, an endogenous organic anion, binds to albumin for transport to the liver, where it is conjugated with glucuronic acid and excreted in bile. It is primarily produced from the breakdown of red blood cells and heme, with lesser contributions from the degradation of myoglobin, cytochrome, catalase, and peroxidase.

The acetaminophen-intoxicated rats (Group 2) exhibited a significant rise ($P<0.001$) in both total bilirubin and total protein levels. In contrast, pretreatment with Silymarin (Groups 3 4and 5) reversed these changes to near-normal levels.

The oxidative stress in the liver was evaluated by measuring SDH reactive substances and antioxidant defense enzymes SOD. Antioxidant enzyme levels, including Superoxide Dismutase or Sodium bi carbonate range (SOD), was assessed in normal, APAP-induced, and Silymarin-treated animals. In APAP-induced liver toxicity (Group 2), SOD level was significantly reduced.

The liver, the largest organ in the vertebrate body, plays a central role in xenobiotic metabolism and excretion. Liver injury can result from exposure to toxic chemicals, drugs, or viral infections. Toxins absorbed from the intestines reach the liver first, often leading to various liver disorders, making liver diseases a significant health concern (Karan et al., 1999).

Acetaminophen is known to cause hepatotoxicity in both experimental animals and humans when taken in high doses (Mitchell and Brian, 1988). The signs of acetaminophen-induced hepatotoxicity resemble those of other acute inflammatory liver diseases, characterized by a significant rise in ALT, AST, and ALP enzyme levels (Davidson, 1996). In APAP-induced hepatotoxicity models, increased serum levels of ALT, AST, ALP, and total bilirubin indicate hepatocellular damage, pointing to cellular leakage and the loss of the liver cell membrane's functional integrity (Drotman and Lawhorn, 1978).

Oxidative stress is believed to play a key role in various diseases, including liver damage (Kiso et al., 1984). It arises from an imbalance between antioxidant defenses and the production of reactive oxygen species. Elevated oxygen levels and the production of reactive species, such as superoxide radicals (O^{2-}), *hydroxyl radicals (OH*), and hydrogen peroxide, contribute to oxidative stress (Zhu et al., 2004).

4.5 Discussion of Liver Mitochondrial Studies (TCA cycle enzyme) in Albino Rats

Oxidative stress compromises cellular integrity when antioxidant defenses are overwhelmed and unable to neutralize free radicals (Ishii et al., 2002). Acetaminophen administration triggers oxidative stress by increasing the production of reactive oxygen species and/or reducing the levels of endogenous antioxidants. The harmful effects of O^{-2} and OH radicals during oxidative stress can be mitigated by antioxidant enzymes like SOD.

SOD is a key intracellular enzyme that protects against oxygen radicals by catalyzing the

removal of superoxide radicals. Acetaminophen administration has been shown to increase superoxide production in liver mitochondria. This heightened superoxide generation promotes lipid peroxidation and induces mitochondrial dysfunction in rats exposed to acetaminophen intoxication. Superoxide radicals generated by the respiratory chain are quickly dismutated by mitochondrial SOD, producing H_2O_2 (Kowaltowski et al., 1999, Yen et al., 1999) that located in the mitochondrial matrix, which is a primary antioxidant enzyme that scavenges superoxide anions. A decrease in SOD activity was observed in rats intoxicated with acetaminophen, likely due to the increased production of superoxides.

The activities of TCA cycle enzymes, including SDH, and respiratory chain marker enzymes (NADH dehydrogenase and cytochrome oxidase), were analyzed in the liver mitochondria of control and experimental rat groups. A significant decrease ($P<0.001$) in these enzyme activities was observed in the acetaminophen-intoxicated group (Group 2) compared to the control group (Group 1). However, a significant increase in enzyme activities was noted in groups 3, 4, and 5. Specifically, the activities of TCA cycle enzymes SDH and respiratory chain marker enzymes (NADH dehydrogenase and cytochrome oxidase) were significantly reduced ($P<0.001$) in the acetaminophen-treated group compared to the normal animals (Group 1). In contrast, animals treated with the standard drug, silymarin, showed a significant ($P<0.001$) increase in the activities of TCA cycle enzymes SDH and respiratory chain marker enzymes (NADH dehydrogenase and cytochrome oxidase).

APAP-induced liver injury is thought to result from the reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI), produced by cytochrome P-450. NAPQI reacts with sulfhydryl groups and protein thiols. At high doses of APAP, NAPQI initially depletes intracellular glutathione (GSH) in hepatocytes and subsequently alkylates cellular macromolecules (Nelson et al.,2003).

SDH contains multiple cysteine-rich sulfur clusters and can be inhibited by agents that modify sulfhydryl groups. NAPQI directly interacts with the sulfhydryl groups on SDH, leading to a loss of its activity (Burcham et al.,1990). The observed reduction in SDH activity may be attributed to a decrease in succinyl CoA levels.

Impaired activities of TCA cycle enzymes in the liver suggest a disruption in glucose oxidation via the TCA cycle. This may be due to the reduced availability of acetyl CoA from pyruvate, resulting from impaired glycolysis during liver injury. Rats pretreated with PG extracts showed significant protection against the impairment of TCA cycle enzyme activities, likely by preventing excessive NAPQI generation. This protection is accompanied by an improvement in the mitochondrial antioxidant defense system, which helps safeguard critical nucleophilic sites on the enzymes from toxic electrophilic metabolites.

5. CONCLUSION

The study indicates that PG exerts dose-dependent histopathological changes in the liver and kidneys of male albino Wistar rats. While low doses resulted in minimal alterations, high doses caused significant tissue damage. Overall, these findings confirm that APAP induces significant liver toxicity, characterized by elevated liver enzymes, decreased protein levels, and oxidative stress markers. Silymarin offers substantial hepatoprotection, as does PG, which shows dose-dependent efficacy in reducing APAP-induced liver toxicity and supporting liver health. These findings suggest that while PG has potential therapeutic benefits, its safety profile needs careful consideration, particularly concerning dosage. Further studies are warranted to elucidate the mechanisms underlying these histopathological changes and to determine safe dosage ranges for potential therapeutic applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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