International Journal of Medicine and Pharmaceutical Research
Home Page: https://pharmaresearchlibrary.org/journals/index.php/ijmpr
CODEN (USA): IJCPNH | ISSN: 2321-2624 | Publisher: Pharma Research Library
DOI: https://doi.org/10.30904/j.ijmpr.2025.4834
Int. J. Med. Pharm. Res., 2025, 13(1): 82-90

Evaluation of Hepatoprotective and Anti-Oxidant Activity of Synthetic Piperonal Dervatives

A. Mamatha*[1], Ch. Suresh[2]
[1]Assistant Professor, Department of Pharmacology, Vijay College of Pharmacy, Nizamabad–503003, Telangana, India
[2]Professor and Principal, Vijay College of Pharmacy, Nizamabad–503003, Telangana, India

A B S T R A C T

The present study was undertaken to evaluate the hepatoprotective and antioxidant activity of newly synthesized piperonal derivatives. Three test compounds (Test compound 1, 2, and 3) were assessed against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Oral administration of the derivatives at 100 mg/kg significantly ($p < 0.01$) reduced elevated serum markers of hepatic damage, including SGOT, SGPT, ALP, total and direct bilirubin, as well as malondialdehyde (MDA). Concurrently, treatment restored protective biochemical parameters such as total protein, glutathione (GSH), and catalase. Histopathological analysis supported the biochemical findings, showing regeneration of hepatocytes and preservation of liver architecture, closely comparable to that observed with the standard hepatoprotective drug, silymarin. Among the derivatives, Test compound 3 demonstrated superior activity, likely due to the presence of an amine substituent contributing to its enhanced pharmacological profile. Overall, the findings indicate that synthetic piperonal derivatives, particularly Test compound 3, exhibit strong antioxidant and hepatoprotective potential and may serve as promising candidates for further therapeutic development.

Keywords: Piperonal derivatives, Hepatoprotective activity, Antioxidant activity, Carbon tetrachloride (CCl_4), Oxidative stress, Glutathione (GSH), Catalase, Malondialdehyde (MDA), Silymarin, Liver histopathology

A R T I C L E I N F O

Corresponding Author
A. Mamatha
Assistant Professor, Department of Pharmacology,
Vijay College of Pharmacy,
Nizamabad–503003, Telangana, India

Article History:
Received : 27 April 2025
Revised : 25 May 2025
Accepted : 29 June 2025
Published : 23 July 2025

Copyright© 2025 The Contribution will be made Open Access under the terms of the Creative Commons Attribution-NonCommercial License (CC BY-NC) (http://creativecommons.org/licenses/by-nc/4.0) which permits use, distribution and reproduction in any medium, provided that the Contribution is properly cited and is not used for commercial purposes.

Citation: A. Mounika, *et al.* Formulation and In-vitro Evaluation of Immediate Release Tablets Containing Febuxostat. Int. J. Med. Pharm. Res., 2025, 13(1):82-90.

CONTENTS

1. Introduction . . . 82
2. Methodology. . . 83
3. Results and Discussion . . . 85
4. Conclusion. . . 89
5. References. . . 90

1. Introduction

Liver is the largest metabolic organ in the body, accounting for a wide range of essential physiological functions including metabolism of nutrients, detoxification of xenobiotics, storage of glycogen and vitamins, and synthesis of plasma proteins. Due to its anatomical and functional role, the liver is highly vulnerable to damage caused by toxins, drugs, alcohol, and infections. Liver diseases such as hepatitis, cirrhosis, and hepatocellular carcinoma represent major global health problems, contributing to high morbidity and mortality rates worldwide. It is estimated that nearly 20,000 deaths occur annually due to liver disorders, and hepatocellular carcinoma ranks among the ten most common tumors globally. Despite advancements in allopathic medicine, conventional drugs for liver ailments are often inadequate and may induce adverse effects, creating a need for safer and more effective hepatoprotective agents (Amartya et al., 2009; Shah et al., 2010). One of the central mechanisms contributing to hepatic injury is oxidative stress, which results from an imbalance between the excessive generation

of reactive oxygen species (ROS) and the limited capacity of endogenous antioxidant defenses. Free radicals such as superoxide anion, hydroxyl radical, and hydrogen peroxide are known to induce lipid peroxidation, protein denaturation, and DNA damage, thereby leading to hepatocyte necrosis or apoptosis (Halliwell & Gutteridge, 1989; Maxwell, 1995).

Carbon tetrachloride (CCl_4) is a classical hepatotoxin widely employed in experimental pharmacology, as its metabolism in the liver produces trichloromethyl free radicals, which initiate peroxidative degradation of membrane lipids, impairing membrane integrity and disrupting cellular architecture (Ueno et al., 2000; Foyet et al., 2011). Therefore, the CCl_4-induced hepatotoxicity model remains one of the most reliable approaches for evaluating hepatoprotective and antioxidant activity of new drug candidates.

Antioxidants play a vital role in mitigating oxidative stress and preserving hepatic integrity. Endogenous enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with non-enzymatic antioxidants like glutathione (GSH), vitamin C, and vitamin E, form the primary defense mechanisms against ROS-mediated injury (Naskar et al., 2010). When these protective systems are overwhelmed, exogenous supplementation becomes necessary. Natural and synthetic antioxidants have thus gained significant attention for their potential to prevent oxidative damage and support liver regeneration. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used widely in the food and pharmaceutical industries, but their long-term use is associated with hepatotoxic and carcinogenic risks (Langseth, 1996; Opara, 2004). Hence, the search for safer and more potent hepatoprotective and antioxidant compounds is of paramount importance.

Piperonal (3,4-methylenedioxybenzaldehyde), a naturally occurring aromatic aldehyde, and its derivatives have been investigated for diverse pharmacological activities including antimicrobial, anti-inflammatory, and anticancer effects. Structural modification of piperonal has led to the development of synthetic derivatives with enhanced bioactivity. In particular, these derivatives hold promise as hepatoprotective and antioxidant agents owing to their ability to modulate oxidative stress pathways and restore hepatic function. The present study was designed to evaluate the hepatoprotective and antioxidant potential of newly synthesized piperonal derivatives using in vivo models of CCl_4 induced hepatotoxicity, with emphasis on biochemical, antioxidant, and histopathological parameters.

2. Methodology

Drugs & Chemicals:

The chemicals and reagents used for the study are of analytical grade and were purchased from the local distributors.

***In vitro* anti-oxidant activity:**

Reductive ability:

The reducing power of the synthetic molecules were determined according to the method of Oyaizu *et al.,*1988**.** Extract solution (2mL), phosphate buffer (2mL, 0.2M, pH 6.6) and potassium ferricyanide (2mL, 10mg/mL) were mixed, and then incubated at 50°C for 20min.

Trichloroacetic acid (2mL, 100mg/mL) was added to the mixture. A volume of 2mL from each of the above mixtures was mixed with 2mL of distilled water and 0.4mL of 0.1% (w/v) ferric chloride in a test tube. After 10-min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power.

b) Hydrogen Peroxide Scavenging Activity:

Scavenging activity of Hydrogen peroxide (H_2O_2) by the synthetic molecules were determined by the method of Ruch *et al.,*1989. Test compounds (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2.

c) DPPH Radical Scavenging Activity: (Blois *et al*., 1958)

Principle: This method is based on the reduction of methanolic solution of DPPH by free-radical scavenger. The antioxidants reacts with DPPH (a purple colored stable free radical) and convert it into a colorless 1,1-diphenyl 2-picryl hydrazine.

Method:

To 0.1 ml of 0.1 mM solution of DPPH in methanol add 3.0 ml of Test sample at different concentrations (10-100 µg/ml). After 30 minutes the absorbance was measured at 517 nm. Lower absorbence indicates higher activity.
Ascorbic acid used as reference material. Percentage

Inhibition IC_{50} was calculated in following way:

$$IC_{50} = (A_{blank} - A_{sample} / A_{blank}) \times 100.$$

Experimental Work:

Hepatoprotective Activity against Ccl_4 Induced Hepatotoxic Rats (Sanjay *et al*., 2009)

Procedure: The Male Wistar rats weighing 180-200 g were used. The overnight fasted animals were divided into eight groups (n=5) as follows:

Group I (Normal Control): received normal saline (1 ml/kg, po) daily for 5 days and received liquid paraffin (1 mL/kg, *s.c.*) on day 2 and 3.

Group II (Toxic control): The animals of this group received normal saline (1 ml/kg, po) daily for 5 days and CCl4 (2 mL/kg body weight, *s.c.*) in liquid paraffin (1:1) on day 2 and 3.

Group III (Standard):

The animals of this group received standard drug silymarin (100 mg/kg, *p.o.*) once daily for 5 days and CCl4 (2 mL/kg body weight, *s.c.*) in liquid paraffin (1:1) on day 2 and 3.

Group IV (Test Compound 1):

The animals of this group received Test compound 1 (100 mg/kg, *p.o.*) once daily for 5 days and CCl4 (2 mL/kg body weight, *s.c.*) in liquid paraffin (1:1) on day 2 and 3.

Group V (Test Compound 2): The animals of this group received Test compound 2 (100 mg/kg, *p.o.*) once daily for 5 days and CCl4 (2 mL/kg body weight, *s.c.*) in liquid paraffin (1:1) on day 2 and 3.

Group VI (Test Compound 3): The animals of this group received Test compound 3 (100 mg/kg, *p.o.*) once daily for 5 days and CCl_4 (2 mL/kg body weight, *s.c.*) in liquid paraffin (1:1) on day 2 and 3.

Biochemical estimations:

On the 6th day after overnight fasting all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 7000 rpm for 15 min. at 5°C. The separated serum was used for estimation of AST and ALT (IFCC, UV Kinetic method), ALP (pNPP Kinetic Method), TP (Biuret method), Bilirubin (Mod. Jendrassik and Grof's Method), Lipid peroxidation (thiobarbituric acid), Glutathione and catalase.

Histopathological Investigations:

On the 6th day the animals were sacrificed and abdomen was cut open, the liver was dissected out. Liver was rinsed in water and preserved in 10% formalin solution. The samples were given to the pathological laboratory for further histopathological examination (Lakshmi *et al.*, 2011).

Preparation of tissue homogenate:

The tissues were weighed and 10% tissue homogenate was prepared with normal saline and centrifuged at 3000 rpm for 15 min. The supernatant was collected and the collected supernatant was used for estimation of catalase activity, lipid peroxidation and glutathione levels.

***In-vivo* anti-oxidant activity:**

Catalase activity:

Catalase activity was assayed according to the method of sinha *et al.*,1972**.** To 0.4 ml of hydrogen peroxide (0.2M) was added 1 ml of 0.01 M phosphate buffer(pH 7) followed by the addition of 0.1 ml of clear supernatent of liver homogenate (10% w/v) and gentley swirled at room temperature. The reaction of the mixture was stopped by adding 2 ml of dichromate acetic acid reagent (5 % K_2Cr_2O7 prepared in glacial acetic acid). The changes in the absorbence was measured at 620 nm and recorded after 3 mint interval. Percentage inhibition was calculated by using this equation.

$$\% \text{ inhibition rate} = \frac{\text{Abs of Control-Abs of sample with extract} \times 100}{\text{Abs of Control}}$$

ii. Estimation of Lipid Peroxidation (MDA):

Lipid peroxidation (LPO) was assayed according to the method of Okhawa *et al*. Lipid peroxidation can be defined as oxidative deterioration of lipids containing any number of carbon –carbon double bonds. The processes where by free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage and increased concentration of end products of lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human disease. The amount of lipid peroxidation products present in the serum samples/ pleural fluid was estimated by the thiobarbituric acid reactive substances (TBARS) method, which measures the malondialdehyde (MDA) reactive products by using UV-Visible spectroscopy.

Principle: The reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a secondary product of lipid peroxidation has been widely adopted as a sensitive assay method for measurement of lipid peroxidation in biological fluids. It is widely used as a index of the extent to which lipid peroxidation has progressed. Since the assay procedure estimates the amount of TBA reactive substances e.g. MDA, it is also referred to as TBARS (Thiobarbituric acid reactive substance) test.

Procedure: To 0.5 ml of Liver homogenase, 0.5 ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. Clear supernatant was taken after centrifuging at 3000 rpm for 10 min. To the supernatant, 500µl of 1%TBA solution and 500µl of water was added and this solution was heated for 1hr at 98°C. Cool the solutions to room temperature and kept them in ice for 5 minutes. Then read the pink color at 532 nm using spectrophotometer. Standard graph was plotted using TEP (1, 1, 3, 3-tetra ethoxy propane).

iii. Estimation of Glutathione:

Glutathione forms a colored complex with DTNB, which is measured spectrophotometrically (Ellman G.L., 1959; Beulter *et al*., 1963)

Reagents:

- Sodium phosphate buffer (0.3 M) pH: 0.3 M sodium dihydrogen phosphate (NaH_2PO_4) 4.68 gm/100 ml and 0.3 M disodium hydrogen phosphate (Na_2HPO_4) 5.33 gm/100 ml, were prepared in double distilled water. To 0.3 M disodium hydrogen phosphate solution, sufficient amount of 0.3 M sodium dihydrogen phosphate solution was added to get pH 8 phosphate buffer solution using pH meter.
- 5-5'(dithiobis-2-nitro benzoic acid) DTNB reagent: 39.6 mg of DTNB was dissolved in 100 ml of 1% sodium citrate solution to give a concentration of 1Mm, sodium citrate solution has been selected for convenience, since its pH was appropriate both for solubility and stability of the reagent. The DTNB was stable in this medium for 13 weeks in refrigerator.

Procedure: To 0.5 ml of liver homogenate, 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 minutes. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer and 0.5 ml of DTNB reagent was added. The absorbance of the yellow color developed was measured at 412 nm. The glutathione content was determined from the standard graph by using pure glutathione.

Statistical analysis:

Statistical analysis was carried out using Graph Pad PRISM software (version 6.03). The data were expressed as mean ± SD. All the date were analyzed by one-way ANOVA followed by Dunnet's multiple comparision tests. A p value < 0.05 was considered statistically significant.

3. Results and Discussion

a) Reducing Power Method:

Table.1: Reductive ability of Ascorbic acid, Test Compound 1, Test Compound 2 and Test Compound 3

Conc (ug/ml)	Absorbances			
	Ascorbic acid	Test Com-1	Test com-2	Test com-3
10	0.042	0.121	0.208	0.451
25	0.051	0.351	0.451	0.624
50	0.065	0.5	0.508	0.681
75	0.07	0.589	0.624	0.889
100	0.094	0.684	0.781	0.921

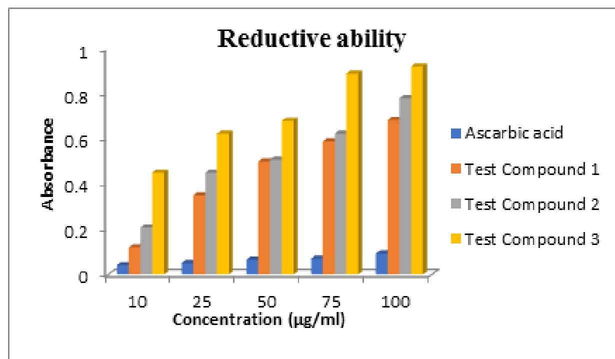

Fig.1: Absorbances of Ascorbic acid, Test compound 1, Test compound 2 and Test compound 3

b) Hydrogen peroxide scavenging activity:

The IC50 value for standard Ascorbic acid was found to be 25.416µg/ml., whereas the IC50 value of Test compound 1, Test compound 2 and Test compound 3 were found to be 58.137µg/ml, 50.868µg/ml and 49.720µg/ml respectively.

Table 2: Percentage scavenging activity of Ascorbic acid, Test compound 1, Test compound 2 and Test compound 3

Conc (µg/ml)	Percentage Scavenging activity			
	Ascorbic acid	Test Com-1	Test com-2	Test com-3
10	41.17	18.52	20.12	25.24
25	69.17	32.01	32.65	38.68
50	89.58	50.491	58.14	58.56
75	94.42	67.4	67.56	70.14
100	97.01	70.17	78.58	79.31
IC_{50} (µg/ml)	25.416	58.137	50.868	49.720

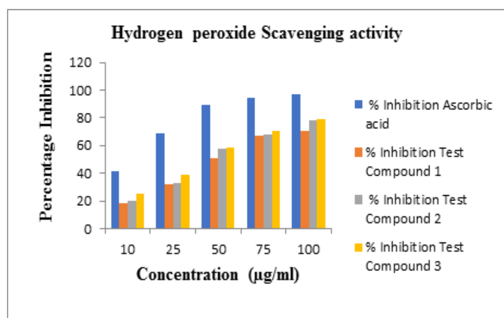

Fig 2: Percentage inhibition of Ascorbic acid, Test compound 1, Test compound 2 and Test compound 3

c) DPPH Method:

The IC_{50} value for standard Ascorbic acid was found to be 7.194µg/ml., whereas the IC_{50} value of Test compound 1, Test compound 2 and Test compound 3 were found to be 46.484 µg/ml, 46.006 µg/ml and 43.115µg/ml respectively.

Table 3: Percentage scavenging activity of Ascorbic acid, Test compound 1, Test compound 2 and Test compound 3

Conc (ug/ml)	Percentage Scavenging activity			
	Ascorbic acid	Test Com-1	Test Com-2	Test Com-3
10	73.35	28.764	38.823	37.352
25	87.80	34.705	44.4117	48.4117
50	89.43	42.058	60.4117	61.764
75	90.53	59.411	71.823	74.823
100	93.55	68.53	74.4117	78.529
IC_{50} (µg/ml)	7.194	46.484	46.006	43.115

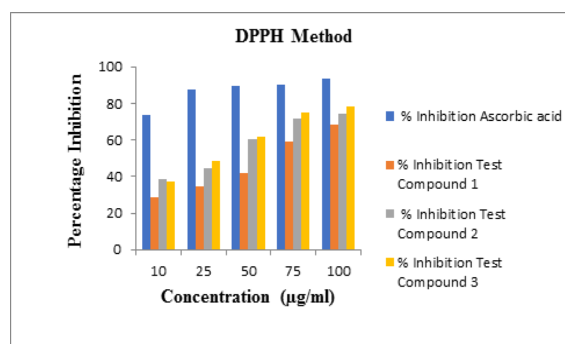

Fig 3: Percentage inhibition of Ascorbic acid, Test compound 1, Test compound 2 and Test compound 3

In-vivo anti-oxidant activity:

a) Lipid Preoxidation:

For the estimation of malondialdehyde levels, standard graph of TEP (1,1,3,3-tetra ethoxy propane) was plotted between the concentration and absorbance values.

Table 4: standard graph of TEP

Conc. (µg/ml)	Absorbance
10	0.125
30	0.375
50	0.625
70	0.875
100	1.15

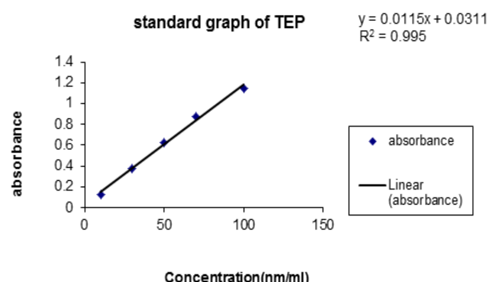

Fig 4: Standard graph of 1,1,3,3-tetra ethoxy propane (TEP)

Table 5: Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum MDA levels in CCl4 induced hepatotoxic rats.

Group (n=5)	Treatment	MDA (nmol/gm)
I	Nomal Control	1.69±0.30
II	Toxic control	22.07±2.11
III	Standard Silymarin	2.50±0.87
IV	**Test Com-1** 100mg/kg	5.63±1.99
V	**Test Com-2** 100mg/kg	4.82±0.55
VI	**Test Com-3** 100mg/kg	4.41±1.55

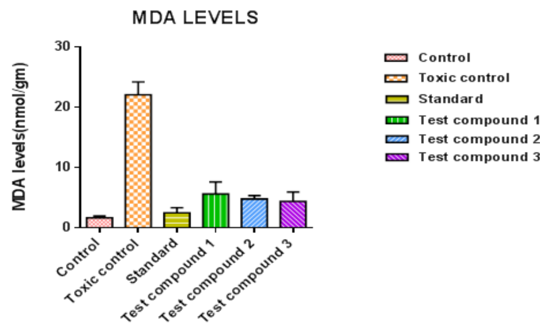

Fig 5: Effect of Test compound 1, Test compound 2 and Test compound 3 on MDA levels in CCl_4 induced hepatotoxic rats

Table 6 : Effect of Test compound 1, Test compound 2 and Test compound 3 on catalase activity compared to the disease control group.(Mean ± SD, n=5).

S.No	Groups	%H_2O_2 Scavenging
1	Normal Control	79.28±8.43
2	Toxic control	20.08±1.15
3	Standard Silymarin	71.43±8.34
4	Test Compound 1 100mg/kg	67.45±3.31
5	Test Compound 2 100mg/kg	69.61±4.30
6	Test Compound 3 100mg/kg	70.21±2.79

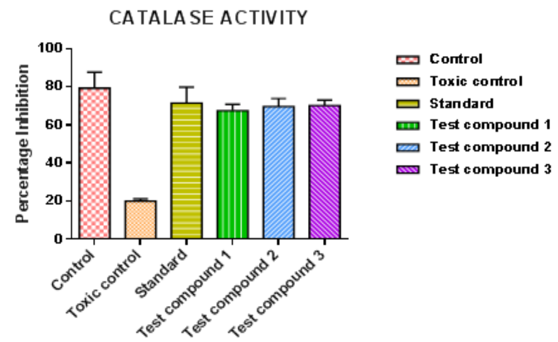

Fig.6: Effect of Test compound 1, Test compound 2 and Test compound 3 on catalase levels in CCl_4 induced hepatotoxic rats

Table 7: Effect of Test compound 1, Test compound 2 and Test compound 3 on glutathione activity

S.No	Groups	% of Total glutathione level
1	Normal Control	24.59±1.11
2	Toxic control	20.96±4.515
3	Standard Silymarin	37.96±4.51
4	Test 1100mg/kg	30.96±0.79
5	Test 2 100mg/kg	36.21±5.03
6	Test 3 100mg/kg	38.01±3.54

Fig 8: Effect of Test com-1, Test com-2 and Test com-3 on Glutathione levels in CCl_4 induced hepatotoxic rats

Groups (n=5)	Dose (mg/kg)	SGOT(IU/L)	SGPT(IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (mg/dl)	Alkaline phosphatase (IU/L)
Control	-----	38.77±15.88	49.79±17.25	1.78±0.27	0.18±0.07	7.82±0.56	34.57±4.53
Toxic control(CCl_4)	1ml/kg	83.71±8.79	91.19±4.89	3.74±0.38	0.83±0.17	3.32±0.38	117.92±7.98
Standard (Silymarin)	100	0.11±19.55*	59.95±24.98*	1.97±0.12**	0.20±0.07*	7.44±0.44**	32.74±1.63**
Test compound -1	100	59±6.83*	57.14±11.66**	2.09±0.35**	0.26±0.22**	6.99±0.72**	64.7±2.83**
Test compound -2	100	49.03±10.34**	52.54±8.35**	1.99±0.20**	0.22±0.08**	7.33±0.55**	52.37±3.72**
Test compound 3	100	47.97±6.32**	47.65±25.06**	1.85±0.09**	0.19±0.09**	7.67±0.37**	28.74±3.70**

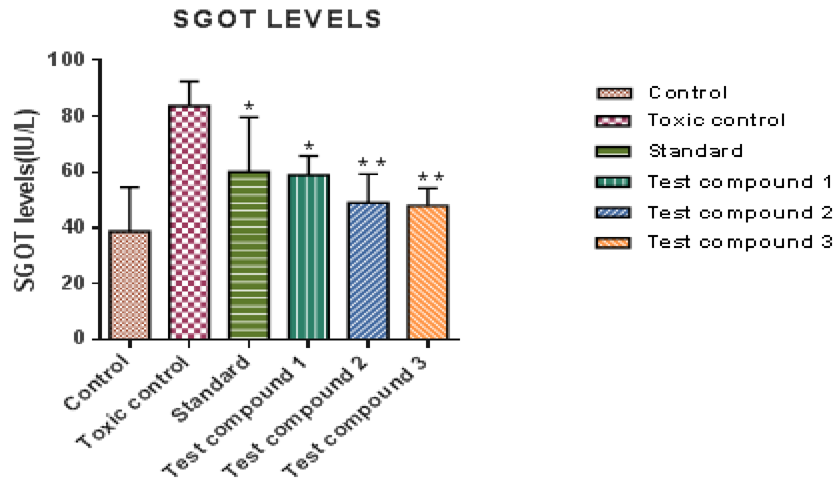

Fig 7 : Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum SGOT levels in CCl4 induced hepatotoxic rats.

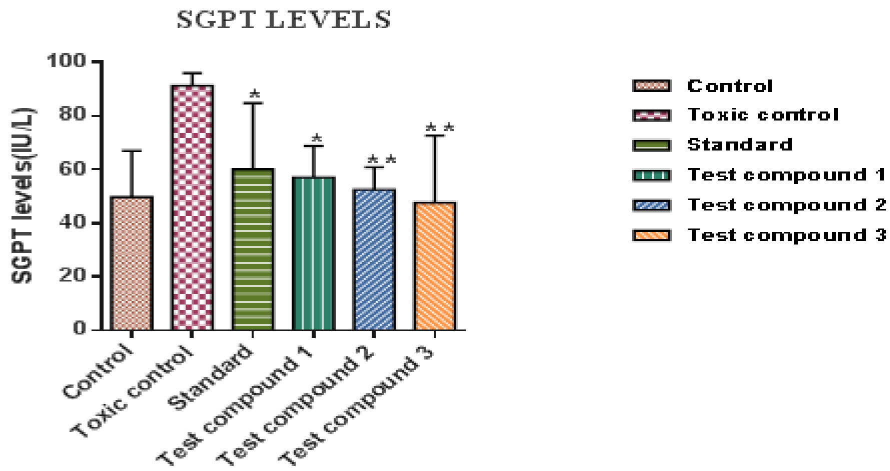

Fig.8: Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum SGPT levels in CCl4 induced hepatotoxic rats

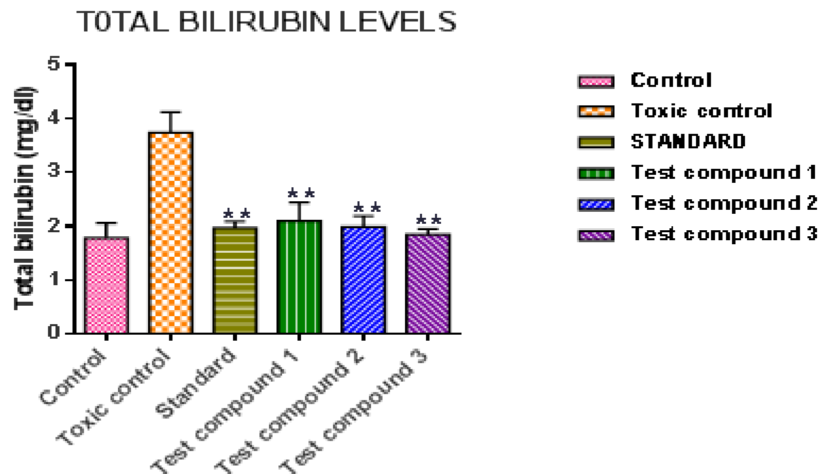

Fig.9: Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum Total bilirubin levels in CCl_4 induced hepatotoxic rats.

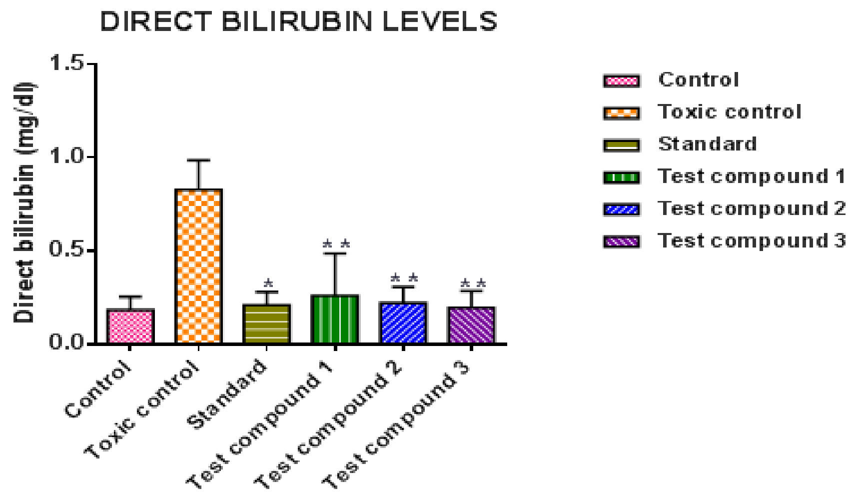

Fig.10: Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum Direct bilirubin levels in CCl_4 induced hepatotoxic rats.

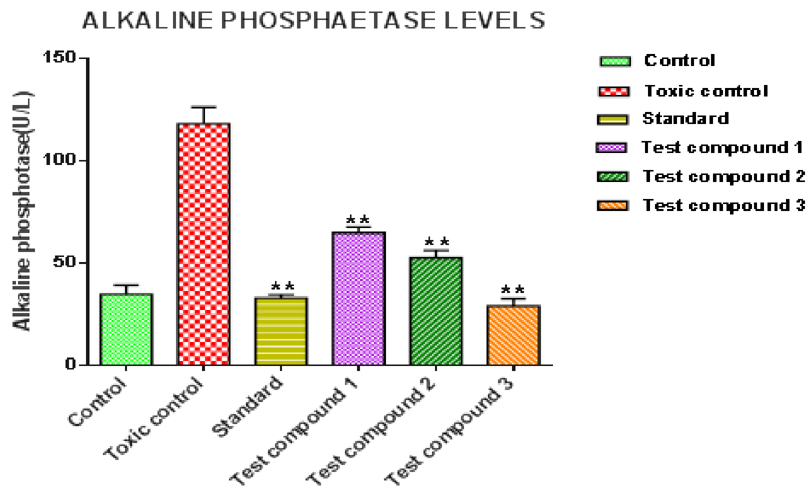

Fig 11: Effect of Test com- 1, Test comp- 2 and Test comp-3 on Serum Alkaline phosphatase levels in CCl4 induced hepatotoxic rats.

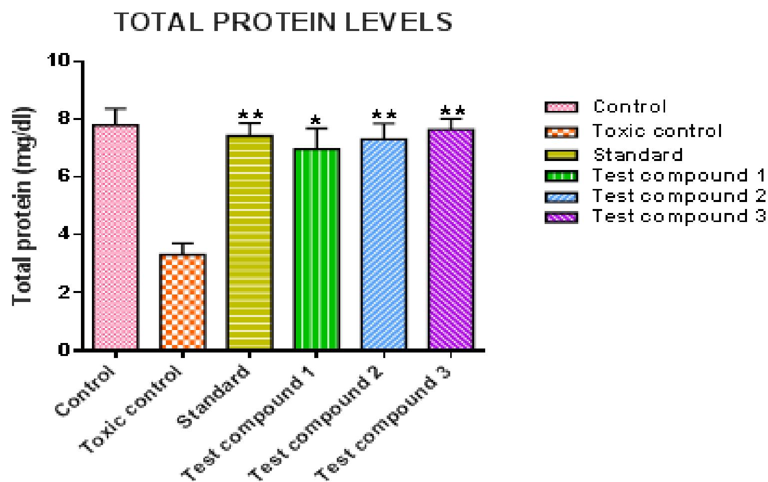

Fig 12: Effect of Test compound 1, Test compound 2 and Test compound 3 on Serum Total protein in CCl4 induced hepatotoxic rats.

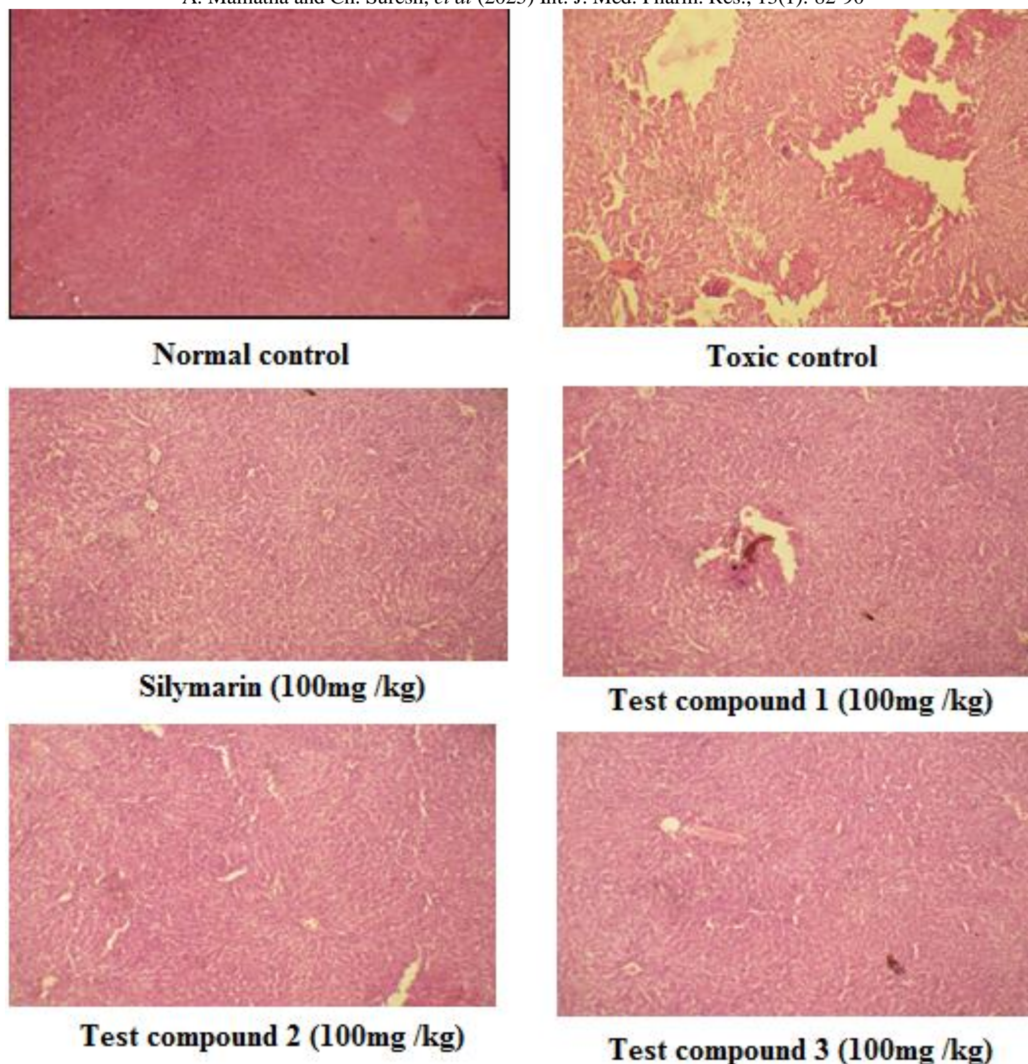

Fig.13: Histopathology of liver sections of CCl4 induced hepatotoxic rats.

Discussion

Carbon tetrachloride (CCl4)-induced hepatotoxicity is a well-established model for evaluating hepatoprotective activity, as its metabolism generates highly reactive trichloromethyl and trichloromethyl peroxy radicals that cause lipid peroxidation, protein damage, and disruption of hepatocyte membranes. In this study, administration of CCl4 significantly elevated serum biomarkers of hepatic injury such as SGOT, SGPT, ALP, bilirubin, and malondialdehyde (MDA), while reducing antioxidant defenses including glutathione (GSH), catalase, and total protein levels. These biochemical changes were further corroborated by histopathological alterations, which revealed necrosis, periportal infiltration, and distortion of normal hepatic architecture in the toxic control group. Treatment with the test compounds (1, 2, and 3) at a dose of 100 mg/kg produced a marked hepatoprotective effect, as evidenced by significant reduction ($p<0.01$) in serum SGOT, SGPT, ALP, total and direct bilirubin, as well as MDA levels compared to the toxic control. Concurrently, levels of total protein, GSH, and catalase were significantly restored, indicating a strong antioxidant defense and stabilization of hepatocellular membranes. The histological evaluation confirmed these findings, showing regenerating hepatocytes and preservation of hepatic architecture in the test compound-treated groups, with results comparable to the standard hepatoprotective drug, silymarin. Aong the test compounds, compound 3 demonstrated superior hepatoprotective and antioxidant efficacy compared to compounds 1 and 2, with liver sections showing nearly normal architecture, closely resembling that of the silymarin-treated group. This suggests that the functional groups present in compound 3 may play a key role in enhancing its free radical scavenging and membrane-stabilizing effects.

4. **Conclusion**

The study demonstrated that the test compounds exerted marked hepatoprotective and antioxidant effects in CCl4-induced liver injury. Treatment significantly lowered elevated hepatic enzymes (SGOT, SGPT, ALP), bilirubin, and lipid peroxidation (MDA), while restoring antioxidant defenses (GSH, catalase) and total protein levels. Histopathological findings further confirmed hepatic protection, showing regeneration of hepatocytes and preservation of liver architecture. Among the derivatives, compound 3 exhibited the strongest activity, with effects

comparable to silymarin. These results suggest that the test compounds, particularly comp-3, may serve as promising candidates for the development of hepatoprotective and antioxidant therapeutics.

5. Bibliography

[1] Kumar V, Cotran RS, Robbins SL. *Robbins Basic Pathology*. 6th ed. Philadelphia: W.B. Saunders; 2000.

[2] Amartya K, Shibnath M, Pranab B. Hepatoprotective medicinal plants: A review. Int J Pharm Sci Res. 2009;1(1):1–5.

[3] Shah VN, Devalia HL. Role of antioxidants in hepatoprotection: A review. J Clin Diagn Res. 2010; 4:2742–2747.

[4] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 2nd ed. Oxford: Clarendon Press; 1989.

[5] Maxwell SRJ. Prospects for the use of antioxidant therapies. Drugs. 1995;49(3):345–361.

[6] Ueno S, Mizuno T, Fukushima H. Mechanisms of carbon tetrachloride-induced hepatotoxicity. Hepatology. 2000;32(2):247–256.

[7] Foyet HS, Tsala DE, Zingue S, et al. Antioxidant and hepatoprotective effects of Acanthus montanus. BMC Complement Altern Med. 2011;11:107.

[8] Langseth L. *Oxidants, Antioxidants, and Disease Prevention*. Brussels: ILSI Press; 1996.

[9] Opara EC. Oxidative stress, micronutrients, diabetes mellitus and its complications. J R Soc Health. 2004;124(1):18–25.

[10] Naskar S, Islam A, Mazumder UK, Saha P, Haldar PK, Gupta M. In vitro and in vivo antioxidant potential of hydromethanolic extract of Phoenix dactylifera fruits. J Sci Res. 2010;2(1):144–157.

[11] Oyaizu, M., 1988.Antioxidantive activities of browning products of glucosamine fractionated by organic solvent and thin layer chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, 771–775.

[12] Ruch, R.J., Cheng, S.J., Klaunig, J.E., 1989. Prevention of cytotoxicity and inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen*, 1003-1008.

[13] Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, 1199-1200.

[14] Sanjay, J., Vinod, K., Dixit., Neelesh, M. And Vijay, A., 2009. Antioxidant and Hepatoprotective Activity Of Ethanolic And Aqueous Extracts Of *Amorphophallus Campanulatus* Roxb.Tubers. *Acta Poloniae Pharmaceutica-Drug Research*, 424.

[15] Lakshmi, B.V.S., Uday Kumar, P., Neelima, N., Umarani, V., Sudhakar, M., 2011. Hepatoprotective activity of Lagenaria siceraria fruit extracts against carbontetrachloride induced hepatic damage in rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 133.

[16] Sinha, A.K., 1972. Colorimetric assay of catalase. *Anal biochem,* 389-394.

[17] Beutler, E., Satish, K., Srivastava. Glutathione metabolism of the erythrocyte the enzymic cleavage of glutathione-haemoglobin preparations by glutathione reductase. *Biochemical journal*, 1970, 353-357.