RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 44 Issue 4: 568-577 (August 2025)

Hepato-protective Effect of Aqueous Extract of Manila Tamarind (*Pithecellobium dulce*) Fruit Pulp against Carbon Tetrachloride Induced Hepatotoxicity in Adult Mice

C. Roselin[1], S. Parameshwari[1]

10.18805/ajdfr.DR-2297

ABSTRACT

Background: Manila Tamarind (*Pithecellobium dulce*) fruit pulp powder is used as a therapeutic agent to treat a range of ailments due to its extraordinary capabilities. The bark and pulp are known for their hemostatic and astringent properties, making them effective remedies for toothaches, bleeding gums and gum problems. This study aims to investigate the hepatoprotective effect of *Pithecellobium dulce* fruit pulp in laboratory animals.

Methods: To induce liver injury, experimental animals were administered approximately 1 ml of carbon tetrachloride (CCl4) intraperitoneal (i.p) injection in olive oil. The animals were then given 50 mg, 300 mg and 2000 mg/kg body weight (bw) of aqueous extract of Manila tamarind fruit solution *via* oral gavage tube. Control animals received distilled water as a vehicle. The study measured serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), triglyceride and bilirubin levels to assess the hepatoprotective effect.

Result: The aqueous extract of the fruit pulp of Manila tamarind significantly reduced serum SGOT, SGPT, ALP, triglyceride and bilirubin levels in carbon tetrachloride-induced hepatotoxicity. Mannose exhibited the strongest binding affinity (-6.2 kcal/mol), followed by Hexyl (-5.1 kcal/mol) and Pyran-4-one (-4.9 kcal/mol), highlighting their potential for therapeutic applications. These findings provide a foundation for further pharmacological research on Manila Tamarind phytochemicals. The results prove that the aqueous extract of the fruit pulp of Pithecellobium dulce possesses a hepatoprotective effect against liver injury.

Key words: Aqueous extract, Carbon tetrachloride, Hepatotoxicity, *Pithecellobium dulce*, SGOT, SGPT.

INTRODUCTION

Human organ liver is an important part that supports nearly all other organs within the body. It is a metabolic organ which performs key functions such as drug metabolism, synthesis of plasma proteins and elimination of metabolic waste products. Because of its advantageous location and diverse functions, it is the principal organ for drug metabolism and elimination (Robin *et al.,* 2012). Liver toxicity can result from the direct attack of xenobiotics/ endobiotics, generation of reactive metabolites and immune system-mediated autoimmune reactions. These elements particularly affect hepatocytes, biliary epithelial cells and the liver vasculature. Chemicals have the potential to induce a hepatotoxic response, which depends on the concentration of the toxin, the specific enzymes that are produced and the gradient of substance concentration in the blood around the acinus (Gulati *et al.,* 2018). Hepatotoxicity is characterised by elevated blood triglycerides, total cholesterol, bilirubin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) which are invariably linked with cellular necrosis, there is an elevation in tissue lipid peroxidation and a reduction in tissue glutathione (GSH) levels (Ganapathi *et al*., 2024; Sul *et al*., 2021). Currently, there are very few hepatoprotective drugs available for the treatment of liver problems, all of which are derived from natural sources (there is not a single successful allopathic drug).

[1]Department of Nutrition and Dietetics, Periyar University, Salem-636 011, Tamil Nadu, India.

Corresponding Author: S. Parameshwari, Department of Nutrition and Dietetics, Periyar University, Salem-636 011, Tamil Nadu, India. Email: sparameshwari2009@gmail.com

How to cite this article: Roselin, C. and Parameshwari, S. (2025). Hepato-protective Effect of Aqueous Extract of Manila Tamarind (*Pithecellobium dulce*) Fruit Pulp against Carbon Tetrachloride Induced Hepatotoxicity in Adult Mice. *Asian Journal of Dairy and Food Research*. **44(4):** 568-577. doi: 10.18805/ajdfr.DR-2297.

Submitted: 22-11-2024 **Accepted:** 09-02-2025 **Online:** 21-03-2025

Pithecellobium dulce, commonly referred to as Manila tamarind, is a fruit that enjoys widespread recognition in rural India for its extensive medicinal properties. This fruit is a rich source of various nutrients, antioxidants and bioactive compounds, making it a valuable addition to traditional medicine. Belonging to the Leguminosae family, the fruit is originally native to Neotropics. However, it has successfully spread beyond its native habitat and is now found in many regions, particularly throughout India and tropical Africa. Its adaptability has allowed it to thrive in coastal areas, where it has become naturalized.In addition to its presence in India and Africa, *Pithecellobium dulce* has also established itself as an invasive species in Greater Antilles. Its invasive nature is also noted in parts of the United States, such as Florida and Hawaii. However, in regions where human and animal

populations exert pressure, the spread of this species is less pronounced, indicating that ecological factors play a significant role in its distribution. The fruit's medicinal properties are attributed to its composition, which includes a variety of nutrients and antioxidants that contribute to its health benefits. These properties have made Pithecellobium dulce a subject of interest for further pharmacological research, particularly in the context of its potential therapeutic applications (Roselin and Parameshwari, 2022; Sneha *et al.,* 2020).

Pithecellobium dulce, commonly known as Manila tamarind, is a fruit that holds significant cultural and medicinal value, particularly in rural India. Locally referred to as "jangal jalebe," it is recognized by various names across different languages, including 'vilayati babul' in Hindi and 'kodukkapuli' in Tamil. This fruit is characterized by its bitter flavor, which is a distinctive feature that sets it apart from other fruits (Raju and Jagadeeshwar, 2014). *Pithecellobium dulce* is used as medicine for treating health problems due to its remarkable therapeutic nature. The wood pulp of the fruit are known for their astringent and hemostatic qualities, making them effective in addressing issues like bleeding, toothaches and gum diseases. Bark extracts are utilized to alleviate conditions such as constipation, dysentery, chronic diarrhea and tuberculosis. The leaves are beneficial for treating indigestion, preventing spontaneous abortion, managing gall bladder disorders and healing both open and closed wounds. Ground seeds are employed in the treatment of ulcers. Furthermore, research suggests that Pithecellobium dulce may be effective against a wide range of conditions, including conjunctivitis, eczema, chronic inflammation, bilious disorders, sore throat (Kaushik *et al.,* 2018). The leaves of the plant serve as fodder and the fruit is edible to eat. Additionally, it is a protein source and has the ability to fix atmospheric nitrogen (Hiwale, 2015).

The fruit is rich in various nutraceutical properties, such as antibacterial, antioxidant, anti-inflammatory, hepatoprotective, anti-ulcer and anti-diabetic effects. However, a significant amount of waste is generated due to the fruit's high perishability and the absence of effective preservation methods or value-added processes. To address this issue, modern technologies are being employed to incorporate desirable characteristics into a range of products (Sul *et al*., 2021). According to the study of Manna *et al.* (2011), the liver demonstrated protection against damage induced by CCl_4 both prior to and following the administration of the aqueous extract of tamarind pulp. Histological examinations further corroborated these findings (Pampori *et al.*, 2024). The study compared the antioxidant properties to antioxidant vitamin C, concluding that the aqueous extract of tamarind pulp effectively protects the liver in murine models from oxidative damage caused by CCl_4. Hence, the present study is helps in identifying the hepatoprotective effect of an aqueous extract of Manila tamarind (*Pithecellobium dulce*) fruit pulp.

MATERIALS AND METHODS

Selection of animals, housing and feeding

Male Wistar strain albino mice, six weeks old with an average weight of 120 g, were obtained from the BIOGEN research animal facility in Bangalore. They were picked at random and given identification labels. Prior to dosing, they were caged and adapted for a week. The light/dark cycle was repeated every 12 hours and heat index, or apparent temperature were kept constant at about 223°C and 40-60%, respectively, for the duration of the study. The experimental animals were fed commercially available normal rat chow (Tetrogon Chemie Pvt. Ltd.) with water accessible at all times. The study was carried out at Srimad Andavan Arts and Science College's Animal Laboratory, Affiliated to Bharathidasan University, Tiruchirappalli and supervision of Experiment of Animals (CPCSEA).

Collection and aqueous extraction of Manila tamarind (Pithecellobium dulce) fruit

The Manila tamarind fruits were harvested in bulk from a farm located in the Tiruchirappalli District of Tamil Nadu. After being detached from their stalks, the fruits were thoroughly washed under running water to eliminate any dirt or contaminants. Once the seeds were removed, the fruits were weighed and laid out on parchment paper for three hours to allow excess water to drain at room temperature. Subsequently, dried in a hot air oven to ensure complete moisture removal, with both the drying temperature and duration recorded. The dried fruit pulp was then powdered using a grinder and passed through a 1 mm mesh for sieving, with the final weight noted. For the aqueous extraction process, approximately 2.5 g of the powdered Manila tamarind fruit was mixed with 7.5 mL of distilled water. This mix was undisturbed till 24 hours at temperate in a sterile flask covered with aluminum foil to prevent evaporation, before being filtered using Whatman No. 1 filter paper.

Liver toxicity induction

All experimental animals, except for the control group, received approximately 1 mL of CCl_4 intraperitoneally (i.p.) using an olive oil vehicle. The rats were weighed weekly throughout the duration of the study. Before and after CCL_4 induction they had ease of space to rat chow (pelleted) and water. The liver function and antioxidant status of the rats were monitored for 15 days following the injection.

Trial design

Randomly allocated thirty six Wistar albino rats to six distinct groups, comprising three treatment groups, two standard control groups and one normal control group. Group I was control group, had unrestricted access to water and standard rat chow. Group II functioned as the CCl_4-induced standard control, receiving saline as a vehicle. The hepatoprotective standard control for Group III was managed silymarin of dosage 50 mg/kg body weight *via* oral gavage. Group IV

received the Manila tamarind aqueous fruit extract as a hepatoprotective treatment of 100 mg/kg body weight. Group V, designated as the second hepatoprotective treatment group, was administered a higher dosage of 200 mg/kg body weight aqueous fruit extract. The sixth group with a high dosage of 400 mg/kg body weight of the fruit extract, serving as hepatoprotective treatment group III.

Blood sample collection

Samples were collected from orbital venous plexus bleeding using capillary tubes while the animals were under mild anesthesia with ether. This procedure was conducted between 09:00 and 12:00 hours, following an 18-hour fasting period after the last treatment dose. For the subsequent biomarker analysis, blood was collected in fresh vials containing EDTA as an anticoagulant and serum was separated using a cooling electric centrifuge (REMI cooling centrifuge-VCBF-1322) at 3000 rpm for 10 minutes. The study was carried out from 2022 to 2023.

Evaluation of the effect of Manila tamarind aqueous fruit powder extract in hepatotoxic adult male mice

Measurement of animal weight (body and liver)

The weight of each individual sample from all groups was measured weekly using an electronic balance. Following this, the liver and pancreas were carefully cleaned and weighed separately in accordance with the standard operating procedure (SOP) for conditional dissection.

Estimation of the lipid profile

The lipid profiles were evaluated after 15 days of the study period. Total cholesterol was assessed by Trinder and Webster (1984) using the CHOD-PAP method, HDL by Lopes-Virella *et al*. (1977) using the precipitation method and triglycerides by Henkel and Stoltz (1982) using the Enzymatic -GPO method. The Friedewald equation was also used by Trinder and Webster to determine total cholesterol.

Effects of Manila tamarind (*Pithecellobium dulce*) fruit aqueous extract on adult male mice antioxidant status

Determination of lipid peroxidation by 2-thiobarbituric acid reactive substances (TBARS)

To determine lipid peroxidation using 2-thiobarbituric acid reactive substances (TBARS), 1 ml of 8.1% sodium didecyl sulfate (SDS), 2 ml of 20% acetic acid and 1 ml of 0.75% thiobarbituric acid (TBA) were added to each 1 ml of tissue homogenates and plasma samples. The mixture was then heated for 30 minutes and centrifuged at 14,000 rpm for 10 minutes. The absorbance of the malondialdehyde (MDA)-TBA adduct was measured calorimetrically at 533 nm using a spectrophotometer (Bidlack and Tappel, 1973). MDA values were calculated and reported as TBARS values, based on a standard curve prepared with tetrameth oxypropane and TBA.

Superoxide dismutase (SOD)

First, 50 mM sodium carbonate buffer was added to 25 µL of the supernatant solution obtained from centrifuged liver homogenate. In a total volume of 2 ml buffer medium, add 0.1 mM epinephrine. At 480 nm, absorption was measured (Sun and Zigman, 1978).

Estimation of LFT

The serum concentrations of bilirubin, alkaline phosphatase, glutamic-oxaloacetic transaminase (SGOT) and glutamine-pyruvic transaminase (SGPT) were measured using standard techniques.

Liver histopathology

The liver and pancreas from both control and CCl_4-treated rats were excised following euthanasia. The tissues were meticulously cleaned to remove any connective tissue and subsequently fixed in 10% formaldehyde buffer for a minimum of 24 hours. After initial fixation, the tissues were transferred to 10% neutral buffered formalin for further preservation before being embedded in paraffin. Using a microtome, the samples were longitudinally and serially sectioned at a thickness of 4 micrometers. Deparaffinization occurred with xylene, rehydrated and stained with hematoxylin and eosin. Finally, slides examined under a microscope equipped with a camera to capture images for analysis (Kafali *et al*., 2004; Henkel and Stoltz, 1982).

Preparation and analysis of phytochemicals - Docking simulations

The study's methodology focused on the preparation and analysis of phytochemicals extracted from the aqueous extract of Manila tamarind. Molecular structures of significant compounds, including furanones, lactones and sugars, were either obtained or modeled to support docking analysis. Docking simulations were performed through virtual screening (PyRx tool), that includes energy minimization and ligand optimization to improve docking accuracy. Target residues for docking were selected based on active sites reported in the literature, ensuring the biological relevance of the findings. The ligands were then docked into the binding pockets of these targets and their binding affinities were calculated in kcal/mol to evaluate the strength of the interactions. Furthermore, the types of bonds formed-such as hydrogen bonds, CH bonds and alkyl bonds-were examined to gain insights into the stability and nature of these interactions. Ultimately, ligands with the lowest binding energy values were prioritized as potential candidates for further pharmacological investigation.

Analysis of data

The statistical analysis was conducted using SPSS version 16.0. To assess the significance of the results, a one-way analysis of variance (ANOVA) was employed. This method allows for the comparison of means across multiple groups to determine if there are any statistically significant differences. A p-value of less than 0.05 ($p<0.05$) was used as the threshold to indicate statistical significance. This means that any observed differences were considered statistically significant if the probability of obtaining the

observed results, assuming the null hypothesis is true, was less than 5%.

RESULTS AND DISCUSSION

Aqueous extract-yield results

Aqueous extract in Manila tamarind yielded 27.5%. Phytochemical analysis indicated that the fruit contains various compounds, including glycosides, carbohydrates, tannins, alkaloids, sterols, flavonoids, saponins and phenols. In this study, damage of liver was made for rats using carbon tetrachloride (CCl_4). CCl_4 is a well-known hepatotoxic agent that leads to liver fatty degeneration and hepatocellular necrosis, ultimately resulting in liver fibrosis, making it a widely recognized model for studying the causes of human liver fibrosis (Nagmoti *et al.,* 2015; Forbes and Newsome, 2016).

Toxicity testing

Conduct of acute toxicity testing on selected albino mice via the oral route. As shown in Fig 1, the histopathology report revealed of liver tissue of experimental rats. The control group was given a 5 mL saline solution, while treatment groups I, II and III were administered doses of 50 mg, 300 mg and 2000 mg/kg body weight of the Manila tamarind fruit aqueous extract, respectively. The mice were monitored over a period of fourteen days. No mortality was observed and the rats displayed normal breathing, consistent food intake, no significant skin alterations and stable body weight. Similar results regarding the acute toxicity of Manila tamarind fruits were reported in previous studies. Notably, treatment group III, which received the highest dose of 2000 mg/kg body weight, did not show any signs of drowsiness, sedation, or coma. Doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight were further investigated.

Effects of Manila tamarind fruit pulp extract on liver weight and lipid profile in rats exposed to carbon tetrachloride

Table I shows the effects of manila Tamarind fruit pulp extract on liver weight and lipid profile in rats exposed to carbon tetrachloride (CCl_4). The results indicates that the rats were monitored for any changes in liver weight after receiving the aqueous extract of the manila tamarind fruit pulp depicted. The administration of Manila tamarind fruit extract resulted in a notable increase in liver weight, rising from 2.97 g to 3.61 g, which is nearly comparable to the normal control group I (4.13 g), as the dosage increased from 50 mg/kg body weight to 400 mg/kg body weight. In contrast, the injection of carbon tetrachloride (CCl_4) in rats reduced liver weight and total protein levels. The groups treated with silymarin demonstrated a significant restoration of liver weight (5.62 g), while the test groups receiving supplementation with Manila tamarind fruit extract at doses of 100 mg/kg (Group IV), 200 mg/kg (Group V) and 400 mg/kg (Group VI) exhibited noteworthy increase in liver weight of 6.18 g, 6.98 g and 7.17 g,

Table 1: Aqueous fruit extract of Manila tamarind on protein and lipid profile of rats.

Treatment group	Liver weight (g)	Total protein (g)	Triglycerides (g)	Total cholesterol (g)	HDL-C(g)	LDL-C(g)
I	4.13±0.09	7.21±0.09	78.17±1.01	157.67±2.04	99.0±0.85	56.65±0.56
II	1.54±0.03	4.16±0.07	360.17±2.76	375.83±3.52	48.67±0.88	106.02±0.79
III	2.44±0.02	5.62±0.02	329.33±1.41	300.00±1.39	60.33±0.67	82.31±0.95
IV	2.97±0.03	6.18±0.03	278.17±1.97	224.00±1.06	77.33±0.88	62.87±2.66
V	3.52±0.02	6.98±0.04	160.17±1.28	209.67±3.43	88.33±0.76	58.32±0.24
VI	3.61±0.03	7.17±0.02	138.00±0.93	197.67±1.84	94.17±0.79	57.37±0.17
'f' Value	421.29***	310.56***	4604.00***	1094***	603.87***	261.84***

[1]I-Normal control, II- CCl4-induced standard control, III- Hepatoprotective standard control silymarin (50 mg/Kg/bw), IV- Treatment Group 1 (100 mg/kg/ bw), V - Treatment Group 2- (200 mg/kg/ bw), VI- Treatment Group 3- (400 mg/kg/ bw). Values are expressed as mean ± SE n=6, ***p<0.001.

Table 2: Effect of aqueous Manila tamarind fruit extract on anti-oxidant status.

Treatment group	Lipid peroxide (mM MDA/g)	Super oxide dismutase (mg epinephrine oxidized/g)
I	116.67±12.02	12.47±0.73
II	810.00±8.56	4.13±0.28
III	666.67±9.88	5.25±0.29
IV	450.00±13.42	7.06±0.45
V	220.00±7.30	12.11±0.27
VI	173.33±6.67	11.57±0.35
'f' Value	820.20***	76.24***

I- Normal control, II- CCl_4-induced standard control, III- Hepato protective standard controls.
ilymarin (50 mg/Kg/bw), IV- Treatment Group 1 (100 mg/kg/bw), V- Treatment Group 2- (200 mg/kg/bw), VI- Treatment Group 3- (400 mg/kg/bw). Values are expressed as mean±SE n=6, ***p<0.001.

respectively, compared to the control (4.16 g) Bidlack and Tappel (1973).

Mice subjected to carbon tetrachloride treatment showed a marked increase in lipid profile levels, including triglycerides (360.17 g), total cholesterol (375.83 g) and LDL cholesterol (106.02 g), alongside a significantly low level of HDL cholesterol (48.67 g) when compared to the normal control group (Trinder *et al.*, 1984); Henkel and Stoltz (1982). Silymarin treatment provided strong protection against liver toxicity induced by carbon tetrachloride. Similarly, groups treated with aqueous extracts of Manila tamarind fruits exhibited a robust response that was comparable to the toxic control. An acute liver toxicity study indicated that administering higher doses of the aqueous extract did not result in significant signs of respiratory distress, drowsiness, or mortality Lopes-Virella *et al*. (1977). In this study, the observed decline in liver weight, total protein and HDL cholesterol, along with increases in triglycerides, LDL cholesterol and total cholesterol in rats administered CCl_4, served as indicators of liver injury (Damiris *et al.,* 2020).

Impact of aqueous Manila tamarind fruit extract on antioxidant status in hepatic injury

Table 2 indicates that lipid peroxide were higher in the toxicity-induced group II (810 mM MDA/g) compared to control group I (116.67 mM MDA/g), suggesting the presence of oxidative stress associated with hepatic injury. In contrast, the administration of the aqueous extract of Manila tamarind fruit led to a reduction in lipid peroxide levels, measuring 450 mM MDA/g, 220 mM MDA/g and 173.33 mM MDA/g at doses of 100, 200 and 400 mg/kg body weight, respectively. Inclusion of the aqueous extract resulted in elevated levels of Superoxide Dismutase (SOD), highlighting antioxidant properties of the fruit. Specifically, group VI, exhibited SOD levels of 11.57 mg/g, closely approaching the normal control group's value of 12.47 mg/g. The liver damage induced by CCl_4 has been associated with oxidative stress, which can disrupt cellular homeostasis and accelerate collagen formation through a toxicological cascade. This study demonstrates that the supplementation of an aqueous extract of Manila tamarind fruits possesses significant antioxidant potential, contributing to a reduction in oxidative stress. Consequently, rats treated extract of Manila tamarind may enhance the liver tissue's ability to combat free radicals, as indicated by the results.

Effect of aqueous Manila tamarind fruit extract on bilirubin levels and liver enzymes in toxicity-induced hepatic injury

Table 3 shows the effect of Manila Tamarind fruit pulp extract (MT) on liver enzymes in rats the management of Silymarin resulted in a reduction of total bilirubin levels in the standard

Table 3: Effect of aqueous Manila tamarind fruit extract on liver enzymes.

Treatment group	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	SGPT (mg/dl)	SGOT (mg/dl)
I	0.38±0.00	0.48±0.029	29.93±0.77	32.78±1.08
II	1.28±0.00	1.75±0.025	70.57±0.80	102.94±1.14
III	1.07±0.10	1.50±0.013	57.40±0.49	84.17±0.97
IV	0.97±0.00	1.29±0.005	49.49±0.32	70.56±0.65
V	0.63±0.00	0.81±0.03	42.92±0.68	52.83±0.70
VI	0.52±0.01	0.66±0.078	40.23±0.54	42.16±0.89
'f' Value	70.45***	374.71***	517.45***	829.04***

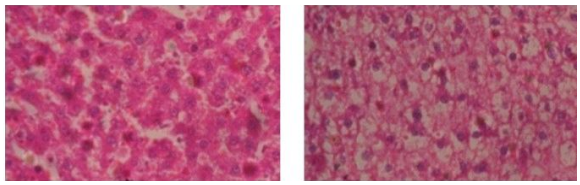
I- Normal control, II- CCl_4-induced Standard Control, III- Hepatoprotective Standard Control Silymarin (50 mg/Kg/bw), IV- Treatment Group 1 (100 mg/kg/ bw), V- Treatment Group 2- (200 mg/kg/ bw), VI- Treatment Group 3- (400 mg/kg/ bw). Values are expressed as mean ± SE n=6, ***p<0.001.

Table 4: Docking analysis through Pyrx virtual screening tool.

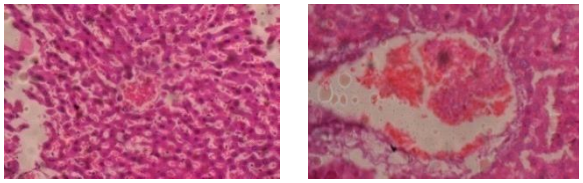
Ligands	Interacting residues	Binding energy	Bond established
2-3H furanone	PHE 114	-4.7	H -Bond
3-2H furanone	SER 229;PRO 93	-4.4	H -Bond,alkyl bond
Butyl lactone	ALA 303	-4.5	H -Bond
Cyclopentene	SER 229;ARG 96	-4.3	H -Bond
Dideoxyribolactone	ARG 96; VAL 228	-4.7	H -Bond, CH Bond
Furanone	ARG 96	-4	H -Bond, CH Bond
Hexyl	SER 229	-5.1	H -Bond
Maltol	PHE 114, TRP 396, PHE 382	-4.8	H -Bond, alkyl bond
Mannose	LEU 98, PHE 382, TPR 396	-6.2	H -Bond, alkyl bond
Methyfuranone	ARG 96; ALA 227; LEU 109, ILW 85	-4.4	H -Bond, CH Bond, alkyl bond
Pyran-4-one	VAL237, ASP300	-4.9	H Bond
Propylacetate	ARG388	-4.3	H -Bond, alkyl bond
Phenol	TYR389, PHE114	-4.4	alkyl bond

control group. Additionally, rats treated with extract of Manila tamarind demonstrated a decrease in both total and direct bilirubin levels. The groups subjected to toxicity showed markedly increased levels of SGPT and SGOT, indicating severe hepatic damage, in contrast to healthy animals. However, treatment with Silymarin led to a significant reduction in SGPT and SGOT levels. Similarly, the rats receiving the aqueous extract of Manila tamarind fruits also exhibited a notable decrease in liver enzyme levels, approaching those of the normal control groups. Following the management of the aqueous extract, the total and direct bilirubin, SGPT and SGOT in the rats decreased, effectively reversing the liver damage caused by carbon tetrachloride. These changes became more pronounced with increasing doses of the aqueous fruit extract. This finding aligns with previous research on the aqueous extracts of the fruits of P. dulce had a significant effect on liver weight and liver volume in rats conducted in Telangana (Raju and Jagadeeshwar, 2014).

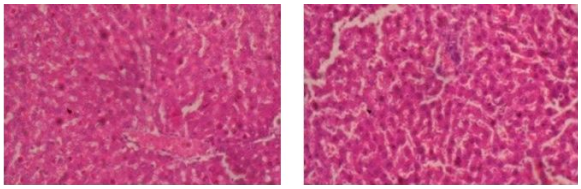
The molecular docking results in Table 4 and Fig 2 to 14 demonstrate the significant bioactive potential of specific



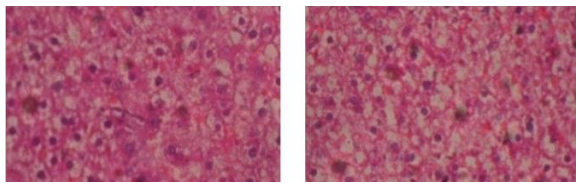
Group I: Microphotograph of Liver Tissue of Rats Administered with Saline



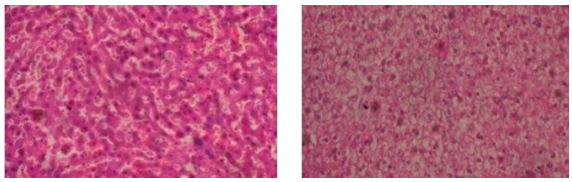
Group 2: Microphotograph of Liver Tissue of Rats Induced with CCl_4 (0.5 ml/rat)



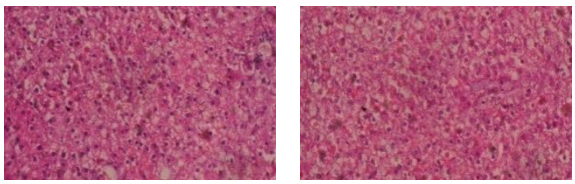
Group 3a: Microphotograph of Liver Tissue of CCl_4 Induced Rats Treated with Aqueous Manila Tamarind Fruit Extract (100 mg/Kgbw)



Group 3b: Microphotograph of Liver Tissue of CCl_4 Induced Rats Treated with Aqueous Manila Tamarind Fruit Extract (200 mg/Kgbw)



Group 4: Microphotograph of Liver Tissue of CCl_4 Induced Rats Treated with Aqueous Manila Tamarind Fruit Extract (400 mg/Kgbw)



Group 5: Microphotograph of Liver Tissue of CCl_4 Induced Rats Treated with Silymarin (25 mg/Kgbw)

Fig 1: Histopathology study of liver tissue of experimental rats.

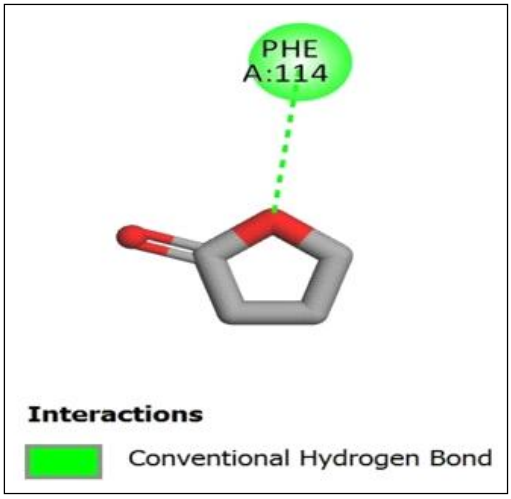


Fig 2: 2-3H furanone.

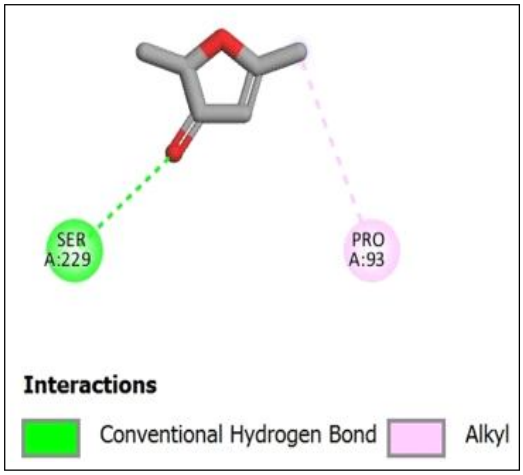


Fig 3: 3-2H furanone.

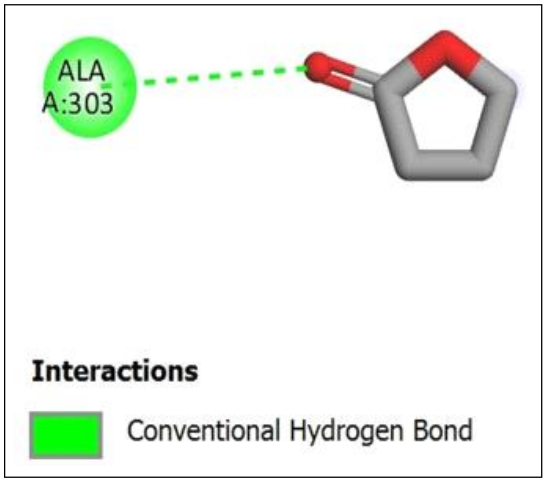


Fig 4: Butyl lactone.

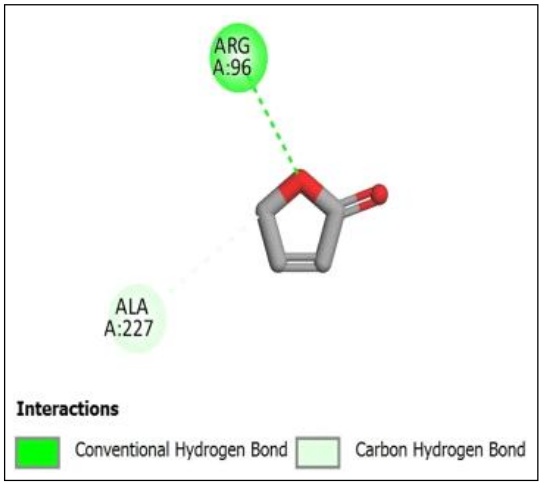


Fig 7: Furanone.

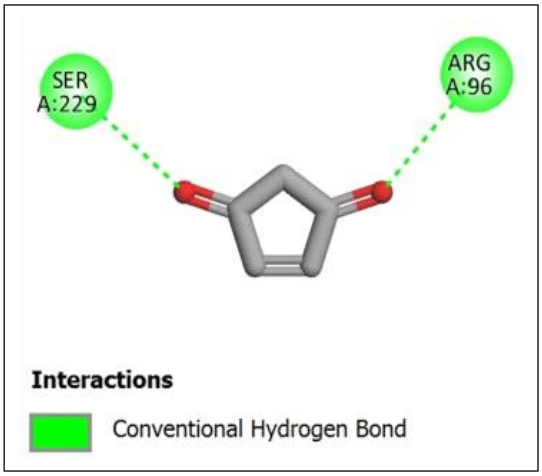


Fig 5: Cyclopentene.

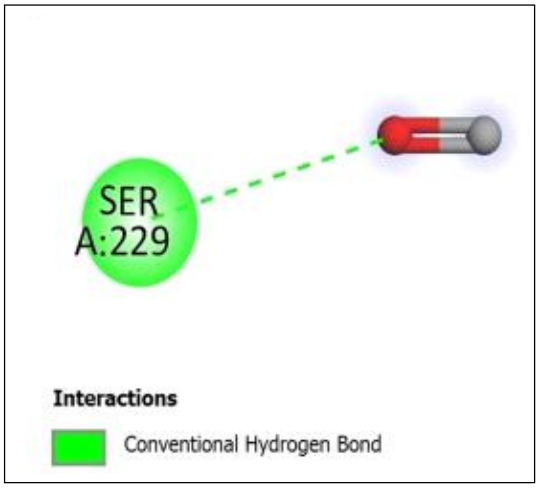


Fig 8: Hexyl.

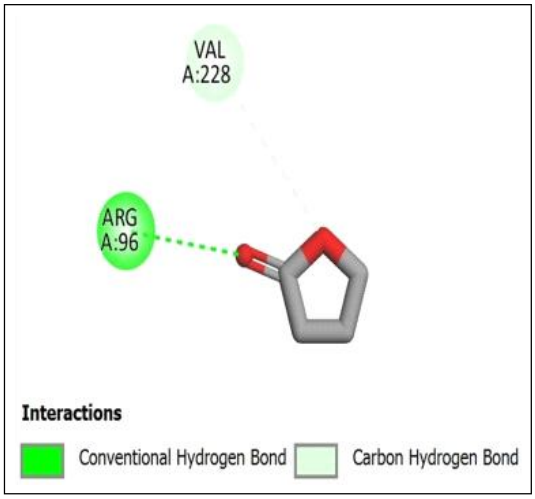


Fig 6: Dideoxyribolactone.

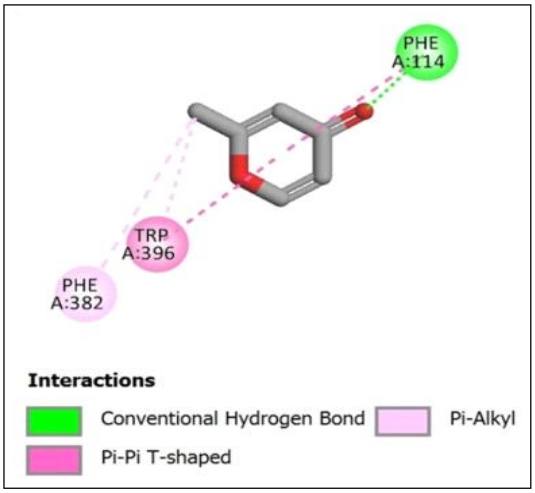


Fig 9: Maltol.

phytochemicals derived from the aqueous extract of *Manila Tamarind*. Among the analyzed compounds, mannose displayed the strongest binding affinity (-6.2 kcal/mol), forming multiple interactions with LEU 98, PHE 382 and TRP 396. This high binding affinity and its ability to establish multiple interactions highlight mannose's potential role in modulating biological pathways, particularly in therapeutic applications targeting enzymes or receptors. Additionally, hexyl (-5.1 kcal/mol) and pyran-4-one (-4.9 kcal/mol) showed notable interactions, further emphasizing their biological relevance. Hexyl demonstrated a strong interaction with SER 229, a residue often involved in enzymatic catalysis, while pyran-4-one interacted with VAL 237 and ASP 300, suggesting its potential for active site inhibition.

Other ligands, such as maltol and 3-2H furanone, exhibited moderate binding affinities. Despite their

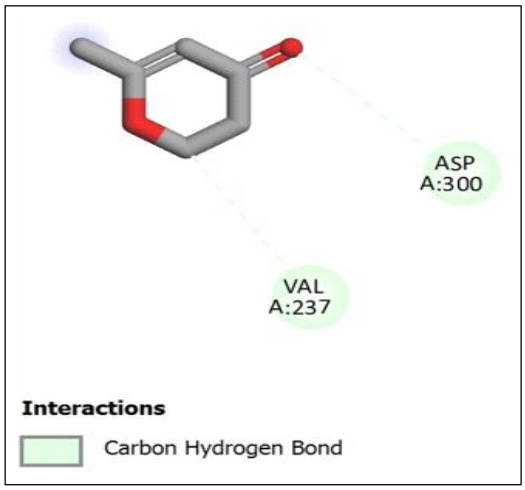


Fig 12: Pyran-4-one.

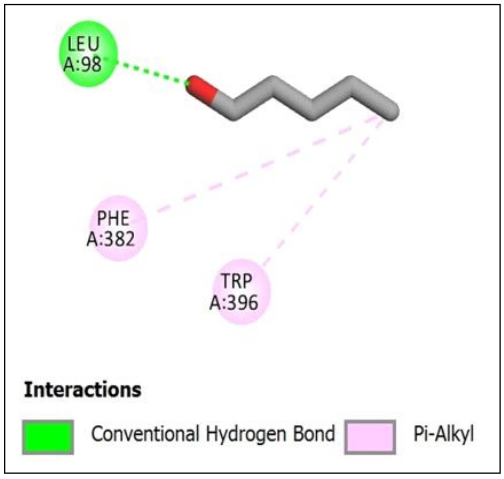


Fig 10: Mannose.

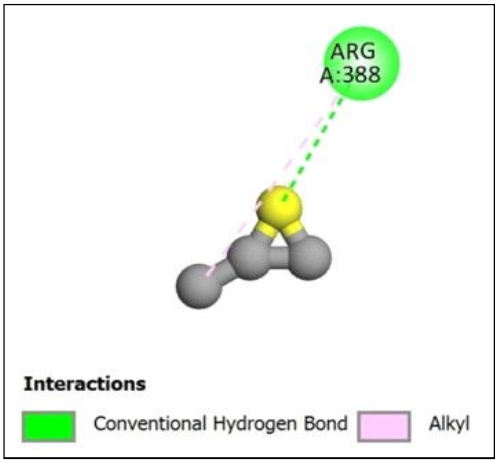


Fig 13: Propylacetate.

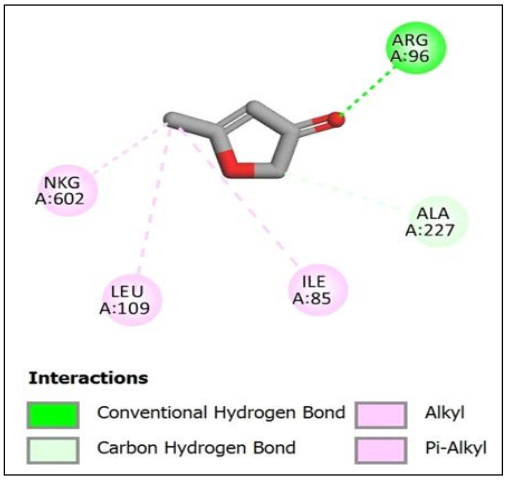


Fig 11: Methyl furanone.

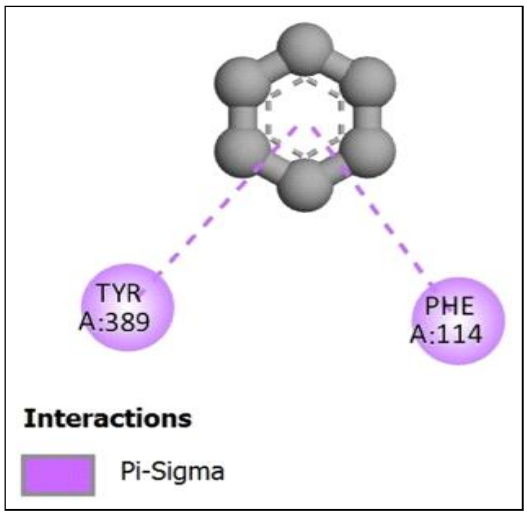


Fig 14: Phenol.

relatively lower binding strength, their interactions with residues like PHE 114 and PRO 93 could still contribute to secondary biological effects or synergistic actions when used in combination with other compounds. The diverse binding affinities and interaction patterns observed in the study underline the versatility of *Manila Tamarind* phytochemicals. These findings align with existing reports on the plant's antioxidant and antimicrobial properties, reinforcing the therapeutic potential of its components.

CONCLUSION

The study findings suggest that the fruit aqueous extracts of manila tamarind (*Pithecellobium dulce*) have hepato-protective properties against carbon Tetrachloride CCl_4 (4) intra peritoneal injection. And also revealed for the first time that acute liver damage caused by carbon Tetrachloride (CCl_4) can be prevented with the supplementation of an aqueous extract of manila tamarind fruits, which may be due to its antioxidant qualities. The molecular docking analysis identified Mannose, Hexyl and Pyran-4-one as promising candidates with strong binding affinities and specific interactions with biological targets. These findings provide a foundation for further exploration of *Manila Tamarind*-derived phytochemicals in drug discovery and development. More research is required to identify the particular chemicals in manila tamarind fruit that are responsible for these hepato protective properties.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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