Assessment of Hepatoprotective Potential of *Manilkara hexandra* STEM Bark: An In-vitro Analysis

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Manilkara hexandra* (Sapotaceae) is tree species, privately known as Khirni, found in Thar Desert districts of northwest and western India. The bark acquired from the stem is utilized as a solution for syphilis, urinary issues, amplification of spleen, gonorrhea, leucoderma, and liver infections. The point of this work is to consider the hepatoprotective impact of unrefined Ethyl acetate removal from the bark portions of *Manikara hexendra*. The Ethyl acetate extricate got from bark portions of *Manikara hexendra* was assessed via cell line study in HepG2 cell line followed in for hepatoprotective movement in rodents by initiating liver harm via paracetamol and carbon tetrachloride.

Results: The Ethyl acetate extricate at an oral portion of 400 mg/kg displayed a critical (P < 0.05) defensive impact. These biochemical perceptions were enhanced by histopathological assessment of liver areas. The action might be a consequence of the presence of flavonoid mixes. Moreover, the intense harmfulness of the concentrates gave no indications of poisonousness up to a portion level of 4000 mg/kg.

Conclusion: It could be inferred that Ethyl acetate concentrate of *Manikara hexendra* has huge hepatoprotective properties.

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ABBREVIATIONS

AST : Aspartate transaminases  
ALT : Alanine amino transferase  
ALP : Alkaline phosphates  
SOD : Superoxide dismutase  
GSH : Glutathione  
MDA : Methylenedioxyamphetamine

1. INTRODUCTION

A few illnesses like AIDS, auto-save infections, hyperglycemia, rheumatoid joint pain, malignant growth, atherosclerosis, waterfalls, and other old matured sicknesses are related to the overabundance of oxidative pressure. Receptive oxygen species (ROS) like hydroxyl extremist (HR), singlet oxygen, peroxides, and superoxides are created in oxidative metabolic response and have significant capacities in cell homeostasis. ROS level can increments fundamentally during the hour of ecological anxieties. It exacts harm to the subcellular organelles and eventually prompts various human infections [1]. Accordingly, regular cell reinforcements have a vital part to kill the overabundance of ROS. Cancer prevention agents, free extreme scroungers, are shaky particles as they contain unpaired electrons and to become stable they take out electrons from different atoms. There are different kinds of free extreme scroungers and cell reinforcements like phenolics, thiols, tripeptide – glutathione, compounds – peroxidase, catalase, superoxide dismutase, and nutrients – E and C that forestall oxidative pressure initiated harm of deoxyribonucleic acids, lipids, and proteins [2]. Numerous scientists have affirmed that phenolic-rich plant items assume a significant job in the anticipation of tumors, cardiovascular, and neurodegenerative infections [3]. There is a positive relationship between the propensity for polyphenolic mixes containing food utilization and the diminished event of degenerative infections. Phenolic acids, tannins, and flavonoids are the principle phenolic mixes. The polyphenols have a few phenolic hydroxyl substituents and have been involved in UV insurance and sickness opposition [4]. They are widely utilized in the staple industry and are considered a significant segment of nutraceuticals. The past investigation reports demonstrated a solid positive connection between the phenolics substance and cancer prevention agent action, as was noticed, in oregano, peppermint, clove, sage, garden thyme, and all flavors [5]. Manilkara hexandra (Family: Sapotaceae) is generally appropriated in South, North, and Central India-primarily in Rajasthan, Gujarat, Madhya Pradesh, and Maharashtra. The bark and leaves of M. hexandra are notable for their few restorative employments. The bark is sweet, sexual enhancer, refrigerant and shows stomachic, astringent, alexipharmic and anthelmintic exercises. It is utilized in the fix of fever, consuming sensation, colic, fart, hyperdipsia, helmithiasis, hyperglycemia, and vitiated states of pitta. The stem bark of M. hexandra is rich in procyanidins, saponins, and flavonoids. It is additionally given to the lactating moms every day once for 3–5 days. The youthful bubbled units are additionally eaten. The leaf methanolic separate portion of M. hexandra is appeared to display better cancer prevention agent potential and in vitro α-amylase inhibitory property than the other concentrate portions. Prior it indicated a focus subordinate expansion in nitric oxide, superoxide, and DPPH free extremist rummaging possibilities of the bark methanolic concentrate of M. hexandra. The progressive leaf methanolic separate portion of M. hexandra contains the most intense cancer prevention agents than the other concentrate parts [6]. In this manner, in the current situation with information, the bark and leaf concentrates of M. hexandra are appealing wellsprings of cell reinforcement mixes. The act of assortment of bark is an issue of worry to plant wellbeing and is more destructive than leaf assortment. Consequently, in the current examination, we expected to build up the reasonableness of the leaves of M. hexandra over the stem barks for the pharmacological exercises. In numerous investigations, the cell reinforcements were segregated with methanol and our previous information additionally demonstrated that the methanolic separate portion had the most elevated in vitro cancer prevention agent exercises (12). Thus in this study, it was aimed at the Hepatoprotective Activity potential of Manilkara Hexendra Bark.

2. METHODS

2.1 Plant Material

The plant Manilkara Hexendra was collected from Rajasthan district, Jaipur in August Month and authenticated in Department of Botany Ch. Charan Singh University, Meerut, and the voucher specimen was deposited for future reference.
2.2 Phytochemical Screening

Preliminary Phytochemical Screening of 70% ethanolic and ethyl acetate extract was carried out by using a standard procedure [6]. Shows the presence of various Phytoconstituent like Carbohydrates, fixed oil, alkaloids, Saponins, flavonoids, tannins, phenol compounds in the extract which are shown in Table 1.

Table 1. Phytochemical screening

| Sl. No. | Constituents       | Tests                      | Ethyl acetate extract | 70 % Ethanol extract |
|---------|--------------------|----------------------------|-----------------------|----------------------|
| 01      | Carbohydrate       | Molish’s test              | _                     | +                    |
|         |                    | Fehling’s test             | _                     | +                    |
| 02      | Fixed oils and fats| Spot test                 | _                     | _                    |
|         |                    | Saponification test        | _                     | +                    |
| 03      | Proteins and amino| Million’s test             | _                     | _                    |
|         | Acids              | Nihydrin test              | _                     | _                    |
|         |                    | Biuret test                | _                     | _                    |
| 04      | Tannins            | Lead Acetate              | +                     | +                    |
|         |                    | FeCl3 Test                 | +                     | +                    |
| 05      | Flavonoids         | Alkali Test                | +                     | +                    |
|         |                    | Shinoda’s test             | +                     | +                    |
| 06      | Saponin            | Foam Test                 | +                     | +                    |
| 07      | Cardenoloids       | Legal test                 | +                     | +                    |
|         |                    | Baljet test                | +                     | +                    |
| 08      | Phytosterol        | Salkowiski test            | _                     | _                    |
|         |                    | Libermann Burchard test    | _                     | _                    |
| 09      | Alkaloids          | Dragendorff’s test         | _                     | _                    |
|         |                    | Mayer’s test               | _                     | _                    |
|         |                    | Wagner’s test              | _                     | _                    |
|         |                    | Hager’s test               | _                     | _                    |

Determination of Total Phenolic and Flavonoids Content: Reagents and Chemicals: Folin-Ciocalteu reagent, gallic acid, and quercetin, aluminum chloride hexahydrate, methanol, and sodium carbonate.

Total Phenolic contents determination assay: The total polyphenol content (μg/mg extract) was analyzed using the Folin-Ciocalteu reagent method [7].

Total Flavonoid contents determination assay: The total flavonoid content (μg/mg extract) was analyzed using the quercetin reagent method [8].

DPPH radical scavenging activity of *Manilkara hexendra*

The radical scavenging activity was done by already predetermined methods via, DPPH radical scavenging assay. The results were expressed as % radical scavenging activity. DPPH assay of Ethyl acetate stem bark extract was estimated by using ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10 –100 μg/ml). The % inhibition curves for ascorbic acid and that for Ethyl acetate stem bark extract was plotted, from which, IC50 value (concentration of extracts that inhibits the formation of DPPH radicals by 50%) of DPPH by ascorbic acid and Ethyl acetate stem bark extract was calculated using calculated by regression equation [10].

II. Pharmacological Activity

Chemicals: Paracetamol, Carbon tetrachloride and Country made liquor.

Extract Preparation: The Bark was kept for air shaded dry 1.5 kg of bark powder was macerated to remove the impurities like fatty substances and further extracted with Ethyl acetate for 5 days by cold maceration method, filter the extract Centrifuge at 10000 rpm/min,
concentrate on Buchi rotary evaporator and further dried in lophilizer freeze drier under reduce pressure, This yield 98.00 gm of solid residue (6.5% w/w).

**Experimental Animals:** All experiments were performed on healthy adult male wistar albino rats weighing 200-250 grams.

**Grouping of Animals:** Five Group of rats, six animal in each group has been used to study the effect of Ethyl acetate extract of *Manilkara hexandra* in three models for the treatment hepatotoxicity.

**Hepatoprotective Assay**

1. **Paracetamol induced Hepatotoxicity**

Paracetamol-induced hepatotoxicity model was adopt for the study [10]. The rats were divided into 5 groups of 6 animals each. Group, I served as a control and received normal saline, 5 mL/kg body weight, daily for 7 days. Group II constituted the hepatotoxic group and was treated with 2gm/kg paracetamol. Group III received the standard drug Silymarin (100mg/kg) daily, Group IV and Group V received 70 ethanolic extracts (100 and 400 mg/kg body weight per day, respectively) suspended in 0.5% sodium carboxymethylcellulose for 14 days. On the 7th day, paracetamol suspension was given orally, 2 g/kg body weight, to all the rats except those in Group I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether. Blood and liver samples were collected for biochemical analysis [11].

**CCl₄ induced Hepatotoxicity**

Carbon tetrachloride (CCl₄) induced hepatotoxicity model was adopted for the study [12]. The rats were divided into 5 groups of 6 animals each. Group, I served as a control and received normal saline 10 ml/kg, i.p once in a day for 7 days. Group II constituted the hepatotoxic group and was treated with 0.5 ml/kg, i.p. Group III received the standard drug Silymarin (100 mg/kg) daily, Group IV and Group V received 70 ethanolic extracts (100 and 400 mg/kg body weight per day, respectively) suspended in 0.5% sodium carboxymethylcellulose for 14 days. On the 7th day, CCl₄ 0.5 ml/kg, i.p, to all the rats except those in Group I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether. Blood and liver samples were collected for biochemical and histological studies.

**Body weight**

Body wt. of individual animals was taken for each group and a record was maintained. Body wt. was taken daily from the starting day of the study till the last dosing was do and also Before sacrificing the animal. If the death of any animal occurs in between the study time, its weight was also to be taken. Any change in the body wt. of the animal was recorded.

**Measurement of ALT, AST, ALP**

Serum ALT, AST, and ALP were assess as per standard kit methods using UV spectrophotometer and the standard kit methods were obtain in detail from the leaflets provide in the commercial kits [13].

**Estimation of glutathione level:** GSH a key antioxidant biomarker is a superoxide radical scavenger where it protects the thiol group required for maintaining the cell integrity against oxidation. Glutathione was estimated [14].

**Estimation of MDA level:** MDA forms a 1:2 adduct with thiobarbituric acid which can be measured by fluorometry or spectrophotometry [15].

**Acute Toxicity Study:** The acute toxicity was performed according to OECD guidelines (OECD 423, 2001). The selected male wistar rats were used for toxicity studies. The animals were divided into three groups of three in each. The animals fasted overnight before the experimental procedure. The acute toxicity study was performed for deciding safe doses for further pharmacological studies along with this any behavioral or physiological changes due to extracting administration were also observed. Extracts were given orally to rats at the graded dose of 1000, 2000, 4000mg/kg body wt. Immediately, after dosing, the animals were observed continuously for the first four hours for behavioral changes and mortality at the end of 24 h and daily up to 14 days for any behavioral change or mortality. Since No mortality was reported even after 14 days. This indicated that the extracts are safe up to a single dose of 4000 mg/kg body weight. Hence the selected doses for the administration in experimental animals were considered 1/10th and 1/5th of the maximum safe dose [16].
Table 2. Grouping of animals

| S. No | Groups | Paracetamol Model | CCl4 Model |
|-------|--------|-------------------|------------|
| 1     | GP 1 (Control) Normal Saline | Normal Saline 5 ml/kg po | Normal Saline 10 ml/kg, i.p. |
| 2     | GP2 (Negative Control) | 2 gm/kg (07 Days) po | 0.5 ml/kg, i.p. (07 Days) |
| 3     | GP 3 (Standard) Silymarin | 100mg/kg (14 Days) po | 100mg/kg (14 Days) po |
| 4     | GP 4 (Extract) | 100 mg/kg (14 Days) po | 100 mg/kg (14 Days) po |
| 5     | GP 5 (Extract) | 400 mg/kg (14 Days) po | 400 mg/kg (14 Days) po |

Table 3. Acute toxicity study

| Groups | Number of animals | Treatment | Route | Dosage | Duration |
|--------|-------------------|-----------|-------|--------|----------|
| 1      | 3                 | Ethyl acetate extract | Oral  | 1000 mg/kg bodyweight | 14 Day |
| 2      | 3                 | of Manilkara | Oral  | 2000 mg/kg bodyweight | 14 Day |
| 3      | 3                 | Hexandra   | Oral  | 4000 mg/kg bodyweight | 14 Day |

3. RESULTS

3.1 Total Phenolic Content Assay of Manilkara hexandra

The absorbance of gallic acid at different concentrations (10-100 μg/ml) was determined (Fig. 1 Tab:4,5). The standard curve of gallic acid is shown in the figure. The Total Phenolic content of Manilkara hexandra bark 70% ethanol extract was found to contain 112.78±0.223 μg/mg of Galic acid. The total Phenolic content of Manilkara hexandra bark ethyl acetate extract was found to contain 132.00±0.384 μg/mg of Galic acid.

![Gallic Acid Absorbance](image)

Fig. 1. Absorbance of Gallic Acid

3.2 Phenolic content of Manilkara hexandra for 70% Ethanol

Table 4. Phenolic content of Manilkara hexandra For 70% Ethanol

| Sample Solution μg/ml | Wt of dry extract gram/ml | Absorbance | Gallic acid Concentration μg/ml | Gallic acid Concentration mg/ml | Total phenol content as gallic acid mg/gm | Mean±SEM |
|-----------------------|---------------------------|------------|--------------------------------|--------------------------------|------------------------------------------|---------|
| 1000                  | 0.001                     | 0.346      | 113                            | 0.113                          | 113.000                                  | 112.78±0.223 |
| 1000                  | 0.001                     | 0.344      | 112.33                         | 0.1123                         | 112.330                                  | μg/mg   |
| 1000                  | 0.001                     | 0.346      | 113                            | 0.113                          | 113.000                                  | gallic acid equivalent dry weight |
|                       |                           | 0.3453333  |                                |                                |                                          |         |
3.3 Phenolic Content of *Manilkara hexandra* for Ethyl Acetate

Table 5. Phenolic content of *Manilkara hexandra* For Ethyl acetate

| Sample Solution µg/ml | Wt of dry extract gram/ml | Absorbance | Gallic acid Concentration µg/ml | Gallic acid Concentration mg/ml | Total phenol content as gallic acid mg/gm | Mean±SEM |
|-----------------------|---------------------------|------------|-------------------------------|---------------------------------|------------------------------------------|----------|
| 1000                  | 0.001                     | 0.403      | 132                           | 0.132                           | 132.00                                   | 132.00±0.384 |
| 1000                  | 0.001                     | 0.405      | 132.66                        | 0.13266                         | 132.66                                   | 132.66±0.384 |
| 1000                  | 0.001                     | 0.401      | 131.33                        | 0.13133                         | 131.33                                   | 131.33±0.384 |

3.4 Total Flavonoid Content

The standard curve of quercetin is shown in the figure. The absorbance of quercetin at different concentrations (10-100 μg/ml) was determined. The total Flavonoid content of *Manilkara hexandra* bark 70% ethanol extract was found to contain 57.33±0.191 μg/mg of Quercetin. The total Flavonoid content of *Manilkara hexandra* bark Ethyl acetate extract was found to contain 88.00±0.57 μg/mg of Quercetin. (Tables 6-8: Fig. 2).

![Fig. 2. Absorbance of Quercetin](image-url)

y = 0.0031x + 0.1408
R² = 0.9977

3.5 Flavonoid Content of *Manilkara hexandra* for 70% Ethanol

Table 6. Flavonoids content of *Manilkara hexandra* for 70% Ethanol

| Sample Solution µg/ml | Wt of dry extract gram/ml | Absorbance | Quercetin Concentration µg/ml | Quercetin Concentration mg/ml | Total phenol content as Quercetin mg/gm | Mean±SEM |
|-----------------------|---------------------------|------------|-------------------------------|---------------------------------|------------------------------------------|----------|
| 1000                  | 0.001                     | 0.331      | 57                            | 0.057                           | 57.00                                    | 57.33±0.191 |
| 1000                  | 0.001                     | 0.313      | 57.66                         | 0.05766                         | 57.66                                    | 57.66±0.191 |
| 1000                  | 0.001                     | 0.312      | 57.33                         | 0.05733                         | 57.33                                    | 57.33±0.191 |
| Mean                  |                           |            |                               |                                |                                          | Quercetin equivalent dry weight |
| SD                    |                           |            |                               |                                |                                          | 0.330     |
| SEM                   |                           |            |                               |                                |                                          | 0.191     |
3.6 Flavonoid content of *Manilkara hexandra* For Ethylacetate

Table 7. Flavonoids content of *Manilkara hexandra* for 70% Ethanol

| Sample Solution µg/ml | Wt of dry extract gram/ml | Absorbance Quercetin Concentration µg/ml | Quercetin Concentration mg/ml | Total phenol content as Quercetin mg/gm | Mean±SEM |
|-----------------------|---------------------------|--------------------------------------|-------------------------------|----------------------------------------|---------|
| 1000                  | 0.001                     | 0.407                                | 89                            | 0.089                                  | 89.000  |
| 1000                  | 0.001                     | 0.404                                | 88                            | 0.088                                  | 88.000  |
| 1000                  | 0.001                     | 0.401                                | 87                            | 0.087                                  | 87.000  |
|                       |                           | Mean                                 |                               |                                        | 88.00   |
|                       |                           | SD                                   | 1.000                         |                                        |         |
|                       |                           | SEM                                  | 0.577                         |                                        |         |

Table 8. Total Phenol and Flavonoids content in both extract

| S. No | Plant extract | *Manilkara hexandra* Total Phenol | Total Flavonoid |
|-------|---------------|-----------------------------------|-----------------|
| 1     | Ethylacetate  | 132.00±0.384                      | 88.00±0.57      |
| 2     | 70% Ethanol   | 112.78±0.223                      | 57.33±0.191     |

3.7 DPPH Radicals Scavenging Activity of *Manilkara hexandra*

The DPPH radical scavenging activity of *Manilkara hexandra* for 70% Ethanolic Extract and Ethyl Acetate extract was determined by using the ascorbic acid solution as standard. The absorbance data was recorded against the selected concentration (10 –100 µg/ml). The IC 50 (µg/ml) for 70% Ethanolic Extract of *Manilkara hexandra* was found to be 92.03% and 85.13% for 70% Ethanol Extract and Ethyl Acetate Extract of *Manilkara hexandra* in comparison to the 37.09% for the standard Ascorbic acid respectively (Fig. 3 Table 9). The study revealed the antioxidant property of *Manilkara hexandra* bark. The 70% ethanol extract of *Manilkara hexandra* shows a higher amount of Phenols and flavonoids content. These phytochemicals are known to possess a good antioxidant property which could further help in protection against hepatotoxicity. This provides supportive evidence for the rationale behind selecting the following extract for further animal activities.

Table 9. DPPH radicals scavenging activity of *Manilkara hexandra*

| Concentration (µg/ml) | Ascorbic acid 8.70±0.11 | Ethyl Acetate Extract 21.80±0.17 | 70% Ethanolic Extract 27.43±0.08 |
|-----------------------|---------------------------|-----------------------------------|----------------------------------|
| 10                    | 33.36±0.46                | 8.70±0.11                         | 4.36±0.33                       |
| 20                    | 41.44±0.71                | 15.46±0.20                        | 11.43±0.33                      |
| 30                    | 46.43±0.46                | 21.80±0.17                        | 17.51±0.04                      |
| 40                    | 53.74±0.09                | 25.36±0.26                        | 21.42±0.06                      |
| 50                    | 56.98±0.17                | 31.46±0.17                        | 27.43±0.08                      |
| 60                    | 62.61±0.43                | 35.53±0.17                        | 32.45±0.07                      |
| 70                    | 67.67±0.56                | 42.23±0.24                        | 38.61±0.04                      |
| 80                    | 72.66±0.85                | 47.36±0.21                        | 43.82±0.06                      |
| 90                    | 77.04±0.50                | 52.50±0.20                        | 48.51±0.04                      |
| 100                   | 81.72±0.21                | 58.23±0.23                        | 53.81±0.05                      |
| IC 50 (µg/ml)         | 37.09                     | 85.13                             | 92.08                            |
3.8 Paracetamol induced Hepatotoxicity

**Bodyweight**

The bodyweight of the animal was decreased in toxic control. The treatment of an animal with the extract showed an increase in body weight. There was no significant decrease in body weight in comparison to the normal control. On administration of Silymarin, the body weight was found to be near normal.

In groups 4 and 5, the effect was found to be in a dose-dependent manner (Table 10 Fig. 4). At a higher dose of extract, a promising effect was seen. The ethanolic extract showed significant activity.

**Bodyweight**

| Table 10. Effect on body weight due to Paracetamol induced Hepatotoxicity |
|-------------------------------------------------|
| Tukey's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
|-----------------------------------|------------|---|-----------------------|---------|----------------|
| Control vs Toxin                  | 91.2       | 84.68 | Yes                   | ***     | 86.72 to 95.68 |
| Control vs Standard               | 2.817      | 2.615 | No                    | ns      | -1.659 to 7.292 |
| Control vs 100 mg                 | 63         | 58.49 | Yes                   | ***     | 58.52 to 67.48 |
| Control vs 400 mg                 | 5.007      | 4.648 | Yes                   | *       | 0.5312 to 9.482 |
| Toxin vs Standard                 | -88.38     | 82.06 | Yes                   | ***     | -92.86 to -83.91 |
| Toxin vs 100 mg                   | -28.2      | 26.18 | Yes                   | ***     | -32.68 to -23.72 |
| Toxin vs 400 mg                   | -86.19     | 80.03 | Yes                   | ***     | -90.67 to -81.72 |
| Standard vs 100 mg                | 60.18      | 55.88 | Yes                   | ***     | 55.71 to 64.66 |
| Standard vs 400 mg                | 2.19       | 2.033 | No                    | ns      | -2.286 to 6.666 |
| 100 mg vs 400 mg                  | -57.99     | 53.84 | Yes                   | ***     | -62.47 to -53.52 |

**Fig. 3. Antioxidant activity of Manilkara hexandra extract**

**Fig. 4. Effect on body weight due to Paracetamol induced Hepatotoxicity**
3.9 Effect on Biochemical Markers

Under the influence of Paracetamol, there is the level of biochemical markers i.e. ALT, AST, and ALP. The administration of the extract to the animals showed a dose depends on the change in the level of ALT, AST, and ALP (Tables 11-13; Figs. 5-7). At a higher dose i.e. 400 mg/kg the results were near to the normal. The level of GSH and SOD (Tab.:14-17 Figs. 8-10) were decreased in toxic control whereas on the administration of extract the levels were revived near to the normal. The level of GSH was increased in toxic control which was significantly altered under the influence of extract.

### Table 11. Effect of ALT due to Paracetamol induced Hepatotoxicity

| Tukey’s Multiple Comparison Test | Mean Diff. | q  | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|----|------------------------|---------|----------------|
| Control vs Toxic                 | -135.3     | 224.2 | Yes ***                | -137.8 to -132.8 |
| Control vs Standard              | -2.248     | 3.726 | No                     | ns      | -4.756 to 0.2594 |
| Control vs MH 100                | -44.52     | 73.77 | Yes ***                | -47.02 to -42.01 |
| Control vs MH 400                | -3         | 4.971 | Yes *                  | -5.508 to -0.4923 |
| Toxic vs Standard                | 133        | 220.5 | Yes ***                | 130.5 to 135.6 |
| Toxic vs MH 100                  | 90.78      | 150.4 | Yes ***                | 88.27 to 93.28 |
| Toxic vs MH 400                  | 132.3      | 219.2 | Yes ***                | 129.8 to 134.8 |
| Standard vs MH 100               | -42.27     | 70.04 | Yes ***                | -44.78 to -39.76 |
| Standard vs MH 400               | -0.7517    | 1.246 | No ns                  | -3.259 to 1.756 |
| MH 100 vs MH 400                 | 41.52      | 68.79 | Yes ***                | 39.01 to 44.02 |

![Manilkara hexandra](image)

**Fig. 5. Effect of ALT due to Paracetamol induced Hepatotoxicity**

### Table 12. Effect of AST due to Paracetamol induced Hepatotoxicity

| Tukey’s Multiple Comparison Test | Mean Diff. | q  | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|----|------------------------|---------|----------------|
| Control vs Toxic                 | -223.2     | 200.3 | Yes ***                | -227.9 to -218.6 |
| Control vs Standard              | -0.7933    | 0.712 | No ns                  | -5.435 to 3.848 |
| Control vs MH 100                | -122.3     | 109.8 | Yes ***                | -126.9 to -117.7 |
| Control vs MH 400                | -4.805     | 4.313 | Yes *                  | -9.447 to -0.1633 |
| Toxic vs Standard                | 222.4      | 209.4 | Yes ***                | 218.0 to 226.8 |
| Toxic vs MH 100                  | 100.9      | 95   | Yes ***                | 96.50 to 105.3 |
| Toxic vs MH 400                  | 218.4      | 205.6 | Yes ***                | 214.0 to 222.8 |
| Standard vs MH 100               | -121.5     | 114.4 | Yes ***                | -125.9 to -117.1 |
| Standard vs MH 400               | -4.012     | 3.776 | No ns                  | -8.437 to 0.4140 |
| MH 100 vs MH 400                 | 117.5      | 110.6 | Yes ***                | 113.1 to 121.9 |
Table 13. Effect of ALP due to Paracetamol induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q  | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|----|------------------------|---------|----------------|
| Control vs Toxic                 | -118.3     | 117.6 | Yes ***                |         | -122.5 to -114.1 |
| Control vs Standard              | -1.647     | 1.637 | No Ns                  |         | -5.827 to 2.534  |
| Control vs MH 100                | -52.32     | 52   | Yes ***                |         | -56.50 to -48.14 |
| Control vs MH 400                | -4.6       | 4.572 | Yes *                  |         | -8.781 to -0.4193|
| Toxic vs Standard                | 116.6      | 115.9 | Yes ***                |         | 112.5 to 120.8  |
| Toxic vs MH 100                  | 65.98      | 65.58 | Yes ***                |         | 61.80 to 70.16  |
| Toxic vs MH 400                  | 113.7      | 113  | Yes ***                |         | 109.5 to 117.9  |
| Standard vs MH 100               | -50.67     | 50.36 | Yes ***                |         | -54.85 to -46.49|
| Standard vs MH 400               | -2.953     | 2.935 | No Ns                  |         | -7.134 to 1.227 |
| MH 100 vs MH 400                 | 47.72      | 47.43 | Yes ***                |         | 43.54 to 51.90  |

Fig. 6. Effect of AST due to Paracetamol induced Hepatotoxicity

Fig. 7. Effect of ALP due to Paracetamol induced Hepatotoxicity
Table 14. Effect of MDA due to Paracetamol induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff       |
|----------------------------------|------------|-----|------------------------|---------|----------------------|
| Control vs Toxic                 | -337.1     | 386.6 | Yes                    | ***     | -340.7 to -333.5     |
| Control vs Standard              | -1.028     | 1.179 | No                     | Ns      | -4.651 to 2.595      |
| Control vs MH 100                | -227       | 260.3 | Yes                    | ***     | -230.6 to -223.3     |
| Control vs MH 400                | -4.55      | 5.219 | Yes                    | **      | -8.173 to -0.9271    |
| Toxic vs Standard                | 336.1      | 385.4 | Yes                    | ***     | 332.4 to 339.7       |
| Toxic vs MH 100                  | 110.1      | 126.3 | Yes                    | ***     | 106.5 to 113.7       |
| Toxic vs MH 400                  | 332.5      | 381.4 | Yes                    | ***     | 328.9 to 336.2       |
| Standard vs MH 100               | -225.9     | 259.1 | Yes                    | ***     | -229.6 to -222.3     |
| Standard vs MH 400               | -3.522     | 4.039 | No                     | Ns      | -7.145 to 0.1012     |
| MH 100 vs MH 400                 | 222.4      | 255.1 | Yes                    | ***     | 218.8 to 226.0       |

Fig. 8. Effect of MDA due to Paracetamol induced Hepatotoxicity

Table 15. Effect of GSH due to Paracetamol induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff       |
|----------------------------------|------------|-----|------------------------|---------|----------------------|
| Control vs Toxic                 | 146.5      | 117.5 | Yes                    | ***     | 141.3 to 151.7       |
| Control vs Standard              | 2.533      | 2.032 | No                     | Ns      | -2.648 to 7.715      |
| Control vs MH 100                | 89.82      | 72.03 | Yes                    | ***     | 84.64 to 95.00       |
| Control vs MH 400                | 7.1        | 5.694 | Yes                    | **      | 1.918 to 12.28       |
| Toxic vs Standard                | -144       | 115.5 | Yes                    | ***     | -149.2 to -138.8     |
| Toxic vs MH 100                  | -56.71     | 45.48 | Yes                    | ***     | -61.89 to -51.53     |
| Toxic vs MH 400                  | -139.4     | 111.8 | Yes                    | ***     | -144.6 to -134.2     |
| Standard vs MH 100               | 87.28      | 70    | Yes                    | ***     | 82.10 to 92.46       |
| Standard vs MH 400               | 4.567      | 3.662 | No                     | Ns      | -0.6149 to 9.748     |
| MH 100 vs MH 400                 | -82.72     | 66.33 | Yes                    | ***     | -87.90 to -77.54     |
Fig. 9. Effect of GSH due to Paracetamol induced Hepatotoxicity

Table 16. Effect of SOD due to Paracetamol induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q     | Significant? | Summary | 95% CI of diff |
|----------------------------------|------------|-------|--------------|---------|----------------|
| control vs toxin                 | 64.57      | 141.1 | Yes          | ***     | 62.66 to 66.47 |
| control vs standard              | 1.8        | 3.933 | No           | ns      | -0.1018 to 3.702 |
| control vs 100                   | 23.8       | 52    | Yes          | ***     | 21.90 to 25.70 |
| control vs 400                   | 2.317      | 5.062 | Yes          | *       | 0.4148 to 4.219 |
| toxin vs standard                | -62.77     | 137.1 | Yes          | ***     | -64.67 to -60.86 |
| toxin vs 100                     | -40.77     | 89.07 | Yes          | ***     | -42.67 to -38.86 |
| toxin vs 400                     | -62.25     | 136   | Yes          | ***     | -64.15 to -60.35 |
| standard vs 100                  | 22         | 48.07 | Yes          | ***     | 20.10 to 23.90 |
| standard vs 400                  | 0.5167     | 1.129 | No           | ns      | -1.385 to 2.419 |
| 100 vs 400                       | -21.48     | 46.94 | Yes          | ***     | -23.39 to -19.58 |

Fig. 10. Effect of SOD due to Paracetamol induced Hepatotoxicity
3.10 Carbon tetrachloride-induced Hepatotoxicity

Bodyweight

The bodyweight of the animal was decreased in toxic control. The treatment of an animal with the extract showed an increase in body weight. No change in the bodyweight normal control was seen. On administration of Silymarin, the body weight was found to be near normal. On administration of the extract, the body weight was found near to normal. At a higher dose of extract, the promising effect was seen (Tables 18-23 Figs. 11-17).

Bodyweight

Table 18. Effect on body weight due to CCL4 induced Hepatotoxicity

| Tukey’s Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|-----|------------------------|---------|----------------|
| Control vs Toxic                 | 97.14      | 70.22 | Yes                    | ***     | 91.39 to 102.9 |
| Control vs Standard              | 3.055      | 2.209 | No                     | ns      | -2.693 to 8.803 |
| Control vs TU 100                | 56.49      | 40.83 | Yes                    | ***     | 50.74 to 62.23 |
| Control vs TU 400                | 4.533      | 3.277 | No                     | ns      | -1.215 to 10.28 |
| Toxic vs Standard                | -94.08     | 68.01 | Yes                    | ***     | -99.83 to -88.34 |
| Toxic vs TU 100                  | -40.65     | 29.39 | Yes                    | ***     | -46.40 to -34.91 |
| Toxic vs TU 400                  | -92.61     | 66.95 | Yes                    | ***     | -98.35 to -86.86 |
| Standard vs TU 100               | 53.43      | 38.63 | Yes                    | ***     | 47.68 to 59.18  |
| Standard vs TU 400               | 1.478      | 1.069 | No                     | ns      | -4.270 to 7.226 |
| TU 100 vs TU 400                 | -51.95     | 37.56 | Yes                    | ***     | -57.70 to -46.20 |

Fig. 11. Effect on body weight due to Paracetamol induced Hepatotoxicity

3.11 Estimation of ALT Level

Table 19. Effect on body weight due to CCL4 induced Hepatotoxicity

| Tukey’s Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|-----|------------------------|---------|----------------|
| Control vs Toxic                 | -135.3     | 224.2 | Yes                    | ***     | -137.8 to -132.8 |
| Control vs Standard              | -2.248     | 3.726 | No                     | ns      | -4.756 to 0.2594 |
| Control vs MH 100                | -44.52     | 73.77 | Yes                    | ***     | -47.02 to -42.01 |
| Control vs MH400                 | -3         | 4.971 | Yes                    | *       | -5.508 to -0.4923 |
| Toxic vs Standard                | 133        | 220.5 | Yes                    | ***     | 130.5 to 135.6  |
| Toxic vs MH 100                  | 90.78      | 150.4 | Yes                    | ***     | 88.27 to 93.28  |
| Toxic vs MH400                   | 132.3      | 219.2 | Yes                    | ***     | 129.8 to 134.8  |
| Standard vs MH 100               | -42.27     | 70.04 | Yes                    | ***     | -44.78 to -39.76 |
| Standard vs MH400                | -0.7517    | 1.246 | No                     | ns      | -3.259 to 1.756  |
| MH 100 vs MH400                  | 41.52      | 68.79 | Yes                    | ***     | 39.01 to 44.02  |
3.12 Estimation of AST Level

Table 20. Effect on AST due to CCL4 induced Hepatotoxicity

| Tukey’s Multiple Comparison Test | Mean Diff. | q     | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|-------|------------------------|---------|----------------|
| Control vs Toxic                 | -173.1     | 171.6 | Yes                    | ***     | -177.3 to -168.9 |
| Control vs Standard              | -2.238     | 2.219 | No                     | ns      | -6.429 to 1.952  |
| Control vs MH 100                | -212.9     | 211.1 | Yes                    | ***     | -217.1 to -208.7 |
| Control vs MH 400                | -6.11      | 6.058 | Yes                    | **      | -10.30 to -1.919 |
| Toxic vs Standard                | 170.9      | 169.4 | Yes                    | ***     | 166.7 to 175.1   |
| Toxic vs MH 100                  | -39.81     | 39.48 | Yes                    | ***     | -44.01 to -35.62 |
| Toxic vs MH 400                  | 167        | 165.6 | Yes                    | ***     | 162.8 to 171.2   |
| Standard vs MH 100               | -210.7     | 208.9 | Yes                    | ***     | -214.9 to -206.5 |
| Standard vs MH 400               | -3.872     | 3.839 | No                     | ns      | -8.062 to 0.3190 |
| MH 100 vs MH 400                 | 206.8      | 205.1 | Yes                    | ***     | 202.6 to 211.0   |

Fig. 12. Effect on body weight due to CCL4 induced Hepatotoxicity

Fig. 13. Effect on AST due to CCL4 induced Hepatotoxicity
### 3.13 Estimation of ALP Level

Table 21. Effect on ALP due to CCL4 induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|-----|------------------------|---------|----------------|
| Control vs Toxic                 | -174.1     | 197.1 | Yes (***)              |         | -177.8 to -170.4 |
| Control vs Standard              | -2.583     | 2.924 | No (ns)                |         | -6.254 to 1.088  |
| Control vs MH 100                | -138.4     | 156.7 | Yes (***)              |         | -142.1 to -134.7 |
| Control vs MH 400                | -5.263     | 5.958 | Yes (**)               |         | -8.934 to -1.592 |
| Toxic vs Standard                | 171.5      | 194.2 | Yes (***)              |         | 167.9 to 175.2   |
| Toxic vs MH 100                  | 35.7       | 40.41| Yes (***)              |         | 32.03 to 39.37   |
| Toxic vs MH 400                  | 168.9      | 191.1 | Yes (***                |         | 165.2 to 172.5   |
| Standard vs MH 100               | -135.8     | 153.8 | Yes (***)              |         | -139.5 to 132.2  |
| Standard vs MH 400               | -2.68      | 3.034 | No (ns)                |         | -6.351 to 0.9910 |
| MH 100 vs MH 400                 | 133.2      | 150.7 | Yes (***)              |         | 129.5 to 136.8   |

![Manilkara hexendra](image)

Fig. 14. Effect on ALP due to CCL4 induced Hepatotoxicity

### 3.14 Estimation of MDA Level

Table 22. Effect on MDA due to CCL4 induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q   | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|-----|------------------------|---------|----------------|
| Control vs Toxic                 | -263.5     | 218.4 | Yes (***)              |         | -268.5 to -258.5 |
| Control vs Standard              | -3.5       | 2.901 | No (ns)                |         | -8.513 to 1.513  |
| Control vs MH 100                | -227.9     | 188.9 | Yes (***)              |         | -232.9 to -222.9 |
| Control vs MH 400                | -7.367     | 6.107 | Yes (**)               |         | -12.38 to -2.354 |
| Toxic vs Standard                | 260        | 215.5 | Yes (***               |         | 255.0 to 265.0   |
| Toxic vs MH 100                  | 35.63      | 29.54 | Yes (***               |         | 30.62 to 40.65   |
| Toxic vs MH 400                  | 256.1      | 212.3 | Yes (***               |         | 251.1 to 261.1   |
| Standard vs MH 100               | -224.4     | 186   | Yes (***               |         | -229.4 to -219.4 |
| Standard vs MH 400               | -3.867     | 3.205 | No (ns)                |         | -8.879 to 1.146  |
| MH 100 vs MH 400                 | 220.5      | 182.8 | Yes (***               |         | 215.5 to 225.5   |
3.15 Estimation of GSH Level

Table 23. Effect on body GSH to CCL4 induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | q    | Significant? P < 0.05? | Summary | 95% CI of diff |
|----------------------------------|------------|------|------------------------|---------|----------------|
| Control vs Toxic                  | 105.1      | 129.4 | Yes                    | ***     | 101.7 to 108.4 |
| Control vs Standard               | 2.512      | 3.093 | No                     | Ns      | -0.8631 to 5.886 |
| Control vs MH 100                 | 42.21      | 51.98 | Yes                    | ***     | 38.84 to 45.59  |
| Control vs MH 400                 | 5          | 6.156 | Yes                    | **      | 1.625 to 8.375  |
| Toxic vs Standard                 | -102.5     | 126.3 | Yes                    | ***     | -105.9 to -99.17 |
| Toxic vs MH 100                   | -62.84     | 77.38 | Yes                    | ***     | -66.22 to -59.47 |
| Toxic vs MH 400                   | -100.1     | 123.2 | Yes                    | ***     | -103.4 to -96.68 |
| Standard vs MH 100                | 39.7       | 48.88 | Yes                    | ***     | 36.33 to 43.08  |
| Standard vs MH 400                | 2.488      | 3.064 | No                     | Ns      | -0.8865 to 5.863 |
| MH 100 vs MH 400                  | -37.21     | 45.82 | Yes                    | ***     | -40.59 to -33.84 |

Fig. 15. Effect on MDA due to CCL4 induced Hepatotoxicity

Fig. 16. Effect on body GSH to CCL4 induced Hepatotoxicity
3.16 Estimation of SOD Level

Table 24. Effect on SOD due to CCL4 induced Hepatotoxicity

| Tukey's Multiple Comparison Test | Mean Diff. | Q    | Significant? | Summary | 95% CI of diff |
|---------------------------------|------------|------|--------------|---------|----------------|
| Control vs Toxin                | 48.02      | 145.8| Yes          | ***     | 46.65 to 49.39 |
| Control vs Standard             | 1.3        | 3.946| No           | ns      | -0.6681 to 2.669 |
| Control vs MH100                | 22.75      | 69.06| Yes          | ***     | 21.38 to 24.12 |
| Control vs MH 400               | 1.567      | 4.756| Yes          | *       | 0.1979 to 2.935 |
| Toxin vs Standard               | -46.72     | 141.8| Yes          | ***     | -48.09 to -45.35 |
| Toxin vs MH100                  | -25.27     | 76.7 | Yes          | ***     | -26.64 to -23.90 |
| Toxin vs MH 400                 | -46.45     | 141  | Yes          | ***     | -47.82 to -45.08 |
| Standard vs MH100               | 21.45      | 65.12| Yes          | ***     | 20.08 to 22.82 |
| Standard vs MH 400              | 0.2667     | 0.8095| No           | ns      | -1.102 to 1.635 |
| MH100 vs MH 400                 | -21.18     | 64.31| Yes          | ***     | -22.55 to -19.81 |

Fig. 17. Effect on SOD due to CCL4 induced Hepatotoxicity

4. DISCUSSION

Some new investigations in the liver and kidney of mice showed that in low centralization of *Manilkara* caused slight impacts in mice [17]. In the Indian arrangement of medication, certain spices are professed to give help against liver problems. The asserted restorative standing must be confirmed logically. In the current examination, one such medication Manikara hexendra was taken for the investigation. The methanolic concentrate of *Manikara hexendra* has a critical (P > 0.05) hepatoprotective impact in the CCl4 model of inebriation in rodents. Our examination of the concentrates demonstrated the presence of triterpenoids and flavonoids in the methanolic removal. As indicated by these outcomes, it possibly theorized that flavonoids, which are available in the methanolic extricate, could be viewed as answerable for the hepatoprotective action. The hepatotoxicity of CCl4 has been accounted for to be because of the arrangement of the profoundly responsive trichloro-free revolutionary, which assaults polyunsaturated unsaturated fats. It produces Hepatotoxicity by adjusting liver microsomal films in test creatures [18]. The impact of CCl4 is for the most part seen after 24 h of its organization. Thus the withdrawal of the blood for biochemical boundaries ought to be completed simply after 24 h of CCl4 inebriation. From Table 1 it is obvious that the Ethyl acetate removal had the option to decrease all the raised biochemical boundaries because of the hepatotoxin inebriation. The degrees of all-out proteins and egg whites were decreased because of the...
hepatotoxin inebriation. The decrease is credited to the harm created and restricted in the endoplasmic reticulum which brings about the deficiency of P450 prompting its practical disappointment with a decline in protein combination and collection of fatty substances. Inebriation with CCl4 additionally brought about restraint of blend of the bile acids from cholesterol which is integrated into the liver or got from plasma lipids, prompting increment in cholesterol levels. Concealment of cholesterol levels recommends the hindrance of the union of bile acids from cholesterol is switched by the concentrate [19]. Decrease in the degrees of SGOT and SGPT towards the ordinary worth means that adjustment of plasma film just as the fix of hepatic tissue harms brought about by CCl4. Decrease of ALKP levels with simultaneous consumption of raised bilirubin level proposes the dependability of the biliary capacity during injury with CCl4. The rise in protein and egg whites levels proposes the adjustment of endoplasmic reticulum promoting protein combination. In the current examination, Ethyl acetate concentrates of Manikara hexendra bark were assessed for its hepatoprotective action utilizing Paracetamol and CCL4 actuated [20]. The harm to the Liver was dictated by biochemical markers (AST, ALT, ALP, SOD, GSH, and MDA level). Further, the body weight was likewise decided. Paracetamol is the most generally utilized harmful control for the investigation of hepatoprotective impacts of the restorative plants removes and drugs [21]. Paracetamol is known for its generally utilized NSAIDs and its drawn-out use causes hepatic injury in man and exploratory creatures by consumption of glutathione and authoritative of harmful metabolite to essential proteins and compounds. The chemical Cytochromes P450 2E1 (CYP2E1) and 3A4 (CYP3A4) causes the transformation of paracetamol to N-acetyl-p-benzoquinone imine (NAPQI) an exceptionally responsive mediator metabolite [22]. In the ordinary course this metabolite, NAPQI is detoxified information with glutathione. Due to paracetamol's harmfulness or CCL4, the sulfate and glucuronide pathways become immersed, and more paracetamol is shunted to the cytochrome P450 framework to deliver NAPQI [23]. This blocks the hepatocellular supplies of glutathione and NAPQI is free for the response with cell layer atoms. These outcomes in hepatocytes harm and demise, for example, intense hepatic necrosis [24]. In such a manner, the diminished degree of AST and ALT towards the ordinary affected by separate shows the plasma film of adjustment [25-26]. Further, this shows the revived hepatic tissue harm brought about by paracetamol. The consequences of biochemical boundaries demonstrated the hepatoprotective movement of Ethyl acetate of bark in portion subordinate way. The phytochemical screening of the concentrates has demonstrated the presence of flavonoids which has additionally indicated its cancer prevention agent exercises.

5. CONCLUSION

Consequently, it may be said that that conceivable activity of hepatoprotector of Manikara hexendra bark might be because of its free extremist rummaging and cancer prevention action. Accordingly the current shows the critical hepatoprotective activity of Manikara hexendra (Sm.) bark extricate against initiated liver damage in the rodents. This additionally underpins its conventional society medication. Thus it could be concluded that Manikara hexendra (Sm.) can be used potentially for its hepatoprotective potential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the animals were procured from the animal house, I.T.S College of Pharmacy, Ghaziabad, India (1044/P0/Re/S/07/CPCSEA, 27th Frb. 2007). All animal procedure was approved by the ethical committee of I.T.S College of Pharmacy, Muradnagar, Ghaziabad.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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