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Effect of quercetinon drug induced hepatotoxicity

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***Abstract*---**Objectives-The present study was designed 1. To achieved maximum yield of bioactive extract from the plant through extraction process. 2. To investigated phytochemicals constituents. 3. To determined pharmacological screening of quercetin against hepatotoxicity. Methods- Crude extracts of *Moringa oleifera* leaves were prepared using various solvents using petroleum ether, chloroform, ethanol, methanol in succession using soxhlet apparatus. The plant extracts were subjected for photochemical analysis, total phenolic content and total flavonoid content. The extracts were then subjected to column chromatography followed by TLC. The isolated compound was subjected to experimental animals and hepatoprotective activity was studied. Results-The ethanol extract showed the presence of higher flavonoid content when compared with other solvent extracts. The ethanol extract was subjected to

fractionalization by column chromatography. The eluted fractions were run in TLC mobile phase with the different solvent ratio. Chromatographic fingerprinting showed the presence of flavonoids. It was found that there was significant decrease in the SGOT, SGPT, ALP and Total Bilirubin level in animal by giving quercetin of ethanolic extract of *Moringa oleifera* plant. Conclusion - The present investigation revealed that the extract containing quercetin of *Moringa oleifera* showed excellent hepatoprotective activity against Paracetamol induced hepatotoxicity.

***Keywords*---**effect quercetinon drug, hepatotoxicity.

Introduction

Hepatotoxicity is liver damage caused by hepato-toxins, which can be caused by medications, chemicals, food, or herbs.[1]In actuality, a number of synthetic hepatoprotective medications are accessible, but their efficacy does not apply to the entire population suffering from this illness. [2]*Moringa oleifera* has a long history in Indian culture; the plant appears in most ancient writings and is renowned for a variety of qualities that are effective in the variety of disease's treatment. The tree is known as "The Miracle Tree" in African culture because every component of the plant has medicinal properties.[3]

MO contains numerous bioactive components including vitamins, phenolic acids, flavonoids, isothiocyanates, tannins and saponins which are present in significant amounts in their leaves, bark, seeds, flowers and roots which are therapeutically useful as antipyretic, antitumor, antioxidant, cardiac stimulants, antidiabetic, antiepileptic, antibacterial, anti-inflammatory, antifungal, hepatoprotective, antispasmodic, antihypertensive, antiulcer, antidiabetic, diuretic and cholesterol lowering effects. [4,5]Although flavonoid components from MO extracts have been found to be powerful antioxidants in vitro and in animals (e.g., rats, goats, and rabbits), few clinical research have looked at their benefits in people. [6]The main bioactive constituents of phenolics are flavonoids like quercetin and kaempferol. Quercetin may have hepatoprotective properties through a variety of methods. [7]The FDA has cleared quercetin and certain of its conjugates for human use. [8]MO is the most cost-effective and reliable way to give proper nutrition as well as the treatment and prevention of a variety of diseases. [9]

Plant profile
Moringa oleifera[10]

FigureParts of *Moringa oleifera*

Taxonomical classification [11] Vernacular name [11, 12]

Kingdom	Plantae
Sub Kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub Class	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	Oleifera

Latin	*Moringa oleifera*
Sanskrit	Subhanjana
Hindi	Saguna, Sainjna
Gujarati	Suragavo
Marathi	Shevga
Unani	Sahajan
Ayurvedic	Akshiva, Haritashaaka, Raktaka, Tikshnagandhaa
English	Drumstick tree, Horseradish tree, Ben tree

Morphology

Wood - Soft and white with a density of 0.5 to 0.7 g/cm3[13]
Leaves - Leaves are bipinnate or more commonly tripinnate, upto 45 cm long. These are compound leaves with the leaflets of 1-2 cm long [12, 13]
Branch - The extended branches grow in a disorganized manner and the canopy is umbrella shaped [13]
Bark - The bark is whitish-gray, thick, soft, fissured and warty or corky, becoming rough. When wounded, the bark exudes a gum which is initially white in color but changes to reddish brown or brownish black on exposure [12]
Leaflets - They are feathery with green to dark green, glabrous and and almost hairless on the upper surface, paler and hairless beneath, with red-tinged mid-veinswith entire (not toothed)margins, and are rounded or blunt-pointed at the apex and short-pointed at the base, 1-2 am long [11, 12]
Twigs - Finely hairy and green, becoming brown[11, 12]
Flower - The fragrant, bisexual, yellowish white flowers are borne on slender, hairy stalks in spreading or drooping axillary panicles 10-25 cm long, 2.5 cm in diameter, with five unequal yellowish-white, thinly veined, spathulate petals, five stamens with five smaller sterile stamens, and a pistil composed of a 1-celled ovary and slender style [11, 12]
Fruits - They are trilobed capsules and are referred as pods. [13] Pods are pendulous, brown, triangular, and so splits into three parts when dry, 30-120 cm long, 1.8 cm wide. Each fruit contain around 26 seeds during their development phase. Immature pods are green in colour. They turn brown on maturity, and

split open longitudinally along the three angles, releasing the dark brown, trigonous seeds.[14]
Seeds- Three-angled, numerous, globular. [15] Seeds are round 1 cm in diameter with a brownish semi-permeable seed hull, with 3 papery wings. Hulls of seed are brown to black but can be white if kernels are of low viability. The white wings of hull are also present which run from top to bottom at 120 intervals. Each tree can produce around 15000 to 25000 seeds/year. Average weight is 0.3 gm/seed. The kernel to hull ratio is 75:25. [12]
Roots - Seedlings develop a swollen, tuberous, white taproot which has a characteristic pungent odor, and very spares lateral roots. Trees grown from seeds develop a deep, stout taproot with a wide-spreading system of thick, tuberous lateral roots. [12]

Biological source

Moringa oleifera, an important medicinal plant is one of the most widely cultivated species [11]

Chemical constituents

Moringa oleifera contains Vitamins, phenolic acids, flavonoids, isothiocyanates, tannins and Saponins such as niazirin, niazirinin, kaempferol quercetin, gallic acid, chlorgenic acid, ellagic acid, aspartic acid, glutamic acid, glycine, threonine alanine, vanillin, leucine, isoleucine, histidine, lycine, phenylalanine, tryptophan, cysteine, methionine, beta sitosterol, etc.[4, 12]

Traditional Uses

Traditionally, the plant is used as an antispasmodic, stimulant, expectorant, diuretic, abortifacient, antifungal, antiparalytic, antibacterial, antipyretic and antiseptic. Pods are antipyretic, anthelmintic, fried pods are used in diabetes. Decoction is used as a gargle in hoarseness and sore throat. Leaf juice is used in hiccough, influenza and catarrhal affections. Root-bark is used as antiviral, anti-inflammatory, analgesic. Stem-bark and flowers are hypoglyceamic. [16]

Research Methodology

Macroscopic, Microscopic Organoleptic characteristics

Loss on Drying[17, 18]

The ground sample was weighed (5 g) in a petri-dish and dried in hot-air oven at 105°C for 4 h. After cooling it was placed in a desiccator, later the loss in weight was recorded. This procedure was repeated till the constant weight was obtained.
Loss on drying (%) =Loss in weight × 100/W
Where,W = Weight of the crude drug in grams

Ash Values
Total Ash [19]

The crude drug powder (2 g) taken in a tared silica dish previously ignited and weighed. Incinerated gradually by increasing the heat, not exceeding dull red heat, until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Total ash value =(Weight after drying-weight of crucible)×100/weight of sample

Acid-Insoluble Ash[20]

2M (70 g/l) HCl (25 ml) added to the crucible containing total ash. The crucible was then added with watch-glass, and the mixture was boiled gently for 5 minutes. The watch-glass was rinsed with hot water (5 ml), and this liquid was added into the crucible. The insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter wastransferred to the original crucible, dried on a hot plate, and ignited to constant weight. The residue was allowed to cool in a desiccator, and weighed immediately.

Acid insoluble ash = (weight after drying-weight of crucible)×100/weight of sample

Water-Soluble Ash[20]

Boil the ash for 5 minutes with 25 ml of *water*, collect the insoluble matter in a Gooch crucible or an ashless filter paper, wash with hot *water*, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash on the dried basis.
Water soluble extractive =
(Weight after drying-weight of petridish)×100/weight of sample × vol. of filtrate

Ethanol-soluble extractive determination[17]

The ground sample (5 g, accurate weighed) was macerated with absolute ethanol (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted was filtered rapidly to avoid loss of ethanol. The filtrate (20 ml) was evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

Water-soluble extractive determination[17]

The ground sample (5 g) was macerated with distilled water (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted wasfiltered rapidly to avoid loss of ethanol. The filtrated (20 ml) was evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

Successive Extraction of *Moringa oleifera Leaves*[21]

The mature leaves of *Moringa oleifera* collected locally were washed with water, shade dried and grounded into fine powder. 300g of the dried leaf powder was extracted using petroleum ether, chloroform, ethanol, methanol, hydroalcohol and water in succession using soxhlet apparatus. The drug was extracted with each solvent till complete extraction was effected (about 50 cycles). Each extract obtained following successive extraction was filtered using Whatman No 1 filter paper, dried to a semisolid mass using water bath and the yield of each extract thus obtained was recorded and stored in a refrigerator at 4ºC till further use.

Phytochemical Screening [20- 24]

A stock concentration of 1 % (W/V) of each successive extract obtained using petroleum ether, chloroform, ethanol, methnol, hydroalcoholic and water was prepared using the respective solvent. These extracts along with positive and negative controls were tested for the presence of active phytochemicals viz:alkaloids, cardiac glycosides, anthroquinone glycosides, carbohydrates, flavonoids, proteins, phytosterols, saponins, triterpenoids and tanninsfollowing standard methods as briefed below.

Alkaloids

Approximately 50 mg of extract was dissolved in 5 ml of distilled water. Further 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below.

Dragendorff's Test

To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.

Mayer's Test

To 1 ml of test solution or filtrate was added a drop or two of the Mayer's reagent along the sides of the test tube. A white or a creamy precipitate confirmed the test as positive.

Hager's Test

To 1 ml of test solution or filtrate, a drop or two of Hager's reagent was added. The formation of yellow precipitate indicated the test as positive.

Wagner Test

Two drops of Wagner's reagent was added to 1ml of the test solution along the the side of the test tube. The formation of yellow or brown precipitate confirmed the test as positive for alkaloids.

Cardiac glycosides

Keller -Killiani test

Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully. The formation of blue colour in acetic acid layer confirmed the test.

Anthraquinone glycosides

Hydroxyanthraquinone Test

To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution. The Formation of red color confirmed the test.

Test for carbohydrates

Molisch's test

To 1 ml of test solution added a few drops of 1 % alpha-napthol and 2-3 ml concentrated sulfuric acid along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test.

Barfoed's test

2ml of reagent was added to 2 ml of the test solution, mixed & kept a in boiling water bath for 1 min. Red precipitate formed indicates the presence of monosaccharides.

Seliwanoffs test

To 3 ml of Seliwanoffs reagent was added to 1 ml of the test sample and heated on a water bath for one minute. The formation of rose red color confirmed carbohydrates.

Fehlings test

Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's(A+B) solution, shooked and heated on a water bath for 10 minutes. The brick red precipitate formed confirmed the test.

Flavonoids

Shinoda test

A few magnesium turnings and 5 drops of concentrated hydrochloric acid was added drop wise to 1 ml of test solution. A pink, scarlet, crimson red or occasionally green to blue colour appeared after few minutes confirmed the test.

Alkaline reagent test

Addition of 5 drops of 5% sodium hydroxide to 1 ml of the test solution resulted an increase in the intensity of the yellow color which became colorless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids.

Lead acetate test

0.5 g of extract was dissolved in 5 ml distilled water, heated for 5 minutes and filtered. The filtrate was allowed stand for 5 minutes to cool and about 2-3 drops of lead subacetate solution was added to the filtrate. A yellow precipitate indicated the presence of flavonoids.

Test for proteins

Biuret test

To 2 ml of the test solution added 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH.Mix thoroughly. Formation of purple or violet color confirmed proteins.

Millon's test

Test solution was treated with Million's reagent and heated on a water bath. The proteins were stained red.

Phytosterols

Liebermann-Burchard's Test

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols.

Saponins

Foam Test

To 2 ml of alcohol diluted with water is added to the 2 ml. of the plant extract, shaked well for 15 minutes. Formation of foam indicates the presence of saponins.

Triterpenoids

Salkowski Test

Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A

red brown color formed at the interface indicated the test as positive for triterpenoids.

Tannins

Residue of each extract (few mg) was taken up separately in water, warmed and filtered. Tests were carried out with the filtrate using the following reagents.

Ferric chloride Test

Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. Formation of blue color indicated the presence of hydrolysable tannins.

Thin Layer Chromatography (TLC) [25, 26]
Chromatographic purification

TLC was carried out to isolate the principle components that were presents in most effective extracts of plants. The TLC was used by different solvent systems.

Method

Each solvent extract was placed to silica gel plates for thin layer chromatography (TLC) analysis. Plates were marked and glass capillaries were used to place the sample. Drawing a light line on the plate and dots to know the place of each extract applied on the plate. The TLC plates were placed in camber containing solvent (mobile phase) and leave for 20 min to develop bands and observed under ultra violet light.

Analyte detection

After the solvent running plates are dried they were later sprayed with iodine vapors for the development of the separated bands and all plates were visualized with the help of UV. The movement of the separated compound was expressed by its retention factor (Rf), values were calculated by formula.

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance traveled by the solvent front}}$$

Determination of Total Phenolic Content[27]

Total phenolic content was determined with the Folin–Ciocalteu reagent. A calibration curve was obtained by using gallic acid as standard. Different concentrations of gallic acid (5–125 mg/l) were prepared. 10 mg sample was diluted in 10 ml methanol on test tube. Both 0.5 mL of standards and samples were taken and mixed with 2.5 ml of Folin–Ciocalteu 50% and 2.5 ml of distilled water. After incubated for 5 min, 2 ml aqueous sodium carbonate solution (7.5%, w/v) was added. The final mixture was shaken and then incubated for 15 min in the dark at room temperature. The absorbance of all standards and samples were

measured at 765 nm using UV-Vis spectrophotometer and the results expressed as milligrams of gallic acid equivalents (GAE) per 100 mg of dry leaf weight.

Determination of Total Flavonoids [28]

Flavonoid content was determined by colorimetric analysis. A mixture of 200 µL extract and 150 µl of sodium nitrite (NaNO2) (5 % w/v), was first incubated for 6 min at room temperature. Next, 150 µl of aluminium chloride hexahydrate AlCl3.6H2O (10 % w/v) was added and incubated for 6 min at room temperature. NaOH (10 % w/v) solution 800 µl was added and incubated at room temperature for 15 min. For blank, extract was replaced with distilled water. Absorbance was read by using UV-Vis spectrophotometer at 510 nm. A standard curve of quercetin dissolved in 80 % ethanol was initially prepared from 0 - 500 µg/ml. Total flavonoid was expressed in mg Quercetin equivalent (QE)/ g dry matter.

Isolation of Quercetin[29]

Ethanolic leaf extract were chromatographed over silica gel column (100-200 mesh) using solvents with increasing polarity. The admixture was packed on a silica gel column (Merck, India) and elution starts with 100% Hexane and then increased the polarity using Chloroform, Ethyl acetate and Ethanol and Methanol in the ratio of 90:10, 80:20, 70:30 and 50:50. All the collected fractions were run for TLC. Based on TLC profile fractions with similar Rf values were pooled into some fractions.

Hepatoprotective Activity

All procedures were approved by Institutional Animal Ethical Committee and were conducted in accordance with CPCSEA guidelines.

Experimental Animals[30]

For the study of Hepatoprotective activity of herbal decoction30 Albino Wistar rats, having average weight 200-250g were used. The rats were divided into five groups, containing six rats in each group. The selected animal washoused in a polypropylene cages at the standard environment at 25ºC - 28ºC, relative humidity of 40-70% in well ventilated room maintained at 12 h light and dark cycle, fed with standard rodent diet and free access to water and subjected to 12 h fasting before the experimentation.

Experimental design[31]

The animals were divided into different group as:

Group I: Normal saline (5ml/kg)i.p. for 7 days
Group II : Normal saline (1ml/kg) i.p. for 7 days + Paracetamol (750mg/kg)p.o. on 7^{th}day
Group III :Silymarin (100mg/kg) p.o. for 7 days + Paracetamol (750mg/kg)p.o. on 7^{th}day

Group IV : Herbal extract (200mg/kg)i.p. for 7 days + Paracetamol (750mg/kg)p.o. on 7thday
Group V : Herbal extract (400mg/kg) i.p. for 7 days + Paracetamol (750mg/kg)p.o. on 7thday

Groups (n-5)	Treatment	Animals used
Group I	Normal saline	6
Group II	Normal saline + Paracetamol	6
Group III	Silymarin + Paracetamol	6
Group IV	Herbal extract + Paracetamol	6
Group V	Herbal extract + Paracetamol	6

Determination of biochemical parameters

The biochemical parameters were estimated after an 18h fast following the last dose, all the rats were sacrificed by using high dose of ketaminethen blood was withdrawn from all groups of rats and allowed to clot. Serum were separated by centrifugation (Remi centrifuge machine) at 2500 rpm at 30°C for 10 min. and used for the biochemical estimation of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), ALP and total bilirubin (TB) by colorimetric methods. [31]

Histopathological studies

For histopathological examination, the animals were sacrificed and livers were dissected out, washed with saline and were transferred and stored in 10% neutral formalin solution. Thick sections (3–4 µm) were prepared and then stained with hematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change. [31, 32]

Results and Discussion

Macroscopic, Microscopic Organoleptic Characteristics

Table no. 1% LOD, Total ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive values

% Loss on drying	Ash value		Extractive value	
	Total ash	Acid insoluble ash	Water-soluble extract	Alcohol soluble extract
7.0 %	10.7 %	1.5 %	27.5 %	20.3 %

Percentage Yield and Weight ofExtracts

Table no. 2 Percentage yield obtained from Moringa oleifera leavesextracts

Solvent used	Petroleum ether	Chloroform	Ethanol	Methanol	Hydroalcoholic	Aqueous
Yield (gm)	9.89	4.9	6.8	3.3	10.4	9.1
Yield (%)	3.29	1.6	2.26	1.1	3.46	3.03

Preliminary Phytochemical Screening

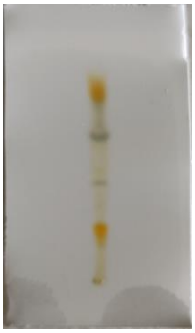
Table no.3 Preliminary phytochemical screening

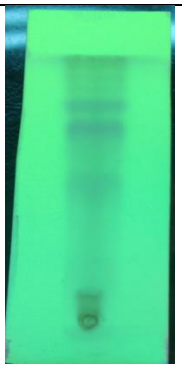
SN.	Tests	Petroleum ether	Chloroform	Ethanol	Methanol	Hydroalcoholic	Aqueous
1	Alkaloids Dragendroff's	-	-	+	+	+	+
	Mayer's	-	-	+	+	+	-
	Hagers	-	-	+	+	+	-
	Wagers	-	-	+	+	+	-
2.	Cardiac glycosides Keller- Killiani test	+	+	+	+	_	-
3.	Anthraquinone glycosides Hydroxyanthraquinone Test	-	+	+	+	+	+
4.	Carbohydrate Molish test	-	-	+	+	+	+
	Barfoed'stest	-	-	+	+	-	-
	Seliwanoffs test	-	+	-	+	-	-
	Fehling's test	-	+	-	+	-	-
5.	Flavonoids Shinoda test	-	-	+	+	+	+
	Alkaline reagent test	-	-	+	+	+	+
	Lead acetate test	-	-	+	+	+	+
6.	Proteins Biuret test	-	-	-	-	-	-
	Millions test	-	-	+	+	+	+

7.	Phytosterols Libermann Burchard	+	+	-	-	-	-
8.	Saponin Foam test	-	-	+	+	+	+
9.	Triterpenoids Salkowski Test	-	-	+	+	+	+
10.	Tannins Ferric chloride Test	-	-	+	+	+	+

Thin Layer Chromatography

Table no. 4Thin layer chromatography of *Moringa oleifera* leaves extract

Sr.no	Solvent system of extract	Ratio	Rf Value	Developed TLC plate
1.	Petroleum ether extract Toluene: ethyl acetate Spraying reagent Iodine chamber	6:4	0.60 0.41	
2.	Ethanolic extract Butanol: acetic acid : water Spraying reagent Iodine chamber	4:5:1	0.40	
3.	Methanolic extract Methanol : Chloroform Spraying reagent Iodine chamber	4:6	0.81 0.51	

Determination of Total Phenolic Content

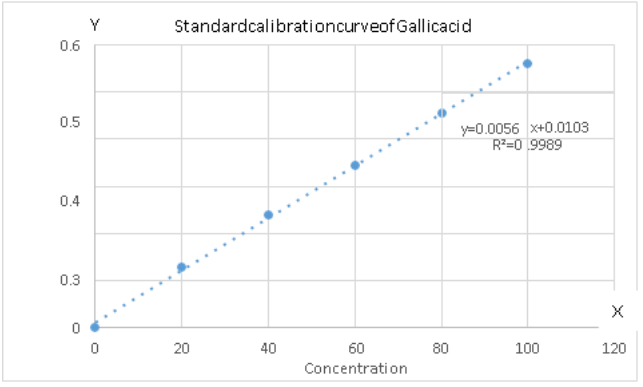


Figure no. 1Standardization of extract to quantify phenolic content

GAE was calculated using the linear regression equation obtained from standard gallic acid graph (R^2 = 0.9989), y = 0.0056x+0.0103. Total phenolic content was found to be 8.56±0.10 and 9.28±0.30 GA/gm in ethanol extract of *Moringa oleifera*.

Determination of Total Flavonoids

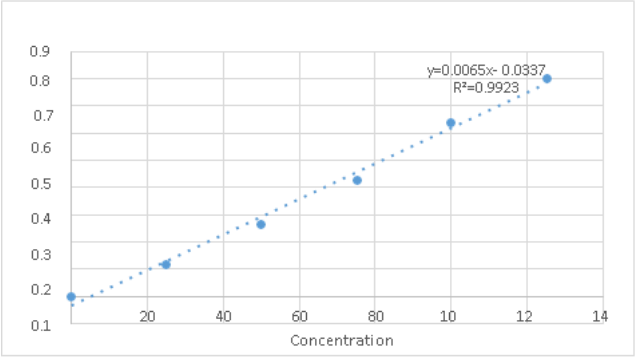


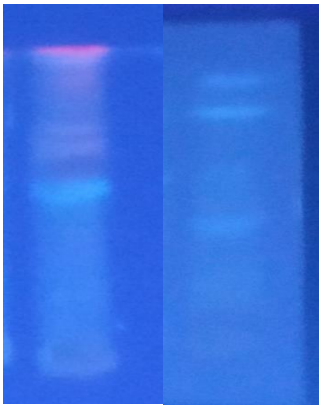
Figure no. 2Standardization of extract to quantify flavonoid content

Quercetin was calculated using the linear regression equation obtained from standard quercetin graph (r^2 = 0.9923), y = 0.0065x+0.0337. Total flavonoid content was found to be 11.65±0.13 and 12.8±0.90 QA/gm in ethanol extract of *Moringa oleifera*.

Isolation of Quercetin

Ethanolic extract was subjected to column chromatography on silica gel (100-200 mesh) eluted with mixtures of chloroform, ethyl acetate, ethanol and methanol of increasing polarity, to obtain fractions for yellow amorphous powder. Column fractions from in the TLC mobile phase solvent ratio of chloroform : ethyl acetate (2.5:2.5) showed Rf value of 0.48 equal to that of standard quercetin. The fractions were then combined and crystallized. This process was repeated several times by using bulk quantity of samples until the desired amount of quercetin has been obtained.

Table no. 5 Thin layer chromatographyof *Moringaoleifera leaves* extractsfordeterminationof Flavonoids

Sr. no.	*Moringa oleifera* extract	Solvent system and visualizing agents	Rf values	Developed TLC plate
1. Determination of flavonoids	TLC 1- Standard quercetin TLC 2- Ethanolic extract of *Moringa oleifera*	Chloroform : ethyl acetate (2.5:2.5) Visualizing agent: AlCl3 observed in UV chamber at 365nm	TLC1 - 0.46 TLC2 - 0.48	 TLC 1 TLC 2

Hepatoprotective Activity

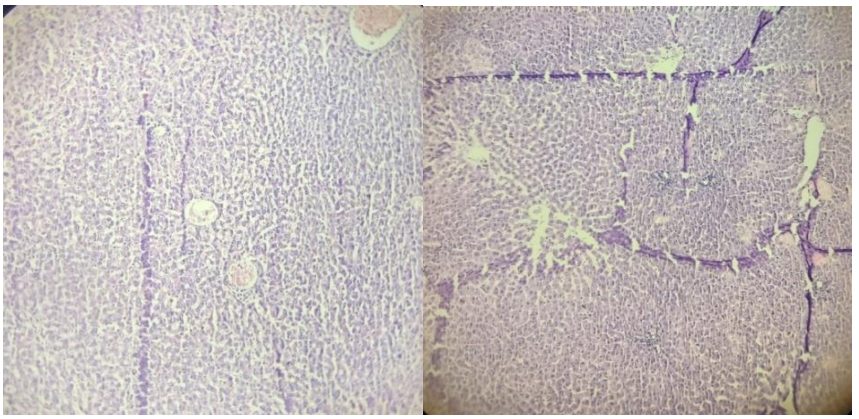
Table no.6 Effect of quercetin in herbal extract on serum biochemical markers in Paracetamol induced hepatotoxicity in rat.

Group Parameters	Normal group	Paracetamol	Paracetamol + Silymarin (100mg/kg)	Paracetamol + Quercetin in herbal extract (200mg/kg)	Paracetamol+ Quercetin in herbal extract (400mg/kg)
SGOT (IU/L)	68.45±2.65	114.8 ± 2.8	72.93 ± 4.43	78.94 ± 2.46	77.68 ± 3.93
SGPT					

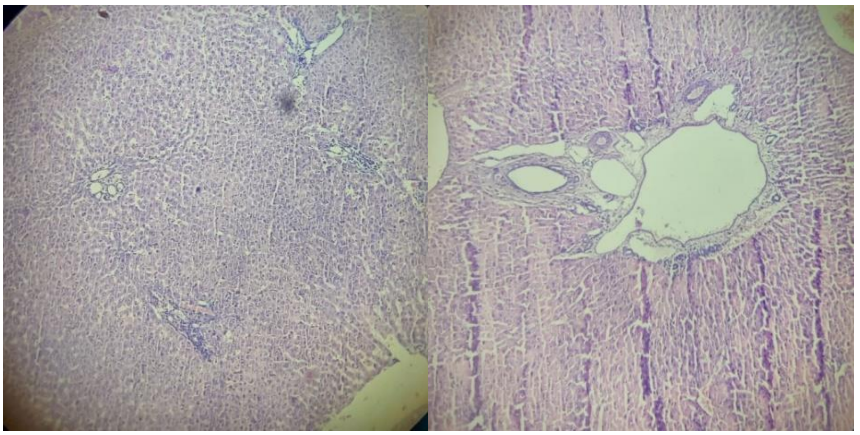
(IU/L)	63.0 ± 2.6	96.53 ± 1.3	69.19 ±2.68	78.8 ± 2.06	69.23 ± 4.25
ALP (IU/L)	59.44±3.45	92.80 ± 3.47	63.93 ± 3.64	68.47 ± 0.18	64.68 ± 5.98
Total bilirubin (mg/dl)	0.118±0.01	0.380 ± 0.01	0.121 ± 0.06	0.149 ± 0.03	0.254 ± 0.09

The serum liver function markers like SGOT, SGPT, ALP and Total bilirubin were significantly elevated in Paracetamol induced hepatotoxic rates shown in (Table no. 6).Administration of decoction reduces the toxic effect of Paracetamolto normal level. The potency with which the high dose (400mg/kg b.w) of decoction acted upon markers was more or less equivalent to silymarin (100mg/kg b.w).

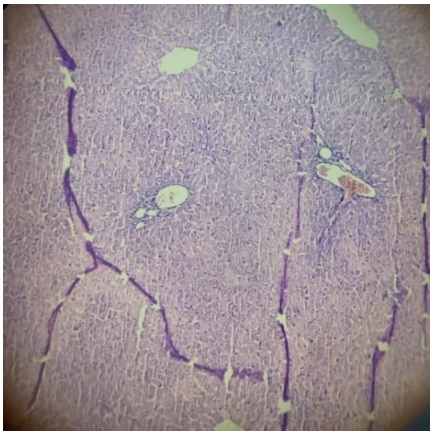
Histopathology



Group I Group II



Group III Group IV



Group V

Figure No.7.7Micrograph showing effect of paracetamol and silymarin on hepatocytes

A. Saline + 5 mg/kg gum acaciatreated group show parenchyma with lobular architecture containing hepatocytes arranged in cords. Individual cells are polygonal with abundant eosinophilic cytoplasm and centrally located pale nucleus. Kupffer cells are located peripherally. The sinusoids appear to be dilated and congested at places on histopathology.
B. Saline + Paracetamol 750 mg/kg treated group show mildly deranged parenchymal architecture. Few hepatocytes appear enlarged. There are small collections of lymphocytes at places especially in the perisinusoidal areas. The sinusoids appear dilated. Kupffer cells are seen.
C. Silymarin 100 mg/kg + Paracetamol 750 mg/kg treated group show parenchyma with loss of lobular architecture at few places. Hepatocytes appear enlarged. There is mild lymphocytic infiltration and more at perisinusoidal area. The sinusoids are dilated and congested. There is periportal fibrosis. Parenchyma shows haemorrhagic areas. Kupffer cells are also seen on histopathology.
D. Quercetin 200 mg/kg + Paracetamol 750 mg/kg treated group show parenchyma with mildly deranged lobular architecture. Few hepatocytes are enlarged and show pkynotic nuclei. There is mild lymphocytic infiltration. The sinusoids appear dilated and there is mild to moderate periportal fibrosis. Kupffer cells are seen on histopathology.
E. Quercetin 400 mg/kg + Paracetamol 750 mg/kg treated group show parenchyma arranged in mostly lobular architecture except at few places where the hepatocytes are enlarged and show pkynotic nucleus. There are also few necrotic hepatocytes. There is mild to moderate lymphocytic infiltration in the parenchyma, at places making small aggregates. There is moderate amount of periportal fibrosis and moderate perisinusoidal lymphocytic infiltration. The sinusoids appear dilated and congested on histopathology.

Summary and Conclusion

Summary

- The present work aimed to study the Hepatoprotective activity of quercetin from *Moringa oleifera* against drug induced hepatotoxicity**.**
- Successive extractions were carried out using different solvent such as petroleum ether, chloroform, ethanol, methanol, hydroalcohol and water using soxhlet apparatus.
- Preliminary phytochemical screening showed the presence of alkaloids, glycosides, flavonoids and phenols.
- Chromatographic fingerprinting showed the presence of flavonoids.
- The total polyphenolic content in ethanol extract was found to be 8.56±0.10 and 9.28±0.30 GA/gm.
- The total flavonoid content ethanol extract was found to be 11.65±0.13 and 12.8±0.90 QA/gm.
- The results suggested quercetin of ethanolic extract of *Moringa oleifera* plant possess hepatoprotective activity against Paracetamol induced liver toxicity of albino wistarrats liver slices.
- The significant decrease in the SGOT, SGPT, ALP and Total Bilirubin level in animal by giving quercetin of ethanolic extract of *Moringa oleifera* plant.

Conclusion

The present investigation revealed that the extract containing quercetin of *Moringa oleifera* showed excellent hepatoprotective activity against Paracetamol induced hepatotoxicity. This was proved by various parameters such as serum biochemical estimation and histopathology. Hence we achieved the aim and objective of the present research work.

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