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RESEARCH ARTICLE

Hepatoprotective activity of methanolic extract of *Canthium dicoccum* in paracetamol-induced hepatotoxicity in Wistar rats

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ABSTRACT

Hepatotoxicity is the liver injury or liver damage caused by exposure to drugs; it is an adverse drug reaction that may be uncommon but serious.

Keywords: Methanolic, Canthium dicoccum, Wistar rats

INTRODUCTION

Plant kingdom is a virtual goldmine of potential dug targets and other active molecule waiting to be discovered. During the past decade, use of traditional medicine has expanded globally and gained popularity. Plants are used as medicine since time immemorial. Plant-based drugs are having a revived interest nowadays because of the deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in pharmaceutical industry as a drug or as a drug carrier or bioenhancers or excipients.

It has been estimated that only 10–15% of around 750,000 existing species of higher plans have been surveyed for biologically active compounds. Modern herbal research is focused mainly on an activity guided isolation or bioassay of the phytoconstituents from crude drugs.

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effects.

Hepatotoxicity

Hepatotoxicity is the liver injury or liver damage caused by exposure to drugs; it is an adverse drug reaction that may be uncommon but serious. Hepatotoxicity mainly occurs due to the alcohol consumption, age, and also due to certain chemical agents. More than 900 drugs, toxins, and herbs have been reported to cause liver injury.

In modern medicine, corticosteroids and immunosuppressants are commonly used to treat liver disease in allopathic form of medicine. However, these drugs are associated with adverse effects such as immunosuppressant and bone marrow depression. In view of severe undesirable side effects of synthetic agents and absence of reliable liver protective drugs in the modern medicine, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the use of traditional herbal medicines which are claimed to possess hepatoprotective activity. About 70–80% of the world populations rely on the use of traditional medicine, which is predominantly based on plant materials. The traditional medicine refers to a broad range of natural health-care practices including Ayurveda, Siddha, Homeopathy, and Unani.

In spite of phenomenal growth of modern medicine, there are few synthetic drugs available for the treatment of hepatic disorders. However, there are several herbs claimed to have possessed beneficial activity in treating hepatic disorders but they need to be validated in the light of science to ensure their ability to conserve therapeutic effectiveness in the formulation form.

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A few reports on the hepatoprotective activity are cited here, for example, *Apium graveolens* Linn. (*Umbelliferae*), *Boerhavia diffusa* Linn. (*Nyctaginaceae*), *Euphorbia antisyphilitica* (*Euphorbiaceae*), *Rubia cordifolia* (*Rubiaceae*), *Solanum lyratum* (*Solanaceae*), and *Tylophora indica* (*Asclepiadaceae*). Hepatoprotective activity of the medicinal plants data is shown in Table 1.

Plant profile

Canthium dicoccum, the Ceylon boxwood also known as "Nalla balusu" in Telugu, belongs to the family *Rubiaceae*. In India, its bark is used for fever, and decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain.

Taxonomy of plant

Botanical name

- *C. dicoccum* (Gaertn.) Merr.,
- *Psydrax dicoccos* is a species of flowering plant in the family *Rubiaceae.*

Family: *Rubiaceae*

***Canthium dicoccum* plant**

Synonyms

- *C. didymum* Gaertn.f.
- *Plectronia dicocca* (Gaertn.) Merr.
- *Plectronia didyma* Elm.
- *Psydrax dicoccos* Gaertn.

Common name

- Bogas (P. Bis.)
- Luing-luing (P. Bis.)
- Malakafe (P. Bis.)
- Tandan (Mag.)
- Ceylon boxwood (Engl.)

Classification

- Kingdom: Plantae
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Asterids
- Order: Gentianales
- Family: *Rubiaceae*
- Sub family: *Ixoroideae*
- Tribe: *Vanguerieae*
- Genus: *Canthium* Lam.

Vernacular names

- Tamil: Nallamandaaram
- Telugu: Nalla balusu
- Malayalam: Nanyul
- English: Ceylon Boxwood
- Kannada: Edrani
- Distribution: Distributed in secondary and primary forests at low altitudes in Benguet, Pangasinan, Zambales, Bataan, Rizal. and Batangas Provinces in Luzon; and in Mindoro, Ticao, Palawan, Negro, Guimaras, and

Table 1: Plant explorations

Name of the plant	Family	Plant part used	Hepatotoxicity inducing agent	Extracts studied
Orthosiphon stamineus	*Lamiaceae*	Leaves	Acetaminophen	Methanolic extract
Baliospermum montanum	*Euphorbiaceae*	Root	Paracetamol	Chloroform and alcohol extract
Tridax procumbens	*Asteraceae*	Leaves	Carbon tetrachloride	Ethanolic extract
Glycyrrhiza glabra Linn.	*Fabaceae*	Root powder	Carbon tetrachloride	Root powder mixed with animal feed
Saururus chinensis	*Saururaceae*	Whole plant	Carbon tetrachloride	Ethanol
Cochlospermum planchonii	*Cochlospermaceae*	Rhizomes	Carbon tetrachloride	Aqueous
Phyllanthus niruri	*Euphorbiaceae*	Leave and fruits	Carbon tetrachloride	Ethanolic and aqueous

Mindanao and also from Southeast China to tropical Asia availability**:** Wild-crafted.

Properties

- Febrifuge, anti-diarrheal.

Parts used

- Bark, roots, leaves

Habit and Habitant

Malakafe is an unarmed, smooth shrub 3–4 m or more in height.

Leaves

They are extremely variable, ovate, elliptic, ovate or somewhat rounded, 5–15 cm long, 1.5–10 cm wide, leathery, shining above, and usually pointed at both ends.

Flowers

They are white, with very slender stalks, 5–10 ml long and borne in compressed, short-stalked cymes. Calyx is cutoff at the end or obscurely toothed. Corolla is bell shaped, with a 4–6 ml tube, and five somewhat pointed lobes.

Fruit

Fruit is rounded, ellipsoid or obovoid, 6–10 ml long, slightly flattened, and obscurely 2-lobed.

Traditional uses

- In India, bark is used for fever
- Decoction of roots used for diarrhea
- Antifungal: Diglycosides, rutin, and its benzoic derivative, 7-O-(5-O-benzoyl-ß-D- glucopyranosyl)-rutin from *C. dicoccum* and kaempferol3-ß-D-rutinoside from *C. rheedii* strongly inhibited all test fungi.

Anti-Inflammatory: Study evaluated an methanolic extract of whole plant of *C. diococcum* for anti-inflammatory activity in Wistar albino rats in various models of anti- inflammatory activity, namely, Carrageenan-induced paw edema, formalin-induced paw edema, fresh egg white-induced paw edema, and cotton pellet-induced granuloma model. Results showed the extract with anti-inflammatory activity and suggest a potential alternative to nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac

- Anti-Diabetic/Nephroprotective: Study evaluated a methanolic extract of *C. diococcum* for antidiabetic in an alloxan-induced diabetic rat model. Results showed a significant drop in fasting blood sugar in a dose-dependent manner, with an effect on the beta-cell population in the pancreas. The extract showed almost equipotent antidiabetic activity compared to standard drug glibenclamide.

MATERIALS AND METHODS

Experimental animals

- White Wistar rats are used
- Either of the sex is used for the study.

White Wister rat

Drugs and chemicals

- Silymarin
- Methanol
- Water
- Paracetamol
- Formalin.

Different types of *in vivo* methods for inducing hepatotoxicity

1. Chemical induced hepatotoxicity
   a. CCL4 induced
   b. Thioacetamide induced.
2. Dimethyl or diethyl nitrosamine-induced drug-

induced hepatotoxicity
   a. NSAIDs induced
   b. Anticancer induced
   c. Antibiotic induced
   d. Anti-TB drugs induced.
3. Non-invasive:
   a. Radiation induced
   b. Metal induced – mercury induced
   c. Diet induced-alcohol induced and high-fat diet induced.

Collection of plant material

The leaves of *C. dicoccum* were collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for further reference.

Preparation of plant extract

The collected plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 100 g of powdered materials were extracted with methanol (70%) using maceration process.

Qualitative phytochemical screening

The phytochemical investigation of extract of methanol exhibited the availability of chemical constituents such as flavonoids, alkaloid, carbohydrates, and phenolic compounds. The following tests were carried out on the standardized herbal extract to detect various phytoconstituents present in them.

Detection of carbohydrates

Small quantity of the extract was dissolved in distilled water and filtered.

The filtrate was subjected to
   a. Molisch's test
   b. Fehling's test
   c. Barfoed's test.

Molisch's test

To the filtrate, few drops of alcoholic α-naphthol were added and 2 ml of conc. sulfuric acid was added slowly through the slides of the test tube. No purple-colored ring was formed at junction of the two layers, which indicates absence of carbohydrates.

Fehling's test

Small portion of the extract was treated with Fehling's solution I and II and then heated on water bath. No brick red-colored precipitate was formed, which indicates absence of carbohydrates.

Barfoed's test

Small portion of the extract was treated with Barfoed's reagent. No red precipitate formed, which indicates absence of carbohydrates.

Test of starch

A small amount of powdered drug was treated with diluted iodine solution. No blue color was observed, which indicates absence of starch.

Detection of proteins and amino acids

Small quantity of extract was dissolved in few ml of water and was subjected to million's, Biuret and Ninhydrin test.

Million's test

The extract was treated with million's reagent. No white precipitate was produced, shows the absence of proteins and free amino acids.

Biuret test

To the extract, equal volume of 5%w/v NaOH and four drops of 1%w/v CuSO4 solution were added. No pink or purple color was formed indicating the absence of proteins.

Ninhydrin test

The extract was treated with Ninhydrin reagent. No purple color was produced, indicating the absence of proteins.

Detection of phenolic compounds and tannins

The decoction was diluted with distilled water and filtered. The filtrates were treated with following reagent.

Ferric chloride test

The filtrate was treated with 5% of ferric chloride solution. Black precipitate was found in the decoction of the plant, indicating the presence of tannins and phenolic compounds.

Test with lead acetate solution

Few ml of filtrate were treated with lead acetate solution. White precipitate was produced in the decoction of plant.

Gelatin test

To the filtrate of decoction, add 1 ml of 1% solution of gelatin. White precipitate was seen, which indicates presence of tannin in plant.

Test for phytosterols

Small quantity of decoction was dissolved in 5 ml of chloroform separately. Then, these chloroform layer subjected to,

a. Salkowski test
b. Liebermann–Burchard's test.

Salkowski test

To 1 ml of the above prepared chloroform solutions, few drops of conc. H2SO4 was added. Red color produced in the lower layer, shows the presence of phytosterols.

Liebermann–Burchard's test

The above chloroform solution was treated with few drops of conc. H2SO4 followed by 1 ml of acetic anhydride solution. Green color was produced, shows the presence of phytosterols.

Test for fixed oils and fats

Spot test

A small quantity of extract was pressed between two filter papers. Oil stain was observed, showed presence of fixed oils.

Saponification

Few drops of 0.5 N alcoholic potassium hydroxide were added to extract along with a few drops of phenolphthalein. The mixture was heated on a water bath for about 1–2 h. Formation of soap or a partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for alkaloids

Small amount of extract was stirred with a few ml of dil. HCl and filtered. The filtrate was tested with various alkaloidal reagents such as Mayer's, Dragendorff's, Wagner's, and Hager's reagent.

Mayer's test

To the small amount of filtrate, add few drops of Mayer's reagent. No white color precipitate was formed, indicating the absence of alkaloids.

Dragendorff's test (Potassium bismuth iodide)

To the small amount of filtrate, add few drops of Dragendorff's reagent. No orange red color precipitate was formed, indicating the absence of alkaloids.

Wagner's test

To the small amount of filtrate, add few drops of Wagner's reagent. No brown color precipitate was formed, indicating the absence of alkaloids.

Hager's test (Picric acid)

To the small amount of filtrate, add few drops of Hager's reagent. No yellow crystalline precipitate was formed, indicating the absence of alkaloids.

Test for glycosides

A small amount of the extract was hydrolyzed with hydrochloric acid for 1 h on a water bath and hydrolysate was subjected to

a. Legal's test
b. Balget's test
c. Borntrager's test
d. Modified Borntrager's test.

Legal's test

To the hydrolysate 1 ml pyridine, few drops of sodium nitroprusside solution were added and then made alkaline with NaOH solution. Pink color was obtained showing the presence of glycosides.

Balget's test

To a solution of extract, sodium picrate solution was added. Yellowish-orange color was obtained showing, the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. Pink color was observed in ammoniacal layer, confirms the presence of glycosides.

Modified Borntrager's test

The extracts were boiled with few ml of dil. HCl and 5 ml of ferric chloride solution. The contents are cooled and shaken with organic solvent. Organic layer was separated and to this equal volume of ammoniacal solution was added. The ammoniacal layer showed pink color. In this test, addition of ferric chloride was added to break the C–C linking of glycosides which is a stronger than C = O linkage.

Test for flavonoids

The extract was dissolved in ethanol and then subjected to the following tests.

Ferric chloride test

To a small quantity of ethanolic solution of extract, few drops of neutral ferric chloride were added. Blackish-red color was observed, showing the presence of flavonoids.

Shinoda's test

To the alcoholic solution, a small piece of magnesium ribbon was added along with Conc. HCl. Magenta color was formed, showing the presence of flavonoids.

Fluorescence test

Alcoholic solution was seen under ultraviolet light. Green color fluorescence was observed, indicating the presence of flavonoids.

Reaction with alkali and acid

With sodium hydroxide solution, the extracts gave yellow color. Extract gave orange color with conc. H2SO4 indicating the presence of flavonoids.

Zinc, HCl reduction test

To a small quantity of extract, a pinch of zinc dust was added. Then, add few drops of conc. HCl. Magenta color was produced, the presence of flavonoids.

Lead acetate solution

To a small quantity of extract, a few drops of 10% lead acetate solution was added. Yellow precipitate was produced, shows presence of flavonoids.

Detection of saponins

The extracts were diluted, with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 min. A 1 cm layer of foam was formed, indicating the presence of saponins.

Detection of coumarins

To a small quantity of extract were dissolved in alcohol and exposed to UV light, shows green fluorescence. To small quantity of extract were dissolved in alcohol and add ferric chloride solution, shows green color, indicating the presence of coumarins.

Constituents

Following are the constituents of the methanol extract of *C. dicoccum*. Major chemical components are

- Ursolic acid
- Spathulenol (20.76%)
- Caryophyllene oxide (19.25%)
- Cedren-13-ol (19.2%)
- Ledene oxide (5.24%)
- M-mentho-4, 8-diene (6.41%) and

- 2-furancarboxaldehyde (4.51%)
- Sitosterol
- Quinovaic acid
- Acetyl quinovaic acid and
- Scopoletin
- Diglycosides
- Flavonol glycoside: Study yielded a new flavonol glycoside, 7-O-(5-O-benzoyl-ß-D- glucopyranosyl)-rutin.

Acute toxicity study

Acute oral toxicity study

The acute oral toxicity procedure was followed according to Organization for Economic Cooperation and Development (OECD) 423 guidelines. The acute toxic class method is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average, 2–4 steps may be necessary to allow the judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data-based scientific conclusion.

The method uses defined doses (5, 50, 300, and 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals which cause the acute toxicity.

Procedure

Male Wistar rats (150–200 g) were used for the study. The starting dose level of MECD was 2000 mg/kg/body weight p.o. as most of the crude extracts possess LD50 value more than 2000 mg/kg/body weight p.o. Hence, the starting dose used was 2000 mg/kg/body weight p.o. Dose volume administered was 1 ml/100 g body weight to the animal, which was fasted overnight with water *ad libitum.* Food was withheld for a further 3–4 h after administration (p.o.) of drugs and observed for the signs of toxicity.

Body weight of the rat before and after termination was noted and any changes in skin, fur, eyes, and mucous membrane and respiratory, circulatory, autonomic, and central nervous system and locomotor activity and diarrhea pattern were observed, and sign of tremors, convulsions, salivations, diarrhea, lethargy, sleep, and coma were noted. The onset of toxicity and signs of toxicity were also noted.

In vivo study of hepatoprotective activity paracetamol-induced hepatotoxicity

The healthy Wistar rats (either sex) weighing between 150 and 200 g were taken. They were fed with standard diet and allowed to access to water *ad libitum*. They were allowed to acclimate for 12 h light/dark cycle before use. Hepatotoxicity was produced by administration of PCM with different dose (2 g/kg, p.o. for 7 days).

Experimental design

The healthy Wistar rats weighing between 150 and 200 g were taken and were divided into five groups having six animals in each (normal control, toxic control, standard, test low dose, and test high dose). The animals were housed in clean polypropylene cages (38 × 23 × 1) with paddy husk bedding and wide square mesh at the bottom to avoid coprophagy having six rats per cage in a well-ventilated animal house and maintained under the standard laboratory conditions of temperature (25 ± 2°C), relative humidity (55 ± 5%), and standard photoperiod of approximately 12 h of light alternating with approximately 12 h of darkness. The animals are fed with balanced rodent pellet diet and potable water. The experimental procedures described were carried out in accordance with the guidelines mentioned in the CPCSEA and IAEC.

Normal control group received saline 1 ml/kg for 1 week, toxic control group received saline 1 ml/kg for 1 week, standard group received silymarin (100 mg/kg, p.o.) once a day for 1 week, and test groups received 200 mg/kg and 400 mg/kg oral dose of methanolic extract, once a day for 1 week. On the 5th day, after the administration of respective treatments, all

the animals of Groups II, III, IV, and V were administered PCM at a dose of 2 g/kg (p.o.). On the 7th day that is, after 48 h of pharmacological treatments, blood was withdrawn by retro-orbital puncture for the estimation of biochemical parameters. After that, animals were sacrificed under ether anesthesia. The liver was collected, washed, and used for histopathological studies.

At the end of experimental period, all the animals were sacrificed under formalin anesthesia. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from 10% formalin anaesthetized rats into heparinized bottles for hematological studies and clean non-heparinized bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Determination of serum biochemical parameters

- Serum bilirubin
- Serum gluconate-oxaloacetate transaminase (SGOT)
- Serum glutamate-pyruvate transaminase (SGPT)
- Serum alkaline phosphatase (ALP) was estimated by standard procedures.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The significance of differences among the groups was assessed using one-way and multiple way analyses of variance (ANOVA). The test followed by Duncan's multiple range test. $P < 05$ was considered as statistical significance.

Anti-arthritic

Study evaluated an ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against egg-albumin-induced arthritis model.

Bioactive components

Study of an ethanolic extract of leaf yielded major chemical constituents, namely, spathulenol (20.76%), caryophyllene oxide (19.25%), cedren-13-ol (10.62%), ledene oxide (5.24%), m-mentho-4, 8-diene (6.41%), and 2-furancarboxaldehyde (4.51%). Some on the constituents provide scientific bases and evidence for antimicrobial, anti-tumor, immunomodulatory, and antioxidant properties of the plant.

RESULTS

Phytoconstituents methanolic extract of *Canthium dicoccum* leaves

Phytochemical constituents	Methanolic extract
Alkaloids	+++
Glycosides	++
Carbohydrates	+
Fixed oils and fats	–
Saponins	+++
Flavonoids and flavones	+++
Steroids	+++
Proteins and amino acids	–
Phytosterols	++
Phenols	–
Triterpenoids	–

The study confirmed the availability of necessity phytochemicals in methanolic extracts. Thus, for the supplementary investigations, methanolic extract of *C. dicoccum* has been preferred.[1-5]

Toxicity study

In the existing examination, the methanolic extract of *C. dicoccum* was evaluated for investigation of toxicity. For the purpose of LD_{50} dose, methanolic extract was administered up to dose 2 g/kg b.w. and extract did not foundation any sort of mortality, hence, 1/5th (400 mg) and 1/10th (200 mg) of greatest dose given were preferred for the existing investigation.

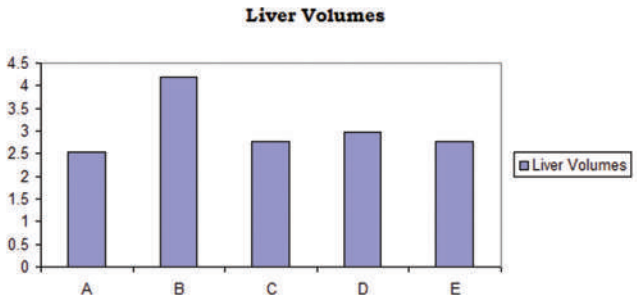
Hepatoprotective activity

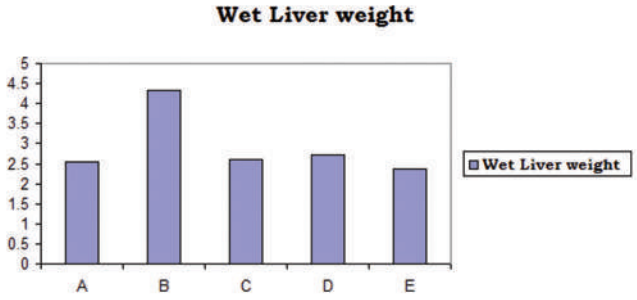
Physical parameters

Weight of wet liver and volume of wet liver

Administration of paracetamol in rat's results in liver swelling which was proved through climb in the weight (w) and volume (V) of wet liver. The administered groups by Liv 52 (std. group) and *C. dicoccum* methanolic extract demonstrate marked return of wet liver volume (V) and wet liver weight (W) similar to normal control.

The MECD at 200 mg/kg b.w and 400 mg/kg b.w displays decline of wet liver volume (V) and wet liver weight (W) markedly at $P < 0.05$. The results are shown in Table 2.

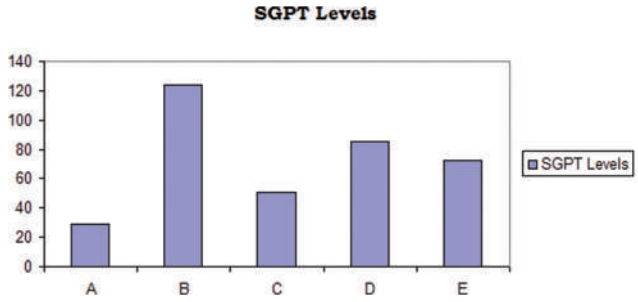




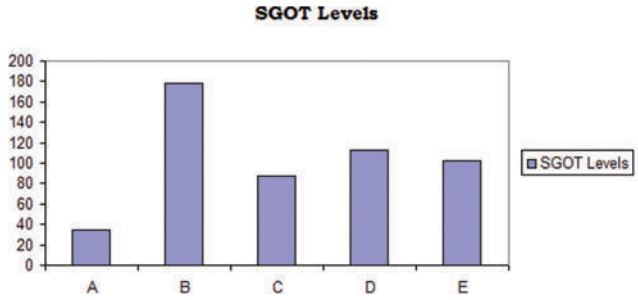
Effect of methanolic extract of *Canthium dicoccum* on wet liver weights in paracetamol-induced hepatotoxic rats.

Biochemical parameters

Therapy with paracetamol evolution into a marked hepatic hurt of liver demonstrate as increased levels of serum of hepatodefinite enzymes such as SGPT, SGOT, and ALP as compared to group of normal control. Former administration of Liv 52 and extract of methanolic had display greatest protection on paracetamol provoke toxicity to cells of liver. Test demonstrated a marked lessen in increased serum levels of enzyme with extract administer animals as compared to animals of group of toxic control which is proven in below table.[6-15]



Effect of methanolic extract of *Canthium dicoccum* on SGPT levels in paracetamol-induced hepatotoxic rats.

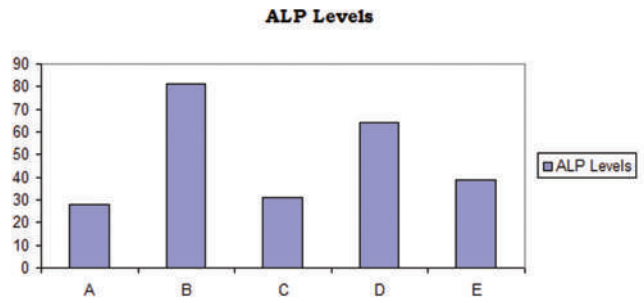


Effect of methanolic extract of *Canthium dicoccum* on SGOT levels in paracetamol-induced hepatotoxic rats.

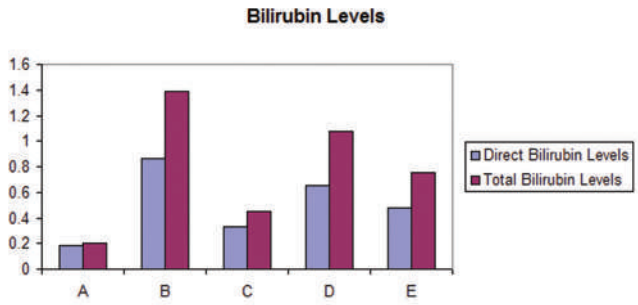
Table 2: Effect of methanolic extract of *Canthium dicoccum* on wet liver weight and wet liver volume in paracetamol-induced hepatotoxic rats.

Group	Treatment	Dose	Wet Liver weigh (g/100 g)	Liver volumes (ml/100 g)
A	Normal control	10 ml/kg p.o	2.25±0.55	2.54±0.51
B	Paracetamol control	2 g/kg, p.o. 5th day	4.37±0.078	4.25±0.06
C	Standard (Liv 52)	5 mL/kg. p.o.+2 g/kg p.o 5th day	2.28±0.13*	2.66±0.27*
D	MECD	200 mg/kg, p.o+2 g/kg	2.74±0.14*	2.86±0.046*
E	MECD	400 mg/kg, p.o+2 g/kg	2.44±0.21*	2.68±0.17*

Values are mean±SEM (n=6) one-way ANOVA. Where, *represents significant at P<0.05, **represents highly significant at P<0.01, and ***represents very significant at P<0.001. All P-values are compared with toxicant



Effect of methanolic extract of *Canthium dicoccum* on ALP levels in paracetamol-induced hepatotoxic rats.



Effect of methanolic extract of *Canthium dicoccum* on direct bilirubin and total bilirubin levels in paracetamol-induced hepatotoxic rats.

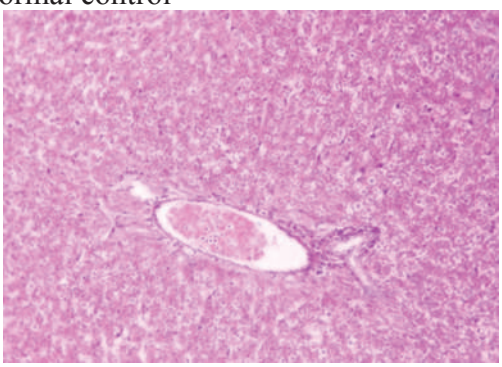
Direct bilirubin and total bilirubin

Boost in levels of total and direct bilirubin following therapy of paracetamol display its hepatotoxicity. Former administration with Liv 52, methanolic extract markedly lessen levels of direct and total bilirubin as compared to group of disease control demonstrating hepatoprotective result of methanolic extract of *C. dicoccum* it may be viewed in the below table.

Effect of methanolic extract of *C. dicoccum* on direct bilirubin and total bilirubin levels in paracetamol-induced hepatotoxic rats.

Histopathological study

1. Normal control



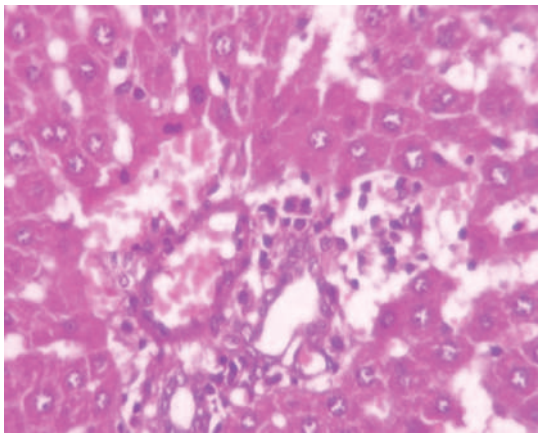
Group	Treatment	Dose	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
A	Normal Control	10 ml/kg p.o	28.96±0.80	35.10±1.45	27.94±1.15
B	Paracetamol control	2 g/kg, p.o. 5th day	124.1±1.40	178.2 ±1.42	80.95±1.42
C	Standard (Liv 52)	5 mL/kg. p.o.+2 g/k, p.o. 5th day	51.08±0.04***	87.06±0.73***	31.12±2.12***
D	MECD	200 mg/kg, p.o+2 g/kg	76.03±0.57*	103.08±0.72*	63.0±2.15*
E	MECD	400 mg/kg, p.o+2 g/kg	73.2±0.07**	104.1±0.60*	39.1±0.98***

Values are mean±SEM (n=6) one-way ANOVA. Where, *represents significant at P<0.05, **represents highly significant at P<0.01, and ***represents very significant at P<0.001. All values are compared with toxicant, SGPT: Serum glutamate-pyruvate transaminase, SGOT: Serum gluconate-oxaloacetate transaminase, ALP: Serum alkaline phosphatase

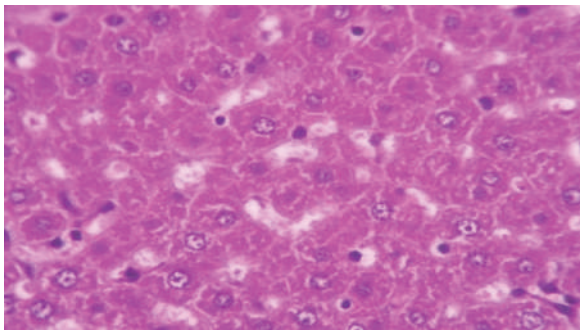
Group	Treatment	Dose	Direct bilirubin levels (mg/dl)	Total bilirubin levels (mg/dl)
A	Normal control	10 ml/kg p.o	0.19±0.0096	0.21±0.01
B	Paracetamol control	2 g/kg ,p.o. 5th day	0.88±0.029	1.41±0.09
C	Standard (Liv 52)	5 mL/kg. p.o. +2 g /kg	0.35±0.017***	0.47±0.06***
D	MECD	200 mg/kg, p.o+2 g/kg	0.57±0.04*	0.96±0.19
E	MECD	400 mg/kg, p.o+2 g/kg	0.45±0.03**	0.73±0.06**

Values are mean±SEM (n=6) one-way ANOVA, Where, * represents significant at P<0.05, ** represents highly significant at P<0.01, and *** represents very significant at P<0.001. All values are compared with toxicant

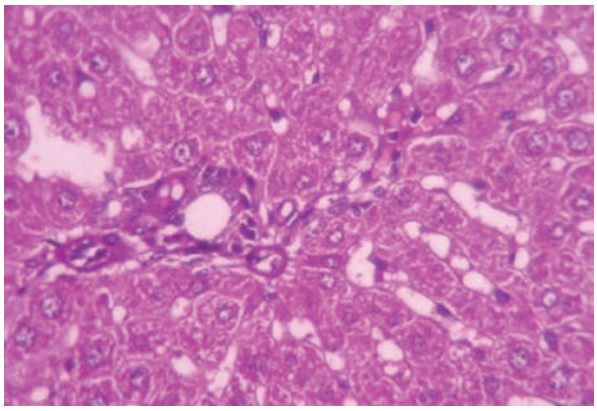
2. Toxicant paracetamol



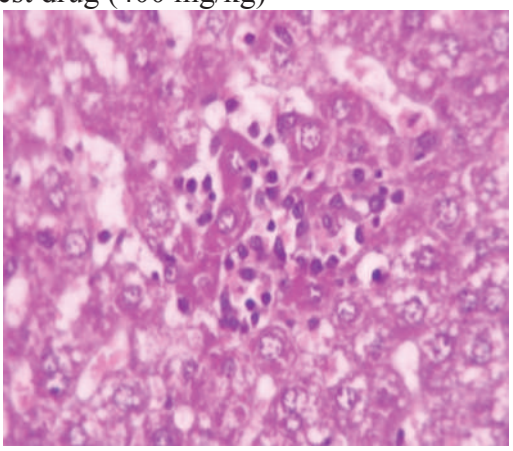
3. Standard (silymarin 200 mg/kg)



4. Test drug (200 mg/kg)



5. Test drug (400 mg/kg)



DISCUSSION

Liver engage in a diverse metabolic role might by means of asset of accessibility of plentiful enzymes and that's why might face to diverse toxicants too, chemicals and pills might hurt it. In our hepatoprotective exploration, paracetamol was exploited as hepatotoxicants to direct injury of liver, since it is applied by means of human beings for either non-medical or medical wants.[16-20]

Paracetamol-induced hepatotoxicity

Liver hurt due to paracetamol in rats was first acknowledged in 2006 and has been roughly and fruitfully exploited by means of various investigators.

Physical parameters

Wet liver weight and wet liver volume

In case of liver toxicity, weight of Wt liver and vol of wet liver are lifted up. In this occasion, water is mount up in the cytoplasm of hepatocytes ensuing to liver cells bulge, guide to lifted total liver vol and mass. It is established that mass and vol of liver are critical parameters in ascertain the hepatoprotective activity of the medicaments. For this reason in this exploration therapy with methanolic extract of the aerial parts of C. *dicoccum* noticeably decline the wt of wet liver and vol of wet liver of rat animals and thus it display noticeably ($P < 0.05$) hepatoprotective effect.

Biochemical parameters

Estimation of serum marker enzymes

Hepatotoxin changes into radicals in hepatic cells by means of enzymes action and these hurt the fatty acids of unsaturated in membranes in accessibility of oxygen to make peroxides of lipid as a result. The functional component of mitochondria of hepatic cells is distorted, advancement to hurt of liver.

SGPT serum amounts may perhaps augment because of injury of the tissues root hepatic necrosis acutely, such as acute cholestasis and viral hepatitis. Paracetamol provoked hurt of liver and

alcoholic cirrhosis may perhaps relation with mild-to-moderate climb of transaminases levels too.

In the present exploration, management with methanolic extract-aerial parts of *C. dicoccum* in rats noticeably ($P < 0.05$ in 200 mg/kg b.w. and $P < 0.01$ in 400 mg/kg b.w.) decline in the raised serum levels of SGPT, sign of hepatoprotective effect.[21]

Liver toxicity augmented the levels of SGOT in serum because of the injury to the tissues grounds acute necrosis, such as chronic acute cholestasis and viral hepatitis. Alcoholic liver harm and cirrhosis may perhaps relations with mild-to-moderate augment of transaminase. In the existing investigation, animals administered with methanolic extract aerial parts of *C. dicoccum* noticeably ($P < 0.05$) declined the SGOT elevated levels in serum, a sign of hepatoprotective effect.

In instance of liver toxicity, levels of alkaline phosphatase are very high might be because of defective hepatic excretion or through raised release of ALP by means of hepatic duct or parenchymal cells. In the present investigation, administration of animals with methanolic extract of aerial parts of *C. dicoccum* noticeably ($P < 0.05$) reduced the ALP levels in serum which is an indicative of hepatoprotective effect.

Few medications (such as probenecid and rifampin) affect the bilirubin net uptake through the cells of liver and might cause a mild unconjugated hyperbilirubinemia. Bilirubin level increases in disorder of hepatocytes, blockage to biliary release into duodenum, in hemolysis and alteration of hepatic uptake, and bilirubin pigment conjugation like in Gilbert's disease. In the present investigation, administration of animals with methanolic extract of aerial parts of *C. dicoccum* noticeably ($P < 0.05$) reduces the levels of bilirubin (total and direct) in serum which is an indicative of hepatoprotective effect.[22-25]

SUMMARY AND CONCLUSION

The existing research was undertaken to assess the hepatoprotective produce of methanolic extract of *C. dicoccum.* LD_{50} analysis was done in albino Wistar rats with methanolic extract of aerial fractions of *C. dicoccum* by means of following OECD guideline No.423 and was revealed as safer up to the dosage amount of 2 g/kg establishing its non-toxic nature. The hepatoprotective effect was explored in paracetamol stimulated hepatotoxic animal model. The physical parameters such as

1. Serum SGOT
2. ALP
3. SGPT
4. Total and
5. Direct bilirubin were evaluated.

Paracetamol provoked hepatotoxicity was noticeably prevented by means of earlier management with methanolic extract of *C. dicoccum*.

1. Drop in wt of wet liver and
2. Volumes of wet liver, decreased in lifted levels of biochemical parameter such as
3. Serum SGOT
4. ALP
5. SGPT
6. Total and
7. Direct bilirubin after management with *C. dicoccum* methanolic extract demonstrated the hepatoprotective effect of extract in existing investigation.

According to enhancement in amount of markers of serum enzyme, factors of physical, and functional, it was established that *C. dicoccum m*ethanolic extract displayed noticeable hepatoprotective effect in the doses employed.

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