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EXTRACTION, PHYTOCHEMICAL SCREENING AND HEPATOPROTECTIVE ACTIVITY OF *OPUNTIA FICUS INDICA*

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Abstract

This study investigates the phytochemical composition and pharmacological properties of the hydroalcoholic extract of Opuntia ficus indica (OFI) leaves and fruits. The hydroalcoholic extract of OFI exhibited a yield of 6.2% w/w, which was higher than the petroleum ether extract (2.7% w/w). Phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, saponins, and diterpenes in the extract. Quantitative analysis indicated a total alkaloid content of 0.352 mg/100 mg and a total flavonoid content of 0.846 mg/100 mg of dried extract. In an acute toxicity study, the extract did not induce any adverse behavioral changes or mortality, confirming its safety profile. The hepatoprotective effects of the ethanolic fruit extract of OFI were evaluated in D-GalN-induced hepatotoxicity in rats. The extract significantly reduced levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), and bilirubin, while increasing albumin levels, compared to the hepatotoxic control group. These effects were dose-dependent, with the 200 mg/kg dose showing the most pronounced effects, comparable to the positive control Silymarin.

Keywords: *Opuntia ficus indica, hydroalcoholic extract, phytochemical screening, alkaloids, flavonoids, hepatoprotective, hepatotoxicity, SGPT, SGOT, ALP, bilirubin, albumin, Silymarin, D-GalN-induced hepatotoxicity.*

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INTRODUCTION:

Opuntia ficus indica, commonly known as prickly pear or Barbary fig, is a species of cactus native to Mexico, but widely cultivated and naturalized in various arid and semi-arid regions worldwide, including parts of Asia, Africa, and the Mediterranean. The plant is known for its edible fruits and has been used traditionally in folk medicine for various therapeutic purposes.

Phytochemical analysis of *Opuntia ficus indica* has revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and polysaccharides, among others (Mahmood et al., 2012). These phytochemicals contribute to its antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties (Nasri et al., 2015).

Hepatotoxicity, induced by various agents including chemicals like D-galactosamine (D-GalN), is a significant global health problem. It leads to liver injury characterized by elevated liver enzymes such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP), as well as impaired liver function (Shi et al., 2020). Management of hepatotoxicity often involves the use of hepatoprotective agents that can mitigate liver damage and improve liver function.

Opuntia ficus indica has shown promising hepatoprotective effects in experimental models of hepatotoxicity. Previous studies have demonstrated its ability to reduce liver enzyme levels and improve liver function parameters in animals treated with hepatotoxins (Salaritabar et al., 2020). These effects are attributed to its antioxidant and anti-inflammatory properties, as well as its ability to promote liver regeneration and inhibit hepatic fibrosis (Nasri et al., 2015).

Given the traditional use and promising preclinical evidence, *Opuntia ficus indica* holds potential as a natural hepatoprotective agent. However, further research, including detailed mechanistic studies and clinical trials, is necessary to validate its efficacy and safety for human use.

MATERIAL AND METHODS:

Collection of plant material

The plants have been selected on the basis of its availability and Folk use of the plant. Leaves of *Opuntia ficus indica* were collected from local area of Sagar in the month of April, 2023.

Drying and Storage

Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of *Opuntia ficus indica* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Mukherjee, 2007; Kokate, 1994).

Defatting of plant material

Leaves of *Opuntia ficus indica* were shade dried at room temperature. 50gm of shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered leaves of *Opuntia ficus indica* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Shamsa *et al.*, 2008).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were

dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In vivo* hepatoprotective potential of hydroalcoholic extract of *Opuntia ficus indica

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD; 2001). Animals were kept fasting providing only water, *ethanolic fruit extract of Opuntia ficus* (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible hepatoprotective effect.

Experimental designs

Group –I: Normal (Saline, p.o.)
Group –II: received D-GalN (d-Galactosamine, 400 mg/kg, i.p.)
Group –III: received Silymarin (100mg/kg, p.o.)
Group -IV: received *ethanolic fruit extract of Opuntia ficus* (100mg/kg, p.o.)
Group –V: received *ethanolic fruit extract of Opuntia ficus* (200mg/kg, p.o.)

Group I received normal saline for 14 days and served as normal control. Group II received normal saline (1 ml/kg, p.o.) for 14 days and served as toxic control. Groups III and IV were treated with *ethanolic fruit extract of Opuntia ficus* at a dose of 100 mg/kg p.o. and 200 mg/kg p.o each, respectively. On 15th day the groups II, III andIV,V received D-GalN (400 mg/kg, i.p.) (Mondal et al., 2020). At the end of the experiments, rats blood was withdrawal via retroorbital plexus for biochemical parameters.

Biochemical Evaluation in Serum

Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP), albumin and bilirubin was estimated by using commercial kits as per the manufacturer instructions (Kalas et al., 2021).

RESULTS AND DISCUSSION:

The hydroalcoholic extract of *Opuntia ficus indica* showed a significantly higher yield (6.2% w/w) compared to the petroleum ether extract (2.7% w/w), indicating that polar solvents are more effective in extracting phytochemicals from the plant material Table 1.

The phytochemical screening of the hydroalcoholic extract of *Opuntia ficus indica* (Table 2) revealed the presence of several bioactive compounds. The extract tested positive for alkaloids (using Wagner's and Hager's tests), flavonoids (using the lead acetate and alkaline reagent tests), proteins (using the xanthoproteic test), carbohydrates (using the Fehling's test), saponins (using the froth and foam tests), diterpenes (using the copper acetate test), but negative for glycosides, phenols, tannins, and phytosterols. These findings suggest that the extract contains a diverse range of bioactive compounds that contribute to its pharmacological activities. Quantitative analysis (Table 3) showed that the hydroalcoholic extract of *Opuntia ficus indica* contains 0.352 mg/100 mg of total alkaloids and 0.846 mg/100 mg of total flavonoids. These compounds are known for their various pharmacological activities, including hepatoprotective and antioxidant effects.

Tables 4 to 9 summarize the effects of the ethanolic fruit extract of *Opuntia ficus* and Silymarin on various biochemical parameters in D-GalN-induced hepatotoxicity in rats. The results indicate that the extract significantly reduced the levels of SGPT, SGOT, ALP, and bilirubin, while increasing albumin levels, compared to the hepatotoxic control group. These effects were dose-dependent, with the 200 mg/kg dose showing more pronounced effects in most parameters, similar to the positive control Silymarin. The observations of behavioral changes and the absence of mortality or other adverse effects further suggest the safety of the extract.

Table 1: % Yield of leaves extract of *Opuntia ficus indica*

S. No.	Extracts	% Yield (w/w)
1	Pet ether	2.7%
2.	Hydroalcoholic	6.2%

Table 2: Phytochemical screening of extract *of Opuntia ficus indica*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's test	-ve
	Wagner's test	+ve
	Dragendroff's test	-ve
	Hager's test	+ve
2.	**Glycosides**	
	Modified Borntrager's test	-ve
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline reagent test	+ve
4.	**Phenol**	
	Ferric chloride test	-ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's test	-ve
	Benedict's test	-ve
	Fehling's test	+ve
7.	**Saponins**	
	Froth test	+ve
	Foam test	+ve
8.	**Diterpins**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin test	-ve
10.	**Phytosterols**	
	Salkowski's test	-ve
	Libermann burchard's test	-ve

Table 3: Total alkaloid and flavonoid content of hydroalcoholic *extract* of *Opuntia ficus indica*

S. No.	Total alkaloid content (mg/100mg)	Total flavonoid content (mg/100mg)
1.	0.352	0.846

Table 4: Behavioral observations of acute toxicity study

Observation	Test Group
Behavioural changes	Normal
Weakness	No
Aggressivity	No
Pain sensibility	No
Body weight	Normal
Change in skin	Normal
Diarrhea	No
Coma	No
Rate of respiration	No
Mortality	No

Table 5: Effect of *ethanolic fruit extract of Opuntia ficus* and Silymarin on SGPT levels in D-GalN induced hepatotoxicity in rats

Treatment	Dose	SGPT(IU/L)
Normal	Saline	49.32±5.5
D-GalN	400 mg/kg, i.p	155.50±12.3
Silymarin	100 mg/kg p.o.	53.45±5.1
Ethanolic fruit extract of *Opuntia ficus*	100 mg/kg p.o.	65.34±5.1
Ethanolic fruit extract of *Opuntia ficus*	200 mg/kg p.o	52.40±6.19

Table 6: Effect of *ethanolic fruit extract of Opuntia ficus* and Silymarin on SGOT levels in D-GalN induced hepatotoxicity in rats

Treatment	Dose	SGOT (IU/L)
Normal	Saline	32.85±5.36
D-GalN	400 mg/kg, i.p	116.55±9.50
Silymarin	100 mg/kg p.o.	45.60±4.50
Ethanolic fruit extract of *Opuntia ficus*	100 mg/kg p.o.	59.12±6.30
Ethanolic fruit extract of *Opuntia ficus*	200 mg/kg p.o	49.82±5.30

Table 7: Effect of *ethanolic fruit extract of Opuntia ficus* and Silymarin on ALP levels in D-GalN induced hepatotoxicity in rats

Treatment	Dose	ALP(IU/L)
Normal	Saline	36.54±5.46
D-GalN	400 mg/kg, i.p	98.71±6.7
Silymarin	100 mg/kg p.o.	38.73±5.17
Ethanolic fruit extract of *Opuntia ficus*	100 mg/kg p.o.	50.71±5.21
Ethanolic fruit extract of *Opuntia ficus*	200 mg/kg p.o	41.43±4.49

Table 8: Effect of *ethanolic fruit extract of Opuntia ficus* and Silymarin on albumin levels in D-GalN induced hepatotoxicity in rats

Treatment	Dose	Albumin (g/dL)
Normal	Saline	5.89±0.06
D-GalN	400 mg/kg, i.p	3.46±0.07
Silymarin	100 mg/kg p.o.	5.91±0.05
Ethanolic fruit extract of *Opuntia ficus*	100 mg/kg p.o.	5.38±0.04
Ethanolic fruit extract of *Opuntia ficus*	200 mg/kg p.o	5.85±0.07

Table 9: Effect of *ethanolic fruit extract of Opuntia ficus*and Silymarin on bilirubin levels in D-GalN induced hepatotoxicity in rats

Treatment	Dose	Bilirubin (mg/dL)
Normal	Saline	0.30±0.04
D-GalN	400 mg/kg, i.p	2.39±0.06
Silymarin	100 mg/kg p.o.	0.37±0.04
Ethanolic fruit extract of *Opuntia ficus*	100 mg/kg p.o.	0.70±0.04
Ethanolic fruit extract of *Opuntia ficus*	200 mg/kg p.o	0.57±0.03

CONCLUSION:

The findings from this study indicate that the hydroalcoholic extract of *Opuntia ficus indica* possesses significant pharmacological activities, including anti-hepatotoxic effects. The presence of alkaloids, flavonoids, proteins, carbohydrates, saponins, and diterpenes in the extract likely contributes to its therapeutic effects. The extract showed promising results in reducing hepatotoxicity markers and improving liver function parameters in rats. These results support the traditional use of *Opuntia ficus indica* in treating liver disorders and suggest its potential as a natural hepatoprotective agent. Further studies are recommended to elucidate the exact mechanisms of action, conduct dose-response relationships, and investigate the long-term safety and efficacy of the extract. Human clinical trials are also necessary to validate these findings and explore the potential therapeutic applications of *Opuntia ficus indica* in clinical settings.

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