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EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF PLANT EXTRACT OF *PEDALIUM MUREX* IN RATS

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ABSTRACT

The liver is a vital organ in the human body that performs a variety of functions including metabolism, immunity, digestion, detoxification, and vitamin storage, to name a few. In the United States, drug-induced liver injury accounts for almost half of all occurrences of acute liver failure. This study was based on the pharmacological screening of antioxidant and hepatoprotective potential of hydroalcoholic leaves extract (HLE) of *Pedalium murex* in Wistar albino rats & in-vitro. The leaves of *Pedalium murex* were obtained from the Unnao reason, UP. It was identified and authenticated by a botanist. The leaves were washed making dust-free and dried in shade at room temperature and extracted by soaking into hydroalcoholic solution: Ethanol + distilled water (1:1) for fifteen days with gradual stirrings. The relative humidity was maintained at 44-56% and are fed with standard rodent diet and water *ad libitum*. Rats were divided into group 1 (normal saline), group 2 (Isoniazid 50mg/kg), group 3 (Isoniazid 50mg/kg + Silymarin 100mg/kg), group 4 (Isoniazid 50mg/kg + HLE of *Pedalium murex* 200mg/kg) and group 5 (Isoniazid 50mg/kg + HLE of *Pedalium murex* 400mg/kg). Activity was evaluated using different parameters i.e., estimation of total antioxidant activity, estimation of SGOT, SGPT, TB and serum lipid levels, determination of SOD activity and assay of lipid peroxidation. In results, P. murex significantly exhibited hepatoprotective effects in all the parameters when compared with Isoniazid control rats. It was found effective at both the doses 200mg/kg and 400mg/kg when tested in albino rats. In conclusion, hepatoprotective action of P. murex might be due to its antioxidant action and healing of hepatocytes (liver cells). It also suggests to identify and isolate the significant responsible chemical constituent that acted in this context of cure.

KEYWORDS: Hepatoprotective, *Pedalium Murex*, SGOT, SGPT, lipid peroxidation.

INTRODUCTION

The liver is a vital organ in the human body that performs a variety of functions including metabolism, immunity, digestion, detoxification, and vitamin storage, to name a few (Saxena et al. 1999).

The aetiology of acute liver failure and the period since the onset of the disease will determine the initial clinical appearance. Acute liver failure can cause the following signs and symptoms (Maher & Schreibman, 2018) -

- Jaundice
- In the upper right abdomen, there is a pain.
- Swelling in the abdomen
- Nausea
- Vomiting
- Malaise
- Dehydration

In the United States, drug-induced liver injury accounts for almost half of all occurrences of acute liver failure (Ostapowicz et al. 2002). Acetaminophen is also a possible cofactor for hepatic damage in patients who are taking the medicine to relieve symptoms of hepatic sickness caused by other factors (Rezende et al. 2003). Alcoholic liver disease, chronic viral hepatitis B & C, non-alcoholic fatty liver disease (NAFLD) and hemochromatosis are the most common chronic liver illnesses in the developed world (Heidelbaugh & Bruderly, 2006).

Silybum marianum (Milk thistle), a member of Carduus marianum family, is an ancient medicinal plant which has been used for centuries for treatment of different diseases such as liver and gallbladder disorders, protecting liver against snake bite and insect stings, mushroom poisoning and alcohol abuse (Kren & Walterova, 2005).

Murex Pedalium is also known as Gokhru and Large Caltrops. This plant is a member of the sesame family, Pedaliaceae. Pedalium murex is one of 14 genera and 70

species in the Pedaliaceae family (Patel et al. 2011; Chaudhary & Kaushik, 2017). Saponins, reducing sugars, phenolic chemicals, alkaloids, triterpenoids, xanthoproteins, and flavonoids can all be found in the root. Flavonoids, alkaloids, resins, steroids, saponins, and proteins are found in the leaves (Bora et al. 2014).

This study was based on the pharmacological screening of antioxidant and hepatoprotective potential of hydroalcoholic leaves extract (HLE) of *Pedalium murex* in Wistar albino rats & in-vitro.

MATERIALS AND METHODS

Requirements

Pedalium murex hydroalcoholic leaves extract (HLE), Silymarin (API), Water bath, distilled water, Wistar albino rats (either sex), rotatory evaporator, weighing machine and ethanol.

Identification and extraction of plant

The leaves of *Pedalium murex* were obtained from the Unnao reason, UP. It was identified and authenticated by a botanist. The leaves were washed making dust-free and dried in shade at room temperature. Then, dried leaves were rendered into coarse powders and then finally into fine ones. The powder was weighed and soaked into hydroalcoholic solution: Ethanol + distilled water (1:1) for fifteen days with gradual stirrings (Khan et al. 2020).

Preparation of animals

Albino rats of either sex weighing 150–200g were obtained from the Animal House, Institute of Pharmaceutical Sciences and Research (IPSR), Unnao. The animals were maintained in proper conditions, at room temperatures of 25±1°C with 12-hour light/dark cycle. The relative humidity was maintained at 44-56% and are fed with standard rodent diet and water *ad libitum*. Animals were kept on fasting but free access to water up to 1 h before starting the study (Bhajoni et al. 2016).

Group design

All the rats are divided into 5 groups (n=6) as followings-

Group 1: rats are given only normal saline once a day for 21 days.

Group 2: rats are given Isoniazid (50mg/kg/day, p.o.) once a day for 21 days.

Group 3: rats are given Isoniazid (50mg/kg/day, p.o.) + Silymarin (100mg/kg) once a day for 21 days.

Group 4: rats are given Isoniazid (50mg/kg/day, p.o.) + HLE of *Pedalium murex* (200mg/kg/day, p.o.) once a day for 21 days.

Group 5: rats are given Isoniazid (50mg/kg/day, p.o.) + HLE of *Pedalium murex* (400mg/kg/day, p.o.) once a day for 21 days.

Methods

Estimation of total antioxidant activity

The total antioxidant activity of fractions was calculated using Prieto et al's method.[8] 0.3 mL sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, to name a few ingredients. Under a water bath, the reaction mixture was incubated for 90 minutes at 95°F. All sample combinations had their absorbance measured at 695 nm. The quantity of ascorbic acid equivalents in milligramme per gramme of extract was used to calculate total antioxidant activity.

Estimation of SGOT, SGPT, TB and serum lipid levels

After 21 days treatment, blood was taken by retro orbital puncture after the animals were anaesthetized with ether anaesthesia. The levels of SGPT, SGOT, TB, TG, and TC were determined using the manufacturer's standard protocols.

The animals were euthanized with an overdose of ether immediately after blood collection, the livers were removed, cleaned in saline, and one lobe of liver was sent for histology. The remaining liver lobes were homogenised, centrifuged, and the supernatant was collected for a study on antioxidants (Nimbakar et al. 2000).

Preparation of tissue homogenate

An overdose of anaesthetic ether was used to scare rats, and the liver was taken after decapitation. The liver was homogenised (1 gm/10 ml PBS, pH 7.8) and centrifuged at 2-8°C for 10 minutes at 15000 rpm. The supernant was used to calculate a variety of biological parameters.

Determination of SOD activity

In a cuvette, 0.5 mL carbonate buffer, 0.1 mL EDTA, and 1.0 mL epinephrine were mixed together. The optical density of produced adrenochrome was measured at 480 nm for three minutes at 30 second intervals. Standard solutions of 0.01 U/ml, 0.1 U/ml, 1 U/ml, and 10 U/ml were used to create the SOD calibration curve. The enzyme activity was measured in units per minute per milligramme of tissue (Misra & Fridovich, 1972).

Assay of lipid peroxidation

1 mL of material was combined with 0.2 mL sodium dodecyl sulphate, 1.5 mL acetic acid in hydrochloric acid, and 1.5 mL thiobarbituric acid in hydrochloric acid. The resulting mixture was heated for 1 hour in a hot water bath at 85°C. At 532 nm, the intensity of pink colour generated was measured against a blank (Ohkawa et al. 1979).

RESULTS AND DISCUSSION

1. Estimation of total antioxidant activity

In estimation of total antioxidant activity, Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) treated animals showed 81.35± 0.76*** µg/ml. Whereas, Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) and Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.) treated rats exhibited 59.43± 0.83** and 72.27± 0.86*** respectively. So, at both the doses it significantly demonstrated anti-oxidant potential

that indicates for its hepatoprotective effect when compared with positive control. Isoniazid (50mg/kg, p. o.) administered rats showed antioxidant activity as 24.19± 0.51* which was lowest.

The following table 1. depicts the anti-oxidant activity of *Pedalium murex*-

Table 1: Estimation of total antioxidant activity of *Pedalium murex.*

Treatment	Total antioxidant activity (Conc. µg/ml)
Normal saline	38.43± 0.62**
Isoniazid (50mg/kg, p. o.)	24.19± 0.51*
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	81.35± 0.76***
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	59.43± 0.83**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	72.27± 0.86***

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

2. Estimation of SGOT & SGPT

P. murex showed a marked decrease in SGOT & SGPT levels at both the doses. Isoniazid (50mg/kg) treated animals showed SGPT and SGOT as 36.27 ±1.25** and 153.15 ±0.41** respectively. Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) administered rats showed a marked decrease in SGPT and SGOT level as 29.82 ±1.15** and 112.16 ±2.31** respectively. Whereas, Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) treated rats exhibited 32.48 ±1.38*** (SGPT) and 128.68 ±4.57* (SGOT)- hepatoprotective effect when compared with control. It showed hepatoprotective potential that might be due to rebuilding of hepatocytes.

The following table 2. depicts the SGOT and SGPT level of *P. murex*-

Table 2: Estimation of SGOT & SGPT of *Pedalium murex.*

Treatment	SGPT Level (Mean+ SEM)	SGOT Level (Mean ±SEM)
Normal saline	22.35±0.31*	104.17 ±1.62***
Isoniazid (50mg/kg, p. o.)	36.27 ±1.25**	153.15 ±0.41**
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	29.82 ±1.15**	112.16 ±2.31**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	32.48 ±1.38***	128.68 ±4.57*
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	30.36 ± 1.36**	124.17 ±3.51*

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

3. Estimation of total bilirubin (TB)

Total bilirubin was estimated as 1.49± 0.81* mg/dl in Isoniazid (50mg/kg) administered rats. Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) given animals exhibited total bilirubin as 0.43± 0.56*** mg/dl and served as standard group. Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) and Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.) exhibited total bilirubin as 0.61± 0.36** mg/dl and 0.49± 0.43*** mg/dl respectively.

P. murex exhibited hepatoprotective effect at both the doses when compared with control group. This action might be due to nourishing the liver cells.

This table 3. depicts the estimation of total bilirubin (TB)-

Table 3: Estimation of total bilirubin (TB) of *Pedalium murex.*

Treatment	Total bilirubin (Conc. mg/dl)
Normal saline	0.37± 0.72**
Isoniazid (50mg/kg, p. o.)	1.49± 0.81*
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	0.43± 0.56***
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	0.61± 0.36**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	0.49± 0.43***

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

4. Estimation of serum lipid levels

In terms of serum lipid levels, *P. murex* demonstrated decreased triglycerides (mg/dl) and total cholesterol (mg/dl) when compared with control group. Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) fed rats showed triglycerides level 73.12 ±1.17** mg/dl and total cholesterol 112.16 ±2.31** mg/dl. Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) and Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.) administered rats exhibited triglyceride and total cholesterol 98.31 ±1.42** mg/dl, 128.68 ±4.57* mg/dl and 81.62 ± 1.32** mg/dl, 124.17 ±3.51* mg/dl respectively.

This action might refer to deplete the cholesterol levels by increasing the circulation and decreasing the Triglycerides and total cholesterol synthesis.

Following table depicts the serum lipid levels of P. murex-

Table 4: Estimation of serum lipid levels of *Pedalium murex*.

Treatment	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Normal saline	67.15±0.36**	48.14 ±0.62***
Isoniazid (50mg/kg, p. o.)	148.24±0.53**	63.17 ±0.47**
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	73.12 ±1.17**	112.16 ±2.31**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	98.31 ±1.42**	128.68 ±4.57*
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	81.62 ± 1.32**	124.17 ±3.51*

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

5. Determination of SOD level

In determination of SOD level, P. murex exhibited SOD level 59.27± 1.32* U/mg of protein in control group. Isoniazid (50mg/kg, p. o.)- hepatotoxicity control group exhibited SOD level 33.19± 0.42** U/mg of protein. Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) fed rats showed increased SOD level as 56.43± 0.36*** U/mg of protein. SOD level as seen as 39.51± 0.61*** U/mg of protein and 48.40± 0.26** U/mg of protein in Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) and Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.) fed rats, respectively.

The following table depicts the SOD level of *P. murex*-

Table 5: Determination of SOD level of *Pedalium murex*.

Treatment	SOD level (U/mg of protein)
Normal saline	59.27± 1.32*
Isoniazid (50mg/kg, p. o.)	33.19± 0.42**
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	56.43± 0.36***
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	39.51± 0.61***
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	48.40± 0.26**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

6. Assay of lipid peroxidation

Normal saline treated animals showed lipid peroxidation as 26.29± .38* moles of MDA/ g of liver whereas Isoniazid (50mg/kg, p. o.) fed rats showed lipid peroxidation 62.92± 0.51** moles of MDA/ g of liver. Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg) showed lipid peroxidation as 34.13± 0.26** moles of MDA/ g of liver. Lipid peroxidation was estimated as 42.36± 0.31** moles of MDA/ g of liver and 37.27± 0.20*** moles of MDA/ g of liver in Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.) and Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.) fed animals respectively.

Following table depicts the assay of lipid peroxidation of *P. murex*-

Table 6: Assay of lipid peroxidation of *Pedalium murex*.

Treatment	Lipid peroxidation (moles of MDA/ g of liver)
Normal saline	26.29± .38*
Isoniazid (50mg/kg, p. o.)	62.92± 0.51**
Isoniazid (50mg/kg, p. o.) + Silymarin (100mg/kg)	34.13± 0.26**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (200mg/kg, p. o.)	42.36± 0.31**
Isoniazid (50mg/kg, p. o.) + HLE of *Pedalium murex* (400mg/kg, p. o.)	37.27± 0.20***

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

In results, P. murex significantly exhibited hepatoprotective effects in all the parameters when compared with Isoniazid control rats. It was found effective at both the doses 200mg/kg and 400mg/kg when tested in albino rats. It decreased lipid peroxidation that indicates its antioxidant action. SOD level was also found lowered in P. murex animals that indicates for their hepatoprotective effect.

CONCLUSION

In conclusion, hepatoprotective action of P. murex might be due to its antioxidant action and healing of hepatocytes (liver cells). Mechanism of action is needed to confirm by the molecular studies that at which receptor subtypes it acts and how its binding efficiency may be increased. Thus P. murex might be useful in the cure of liver diseases or hepatotoxicity and to nourish liver in a dose dependent manner.

It also suggests to identify and isolate the significant responsible chemical constituent that acted in this context of cure. After, suitable dosage form will develop for better patient compliance and bioavailability.

SOURCE OF FUNDING

Nil.

CONFLICT OF INTEREST

None.

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