

Protective Activity of polyherbal formulation in methotrexate Induced Hepatotoxicity in Wistar Albino Rats

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Abstract

Herbal formulations means dosage form consisting of one or more herbs or processed herbs in specified quantities to provide specific nutritional value, treatment and mitigate diseases. Vitis viniferathe fruit is used as food supplement and the seeds and leaves are employed in herbal therapy. The present study was carried out to evaluate the hepatoprotective activity role of seed extracts of Petridafoetida and Vitisvenifera. Hepatoprotective activities of ethanolic and aqueous extracts of Vitisvinifera were examined against methotrexate induced liver damage in waster rats using silymarin as control. Enzyme activities of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) were analyzed. Petridafoetida and Vitis venifera seeds extract of exhibited significant ($P < 0.05$) hepatoprotective activity. Ethanolic seeds extract of Vitis vinifera and Petridafoetida exhibited moderate activity over methotrexate treated animals. Results confirm the traditional - ethno medicinal use of methotrexate as a potential source of hepatoprotective activity.

Keywords: *Hepatoprotective activity, herbal formulation, Methotrexate, SGPT and SGOT.*

Introduction

Paederia foetida Plant is extremely important in society since they are used for food, fodder, fuel, and other medicinal purposes. Herbal plants play an important role in society primary health care¹. The *Paederia foetida* is useful in relieving a number of ailments like rheumatism, paralysis, abscesses, gout, diarrhea, dysentery, infertility, colic and flatulence. Roots are decocted to discharge gas. Fruit is used to treat toothaches and whiten teeth; abdominal pain, abscesses, and arthritis are treated with a decoction of the whole plant. Traditional remedies for diarrhoea² and dysentery are utilized in several Asian countries. Used to treat diarrhea in Bangladesh. Fruit relieved with a poultice of leaves. The roots and bark are used as an emetic³ and to cure piles and liver infections. Toothache-relieving fruits Rheumatism and joint stiffness are treated with this herb in India. To ease distention and flatulence, apply a poultice of leaves to your abdomen^{4,5}. Problems, The root juice is used to treat piles, liver and spleen problems. Asthma. *Vitis vinifera* (Family: Vitaceae) the fruit is used as food supplement and the seeds and leaves are employed in herbal therapy. The most significant application of grape is in wine production followed by raisin and juice. The fruits are vitamins, tonics, anticancer⁶, and hepatoprotective⁷, promote hair growth and prevent ischemic processes⁸. The grape is one of the most delicious, refreshing and nourishing fruits. *V. vinifera* and their pharmacological effects including skin protection, antioxidant, and antibacterial, anticancer, anti-inflammatory and anti-diabetic activities are possess.

Materials and methods:

Plant material:

The Plant *Pteridum foetida* and *Vitis vinifera* were collected from Turari vicinity ITM campus, Gwalior, India and authenticated by the taxonomist at the Department of Botany, Faculty of Science, and ITM University. A voucher specimen was deposited in the herbarium of University for future reference.

Animals:

Hepatoprotective activity was carried out on Albino rats of either sex (110-145 g), CCMB, Tarnaka by the central animal house facility of CCMB, Hyderabad, and the rats were maintained in a 12 h light/dark cycle at $25 \pm 2^\circ\text{C}$. They were allowed free access to a standard pellet diet (Vijaya College of pharmacy Hyderabad, Telangana, India) and water ad libitum. The study was approved by the ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures. (CPCSEA Registration No: 1292/AC/09/CPCSEA).

Herbal formulation:

Formulations	Plants extracts (gm)	
	A	B
HF1	20	20
HF2	10	30
HF3	30	10

A-indicate *Pteridum foetida* plant Extract

B-Indicate *Vitis vinifera* plant seed Extract

Methodology:

Rats will randomly divide into five groups (6 rats per each group) as follows:

1. Group 1: Control group receive normal saline until termination of the experiment.

2. Group 2: Methotrexate (MTX) group will give a single injection of MTX (20 mg/kg,i.p.) on the sixth day.

3. Groups 3:HF1+MTX groups, receive HF1 once daily for 5

consecutive days and single injection of MTX (20 mg/kg, i.p.) on the sixth day.

4. Groups 4:HF2+MTX groups, receive HF2 once daily for 5

consecutive days and single injection of MTX (20 mg/kg, i.p.) on the sixth day.

5. Groups 5:HF3+MTX groups, receive HF3 once daily for 5

consecutive days and single injection of MTX (20 mg/kg, i.p.) on the sixth day.

Biochemical parameters:

Serum separated by centrifugation were used to determined serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and total bilirubin

Histopathological studies:

In histopathological study, on the seventh day, the animals were anesthetized by inhalation of halothane and blood samples will collect from the retro-orbital plexus and allowed to clot. The

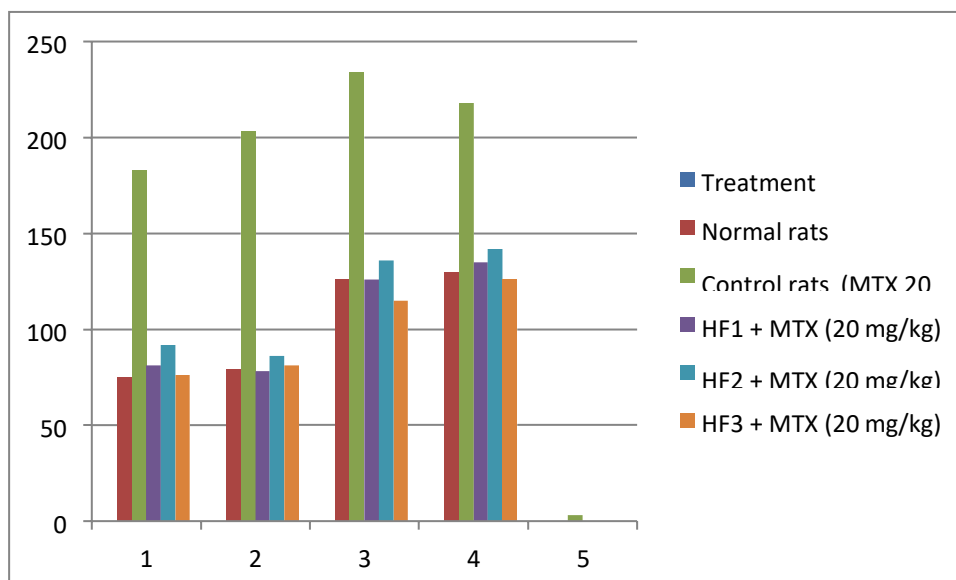
serum samples will obtain and kept at -80°C until needed.

Rats will then sacrificed by overdose of halothane anesthesia with maximal effort doneto minimize pain Liver tissues will dissect and washed with ice-cold saline. Liver homogenates will prepare by homogenization in phosphate-buffered saline (PBS, pH 7.4) then centrifuged to get the supernatants which will keep at -80°C until analyzed. An extra sample of liver will excise and fixed in 10% neutral buffered formalin solution for histopathological study.

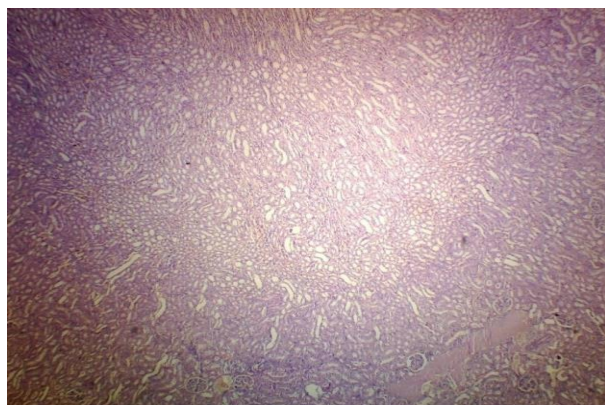
Results and discussion:

The administration of methotrexate led to significant hepatocellular damage as evident from the increase in serum levels of SGOT, SGPT, Alkaline phosphatase(73.35, 81.25, 126.56, 0.30, and 0.49). The administered formulations (HF1 and HF2, HF3 shows effective and significant activity for hepatoprotective activity as compared with standard marketed Product (Liv-52, Himalayan Pharmaceuticals). Treatment of rats with the ethanolic extract of leaves formulations (HF1 and HF2, HF3) at a dose of 20 mg/kg body weight by oral administration exhibited a significant reduction ($P<0.05$) in MTX induced elevation of SGOT, SGPT, ALKP (73.35, 81.25, 126.56, 0.30, and 0.49 units/mL, respectively) bilirubin (0.49mg/dL) and increased the level of TP and TA (0.30 - 0.49g/dL, respectively) as illustrated by below table:

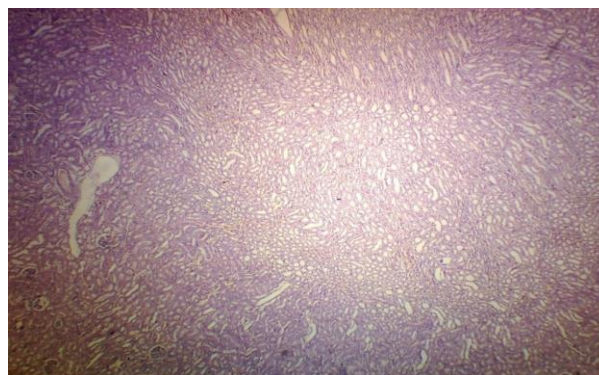
Treatment	SGOT (AST) (U/L)	SGPT (ALT) (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct (mg/dl)	Total (mg/dl)
Normal rats	75.34	79.43	126.81	130.29	0.25	0.51
	+ -	+ -	+ -	+ -	+ -	+ -
	3.47	6.28	2.72	4.83	0.43	0.14
Control rats (MTX 20 mg/kg)	183.12	203.74	234.51	218.42	1.06	3.52
	+ -	+ -	+ -	+ -	+ -	+ -
	5.71*	3.28*	6.59*	7.34*	0.12*	0.28*
HF1+ MTX(20 mg/kg)	81.42	78.63	126.82	135.55	0.32	0.54
	+ -	+ -	+ -	+ -	+ -	+ -
	3.48 ∞ □	6.51 ∞	4.97 ∞	5.21 ∞	0.36 ∞	0.42 ∞
HF2+ MTX(20 mg/kg)	92.15	86.11	136.32	142.85	0.33	0.58
	+ -	+ -	+ -	+ -	+ -	+ -
	2.56 ∞ □	4.47 ∞	3.28 ∞	2.49 ∞	0.58 ∞	0.27 ∞
HF3+	76.35	81.25	115.74	126.56	0.30	0.49
MTX(20 mg/kg)	5.73a	3.38a	5.17a	4.64a	0.25a	0.29a



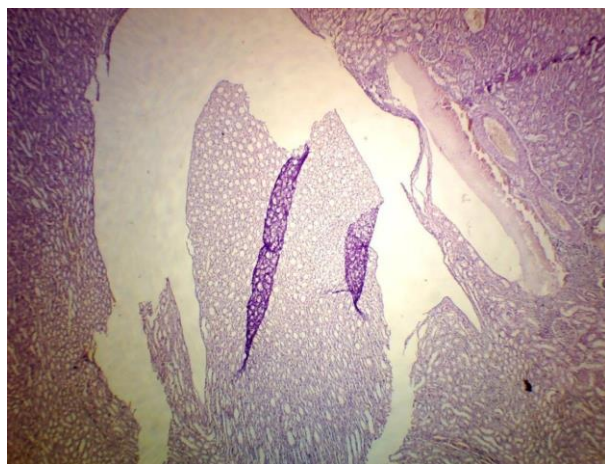
LIVER HISTOPATHOLOGICAL IMAGES:



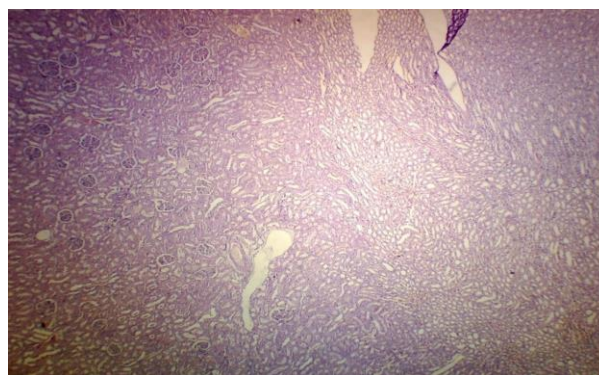
Control group



HF1 group



MTX group



HF2 group:

Conclusion

The above mentioned plant extracts of *Petridafoetida* and *Vetisvenifera* showed significant effects of hepatoprotective activity

as compare to Liv-52 consider as a standard hepatoprotective drugs. No Drug-drug interactions and adverse or untoward reactions have been observed.

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