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HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *FUMARIA PARVIFLORA* AGAINST CCL_4 AND ATT–INDUCED HEPATIC INJURIES IN RATS: A RANDOMIZED CONTROLLED PRECLINICAL TRIAL

Dr.Muthuramu.T[1], Abdurohman Mengesha Yessu[2], Dr. Mujeeb Ur Rahman[3]

[1]*Department of chemistry, Pharmacology and Toxicology, Arba Minch University, Ethiopia.*
[2]*Department of Chemistry Arba Minch University, Ethiopia*
[3]*Department of Pharmaceutical Chemistry, Alwar pharmacy college, Rajasthan.*

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ABSTRACT

Fumeria parviflora is (Fumariaceae) is an annual herb found throughout the world. Traditionally it has great significance in various disorders. Hence, the present study was intended to evaluate methanolic plant extract of Fumeria parviflora was studied by CCl4 at the dose of 1ml/kg of body weight in liquid olive oil in the ration of 1:1 and ATT (isoniazid - 7.5 mg/kg, rifampicin -10 mg/kg and pyrizinamide -35 mg/kg b.w.) induced models. Acute toxicity study was also studied to evaluate the toxicity. There are no toxicity profile was observed in rats after oral administration of the methanolic plant extract at the dose of 2g/kg body weight. The different doses of 300 mg/kg and 500 mg/kg administered with the extract of *Fumeria parviflora* there was significant ($P < 0.001$) reduction in Biochemical parameters with respect to control. *Fumaria parviflora* demonstrated significant hepatoprotective activity as shown by its ability to control the effect was more at dose of 500 mg/kg for methanol fraction Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids and saponins, and terpenoids. It can be concluded that the hepatoprotective activity elucidated by *Fumeria parviflora* could be mainly due to the presences of high value class of compound like phenolic group as the major content in the plant.

Corresponding author
Dr.Muthu Ramu.T
Department of chemistry,
Pharmacology and Toxicology,
Arba Minch University, Ethiopia
muthucology@gmail.com
+251966815965

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INTRODUCTION

Two thirds of the new chemicals identified yearly were extracted from higher plants. 75% of the world's population used plants for therapy and prevention. In the US, where chemical synthesis dominates the pharmaceutical industry, 25% of the pharmaceuticals are based on plant-derived chemicals (Al-Snafi et al., 2017) Annual incidence of Drug-induced liver injury (DILI) has been estimated between 10 and 15 per 10,000 to 100,000 persons taking prescribed medicines (Gupta et al., 2005) . Hence, natural products from medicinal plants need to be investigated by scientific methods for their hepatoprotective activity. The plant of *Fumeria parviflora* belonging to the family of Fumariaceae possess a wide variety of activities like antidiabetic, antiinflammatory, antipyretic, analgesic, prokinetic, laxative, dermatological, antimicrobial, antiparasitic, reproductive, anticholinesterase and smooth muscle relaxant effects. This review was designed to highlight the chemical constituents and the pharmacological effects of *Fumaria parviflora* (Rao et al., 1998).

METHODS

Collection, identification, and authentification of the plant

The plant of *Fumaria parviflora* was purchased from local herbal dealer in Aligarh, India. It was identified and authenticated by Prof Wajahat Ali, Department of Botany, Aligarh Muslim University and a voucher specimen was submitted (Rump 109), Aligarh, India.

Preparation of extract

The granulated, plant of *Fumaria parviflora* (200g) were packed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hrs using 500 ml of methanol (95% v/v) as a solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 45.6g, 15.20% w/w). The extract was suspended in 5% gum acacia and used for further experiments.

Preliminary phytochemical screening

The extract was screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests (Tschrich et al., 1923, Kokate CK et al.,1986).

Procurement of experimental animals

Animals were selected as per the OECD guidelines. Healthy young and nulliporous, non-pregnant Sprague Dawleys female rats weighing from 160 to 180 mg of 8-12 weeks old were selected, because literature survey of lethal dose 50% test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive, were procured from listed suppliers of Sri Venkateswara Enterprises, Bengaluru, India. The animals were fed with standard pellet diet (Hindustan Lever Ltd. Bengaluru) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 hrs of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals fasted for at least 12 hrs before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.- P.Col/02/1686//09/2016/IAEC/ JSPC) after scrutinization. The animals received the drug treatments by oral routs.

OBSERVATIONS

Animals were observed individually for 48 hrs after dosing at the first 30 minutes, periodically and during the first 24 hrs, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. Additional observations were also made if the animals continue to display signs of toxicity. Observations included were changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern. Observations were also made and checked for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Results were tabulated in Table 1.

Table 1: Acute toxicity study of methanolic extract of plant of *Fumaria parviflora* based on OECD guidelines 423.

S. No	Number of animals	Dose in mg/kg	Report
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	No death

Experimental Design (Darbar et al.,2009**)**

The rats were divided into the 7 groups of each containing six rats.
Group I: Control rats, which fed normal diet and water.
Group II: Rats treated with CCl_4 (1ml/kg) once daily for 7 days.
Group III: Rats treated with silymarin (50 mg/kg) + CCl_4 (1ml/kg) once daily for 7 days.
Group IV: Rats treated with *FP* (300 mg/kg, i.p.) + CCl_4 (1ml/kg) once daily for 7 days.
Group V: Rats treated with *FP* (500 mg/kg, i.p.) + CCl_4 (1ml/kg) once daily for 7 days.
Group IV: Rats treated with *FP* (300 mg/kg, i.p.) + ATT (1ml/kg) once daily for 35 days.
Group V: Rats treated with *FP* (500 mg/kg, i.p.) + ATT (1ml/kg) once daily for 35 days.

Statistical analysis

Values were represented as mean ± standard error of mean of three parallel data's.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of methanolic fractions of *Fumaria parviflora* shows the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin, and carbohydrate.

Table No 2: Results of gross behavioral studies in rats on administration of *Fumaria parviflora* 2000mg/kg/p.o.

Observation	Effects								
Gross activity	Upto 3hrs	3 ½hrs	4 hrs	4 ½hrs	5hrs	5 ½hrs	6hrs	12hrs	24hrs
Respiration	+	+	+	+	+	+	+	+	+
Writhing	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-	-	-	-
Sense of touch and sound	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Diarrhoea	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-

\+ Normal - No Effect

Table 3: Effect of *Fumaria parviflora* fractions on liver function test in CCl_4 induced liver toxicity.

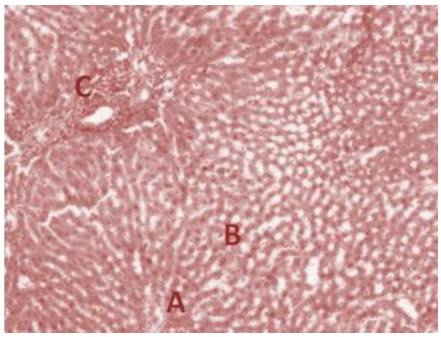
Groups	AST(IU/ml)	ALT (IU/ml)	ALP (KAU/dl)	BILIRUBIN (mg/ml)
Group I	26.8±0.8	30.3±2.8	41.7±0.7	0.57±0.13
Group II	182.6 ±0.9***	159.7±5.0***	81.7±3.3***	0.83±0.06
Group III	54.2±2.3***	56.1±1.8***	51.3±1.2**	0.65±0.02
Group IV	145.3±6.8*	139.3±10.3	69.8±6.4	0.72±0.04
Group V	111.0±12.3***	12.23±11.6**	56.0±6.4**	0.67±0.05
Group VI	134.7±15.0**	127.3±15.2	60.0±4.4*	0.70±0.05
Group VII	88.0±7.4***	91.7±6.1***	56.7±5.5**	0.67±0.05

n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to VII were compared to Group II. *p<0.01 versus CCl_4 group: Significant; **p<0.001 versus CCl_4group: Highly significant data were analyzed by one-way ANOVA followed by Dunnett's t-test.

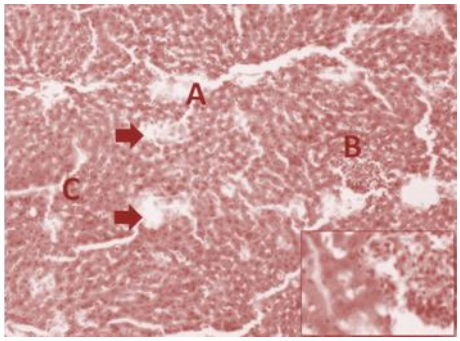
Table 4: Effect of *Fumaria parviflora* fractions on liver function test in ATT induced liver toxicity

Groups	AST(IU/ml)	ALT (IU/ml)	ALP (KAU/dl)	BILIRUBIN (mg/ml)
Group I	28.5±0.98	31.4±2.34	43.3±1.2	0.73±0.04
Group II	169.0±6.7***	170.1±5.6***	80.2±2.7***	2.47±0.49***
Group III	56.4±3.3***	58.3±2.9***	51.4±2.2***	1.37± 0.03***
Group IV	124.0±9.5**	109.7±6.7***	68.3±4.8	2.71±0.14*
Group V	106.2±12.4***	106.5±9.6***	62.5±3.6**	2.2±0.33**
Group VI	121.2± 9.6**	131.5±8.9**	56.3±2.9**	2.21±0.21**
Group VII	104.1±11.2***	98.2±10.0***	56.2±1.5***	2.0±0.21**

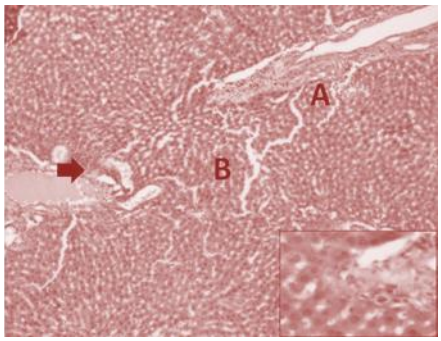
n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to VII were compared to Group II. *p<0.01 versus ATT group: Significant; **p<0.001 versus ATT group: Highly significant data were analyzed by one-way ANOVA followed by Dunnett's t-test.



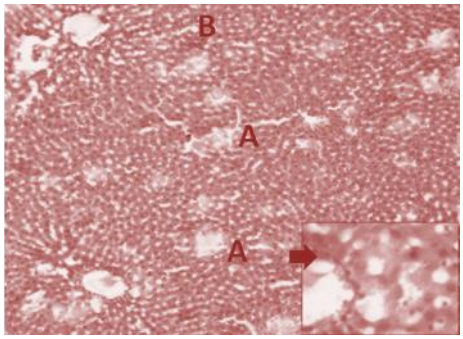
(a) Normal Control.



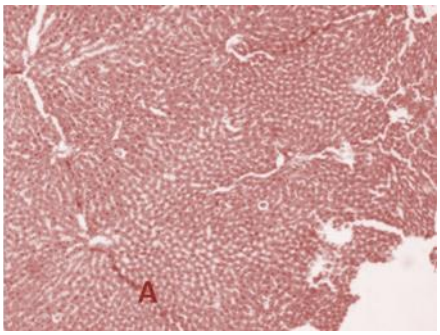
(b) Negative Control.



(c) Positive Control.

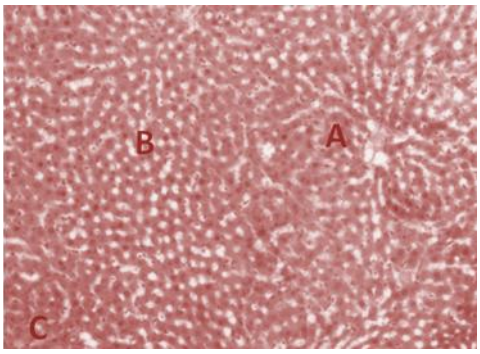


(d) Test Group ($CCl_4$300mg/kg).

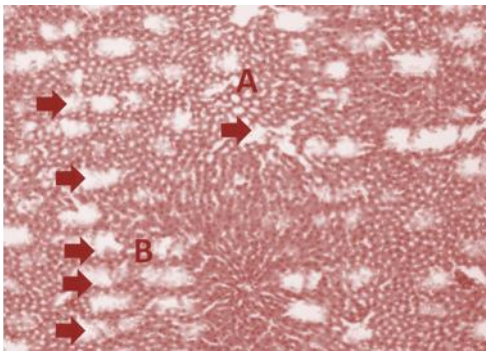


(e) Test Group ($CCl_4$500mg/kg).

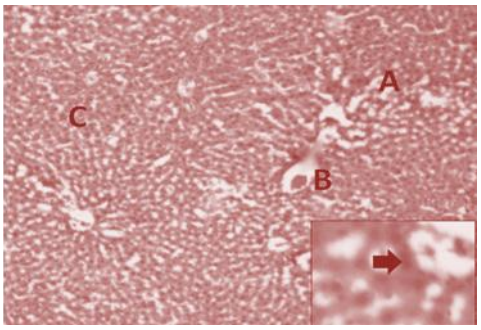
Fig. 1: Histopathological studies of liver (CCl_4 induced), (a) Group I, (b) Group II, (c) Group III,(d) Group IV, (e) Group V.



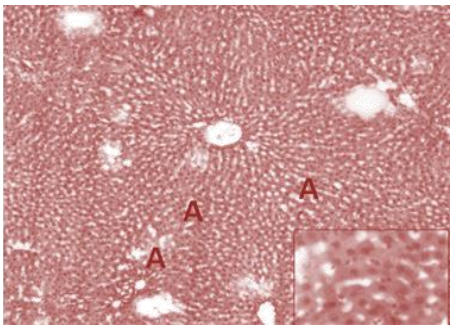
(a) Normal Control.



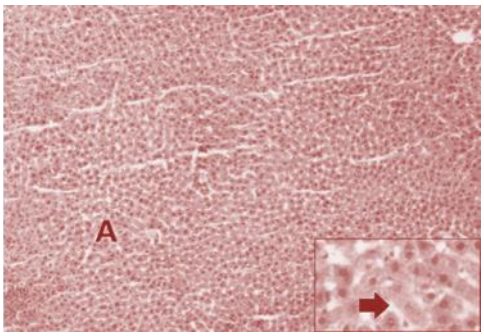
(b) Negative Control.



(c) Positive Control.



(d) Test Group (300mg/kg).



(e) Test Group (500mg/kg).

Fig. 2: Histopathological studies of liver (ATT induced), (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V.

DISCUSSION

The present study reveals the hepatoprotective activity of *Fumaria parviflora* against CCl_4 and ATT-induced hepatic damage in rats. Liver damage is assessed by measuring the levels of serum transaminases like AST, ALT and ALP, which are released into the blood from damaged liver cells. They are also the indicators of liver damage (Shah and Gupte, et al., 2004). The normalisation of the elevated markers after administration of drugs is an indicator of their efficacy for regeneration of liver cells. It has been reported that serum transaminases return to normal level with the healing of liver parenchyma and hepatocytes (Ramakrishna et al.,2011). We found that administration of CCl_4 with or without fractions did increase the weight and volume of the liver relative to the body weight of the rats as compared to the normal control group but the increase was not statistically significant. The weight and volume of the liver are increased in inflammation due to extravasations of fluid in extracellular compartment. The methanol fractions of *Fumaria parviflora* demonstrated significant hepatoprotective activity as shown by its ability to control the rise of serum transaminases. Although the effect was more at dose of 500 mg/kg for methanol fraction 12.23±11.6 IU/ml but with 300 mg/kg dose no significant hepatoprotective activity was observed for liver specific enzyme ALT. There was no significant rise in the bilirubin levels of negative control as compared to normal control group or any significant decrease in the levels of bilirubin in test groups as compared to negative control. The percentage hepatoprotection was good at 500 mg/kg b.w 32.1% for ALT. Our findings are in accordance with the observations. Similarly, the methanol and ethyl acetate fractions of *Fumaria parviflora* showed significant hepatoprotective activity as shown by its ability of control the rise of serum transaminases. Although the effect was more at doses of 500 mg/kg of methanol fraction (124.2± 9.9 IU/ml) There was no significant rise in the bilirubin levels of negative control as compared to normal control group or any significant decrease in the levels of bilirubin in test groups as compared to negative control. Methanol fraction of *Fumaria parviflora* demonstrated significant hepatoprotective activity in ATT induced liver injury, as shown by its ability of limit the rise of serum transaminases. Highly significant decrease was observed in liver specific ALT levels with the fraction at a dose of 500 mg/kg (106.2±12.4 IU/ml). There was a increase in the bilirubin levels in the negative control that was significantly prevented in all test groups as compared to negative control methanol 300 mg/kg 2.71±0.14 IU/ml and 500 mg/kg 2.2±0.33 IU/ml, *Fumaria parviflora* test groups the methanol fraction in the dose of 300 and 500 mg/kg b.w. offered a protection of 36.3% and 45.9% for ALT. (33.2% at 300 mg/kg) and (42.4 % at 500 mg/kg) dose.

CONCLUSION

From the present work, we conclude that species of *Fumaria parviflora* are highly potential in biological activity. The preliminary screening of the samples revealed the presences of high-value class of compound like the phenolic group as the major content in the plant.

ACKNOWLEDGEMENT

All Authors

ABBREVIATIONS LIST

CCl_4 : Carbon Tetrachloride
ATT : Anti tuberculosis Treatment
FP : *Fumaria parviflora*
AST : AspartateAminotransferase
ALT : Alanine Transaminase
ALP : Alkaline Phosphatase

CONFLICT OF INTEREST

NIL

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