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Determination of Hepatoprotective Activity of Methanolic Extract of *Ipomea reniformis*

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Abstract

Objectives: The main objectives of the study were to evaluate investigation of hepatoprotective activity of methanolic extract of *Ipomoea reniformis* (*MEIR*) in experimentally induced hepatotoxicity in rats.

Methods: Hepatoprotective activity of *MEIR* was studied against Paracetamol (3 g/kg p.o.) and ethanol (4 g/kg p.o.) induced hepatotoxicity in rats. Silymarin (100mg/kg p.o.) was used as a standard reference in this study. Parameters evaluated in the study of Serum biomarkers Serum glutamate oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Bilirubin (total &direct) and Total protein and Tissue antioxidant levels Glutathione, Lipid peroxidation (LPO) and histopathological changes of livers were assessed in the above mentioned models.

Results & Conclusion: When compared to PCM and ETH toxicant groups to normal group there were increased in wet liver weight and wet liver volume, (SGOT), Serum glutamate pyruvate transaminase (SGPT), Bilirubin (total & direct) and Glutathione and Total protien levels were markedly reduced. Treated groups showed significant decreased wet liver weight and wet liver volume, SGOT, SGPT, DB, TB, LPO, and increased in GSH, TP levels were markedly increased in Silymarin, *MEIR* low dose (200 mg/kg p.o.) and *MEIR* high dose (500 mg/kg p.o.). Histopathological changes Steatosis, Necrosis etc., were partly or completely prevented in animals treated with the *MEIR.*

Based on improvement in serum marker enzyme levels, physical parameters, Antioxidant parameters, and histopathological studies, it is concluded that the *MEIR* possesses hepato protective activity.

Keywords: Hepatoprotective, *Ipomoea reniformis*, Paracetamol, Ethanol.

INTRODUCTION

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply energy provision and reproduction [1]. The liver is expected to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals.

Liver diseases are largest health problem worldwide and are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Excessive production of reactive oxygen species (ROS) plays an important role in the pathogenesis and progression of various disease involving different organs such as liver [2].

Alcohol and Paracetamol are known to cause liver damage due to production of excessive reactive free radicals which alter the constitution of hepatocytes there by affect the physiological functions carried out by the liver. Ethanol gets converted into acetaldehyde by alcohol dehydrogenase which further gets metabolised in mitochondria to acetate by acetaldehyde dehydrogenase. Along with the formation of highly reactive oxygen and hydroxy ethyl radical, this covalently modifies the proteins, DNA and lipids of hepatocytes [3]. In the absence of a reliable liver protective drug in modern medicine there are a number of conventional medicinal preparations in ayurveda used for the treatment of liver disorders [4]. In the absence of a reliable liver protective drug in modern medicine there are a number of conventional medicinal preparations in ayurveda used for the treatment of liver disorders [5]. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which have stimulated intense research on their potential health benefits. Hence, searching the safe and potent remedies from the herbal origin for the treatment of hepatic disorders has become most fascinating and desired area of research for the pharmacologists. According to the literature survey some medicinal plants such as *Spirulina maxima* [6] *Eclipta alb* [7] *Boehmeria nivea* [8] *Cichorium intybus* [9] *Aegel marmelos* [10] etc, which are traditionally used in the management of liver disorders and reported to possess measurable hepatoprotective effect against various experimental animal models. However, still more numbers of medicinal. plants are needed to be screened for their hepatoprotective efficacy.

Ipomoea reniformis chois (Convolvulaceae) is a perennial, much branched herb (creeper). It is widely distributed all over India. It is also known as *Merremia emarginata*. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the e ye s a nd gu ms . The Methanolic extract of this plant has proven to have anti-inflammatory [11], antidiabetic [12], antioxidant and antiobesity [13] activities while Methanolic extract of this plant has proven to have nephroprotective activity [14].

Methanol extract of this plant might be used for anti oxidant and antiobesity activities with minimal toxicity [15]. Literature reviews indicated that there is no scientific report on liver protective property of the title plant till date. Hence, the present investigation is aimed to assess protective role of *I pomoeareniformis* whole plant extract against paracetamol and alcohol induced liver damage in rats.

MATERIALS AND METHODS

Solvents required for Methanolic Extract of *Ipomoea reniformis*

Methanol, Hexane

Apparatus and Diagnostic kits used for Dissertation work:

Cooling centrifuge, Homogenizer, Incubator.

METHODOLOGY

Methanolic extraction of *Ipomoea reniformis*

The whole plant was washed with distilled water and shade dried for two weeks. After drying, the dried plant material was powdered with mechanical grinder and passes the powder with sieve no. 22 to get uniform particle size. The powder is packed in Soxhlet apparatus for defatting with n-hexane for 3 days. The plant powder (marc) was air dried after defatting. Again it is packed in Soxhlet apparatus for methanol extraction for 18hrs until to get clear solution in syphon tube.

Pharmacological Evaluation

Preparation of dose

Weighed quantity of Methanolic extract of *Ipomoea reniformis* (MEIR) was suspended in water using 0.5% carboxy methyl cellulose and administered orally to experimental animals. Suspension of MEIR was prepared freshly. The MEIR was administered at doses of 200 mg/kg (L.D) and 400 mg/kg (H.D) for each animal as per previous study [6].The experiments were conducted 1 h after the oral administration. In Multiple dose study the animals daily received the suitable oral dose of the MEIR for a period of 14 days. The parameters were assessed on the 14th day after the administration of particular day dose.

Paracetamol induced hepatotoxicity

Experimental design

Animal: Wistar rat, Weight: 150 - 250 g

No. of animals in each group (n): 6

Treatment schedule

Thirty animals were randomly divided equally into six groups of six animals each.

Group 1: (Normal Control) received 0.5% CMC orally for 7 days

Group 2: **(**PCM-induced control) received Paracetamol (3 g/kg p.o.) suspended with 0.5%CMC orally for 7 days

Group 3: (Drug control) received MEIR (400 mg/kg p.o.) for 7 days orally

Group 4: (Low dose) received MEIR (200 mg/kg p.o.) for 7 days and paracetamol (3 g/kg p.o.) on Day 1, 4 and 7 of the treatment period.

Group 5: (High dose) received MEIR (400 mg/kg p.o.) for 7 days and paracetamol (3 g/kg p.o.) on Days 1, 4 and 7 of the treatment period.

Group 6: (Standard) received Silymarin(100 mg/kg p.o.) for 7 days and paracetamol (3 g/kg p.o.) on day 1,4 and 7 of the treatment period.

After 7 days treatment with MEIR, 8th day blood is collected from retro orbital plexus puncture and the rats were sacrified and liver was collected immediately perfused with Phosphate Buffer Solution. Serum was separated by centrifugation. The collectedSerum glutamate oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), direct bilirubin (DB), total bilirubin (TB), total proteins were assayed. In tissue homogenate lipid per-oxidation, glutathione, were assayed and then histopathological studies also carried out.

Ethanol induced hepatotoxicity

Experimental design

Animal: Wistar rat, Weight: 150 - 250 g

No. of animals in each group (n): 6

Treatment schedule

Thirty animals were randomly divided equally into six groups of six animals each.

Group 1: (Control) received 0.5% CMC for 14 days.

Group 2: (Ethanol control) received 99.9% ethanol 4g/kg with corn oil (10 ml/kg/day) p.o. for 14 days.

Group 3: (Drug control) received MEIR (400 mg/kg/day p.o.)

Group 4: (Low dose) received MEIR (200 mg/kg/day p.o.) and 99.9% ethanol (4 g/kg) with corn oil (10 ml/kg/day) for 14 days.

Group 5: (High dose) received MEIR (400 mg/kg/day p.o.) and 99.9% ethanol (4 g/kg) with corn oil (10 ml/kg/day) for 14 days.

Group 6: (Silymarin control) received silymarin (100 mg/kg/day p.o.) and 99.9%ethanol (4 g/kg) with corn oil (10 ml/kg/day) for 14 days.

After 14 days of treatment with MEIR, 15th day blood is collected from retro orbital plexus puncture and rats were sacarified and liver was collected immediately perfused with Phosphate Buffer Solution. Serum was separated by centrifugation. Serum is collected and assayed for Serum glutamate oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT)**,** direct bilirubin, total bilirubin, total proteins were assayed. The tissue homogenate lipid peroxidation, glutathione were assayed and then histopathological studies also carried out.

Parameters evaluated in the study

Determination of wet liver weight

Livers isolated from the animals were washed with alcohol, dried with filter paper strips, weighed on an electronic balance and were expressed with respect to their bodyweight i.e.gm/100 gm

Determination of wet liver volume:

After recording the liver weights weighing, the livers were individually dropped into measuring cylinder containing a fixed volume of distilled water and the volume displaced was recorded and expressed as ml/100g body weight.

RESULTS

ACUTE TOXICITY STUDIES

Each animal received single dose of MEIR (2000 mg/kg) by p.o. After administration of the test compound, animals were observed individually. No, moratality were observed for the period of 14 days. $1/10^{th}$ (200 mg/kg, p.o.) and $1/5^{th}$ (400 mg/kg, p.o.) of the maximum tolerable doses (2000 mg/kg) were taken for the further pharmacological studies.

HEPATOPROTECTIVE ACTIVITY OF MESUOL PARACETAMOL INDUCED HEPATOTOXICITY PHYSICAL PARAMETERS

Effect of MEIR Low Dose, MEIR High Dose and Silymarin on physical parameters in PCM intoxicated rats.

Paracetamol alone (750mg/kg) treated rat showed significant ($p<0.001$) increased the liver weight and volume when compared with normal control animals. Treatment with MEIR 200 ($p<0.01$), 400mg/kg ($p<0.001$) and silymarin 100mg/kg ($p<0.001$) to PCMtreated animals showed significant decreased liver volume and weight when compared with PCM alone treated animals.

SERUM BIOCHEMICAL PARAMETERS

Effect of MEIR Low Dose, MEIR High Dose, and Silymarin on serum parameters in PCM intoxicated rats.

The levels of SGOT, SGPT, DB and TB increased significantly ($p<0.001$) in PCM alone treated, while the content of TP decreased significantly ($p<0.001$) when compared with normal animals. Treatment with silymarin, MEIR low dose, MEIR high dose were found to significant ($p<0.001$) decrease in the levels of SGOT, SGPT, DB, TB and significantly ($p<0.001$) increased the TP levels when compared to PCM alone treated group.

Table 1: Effect of *MEIR* on Wet liver weight and Wet liver volume in Paracetamol induced hepatotoxic rats.

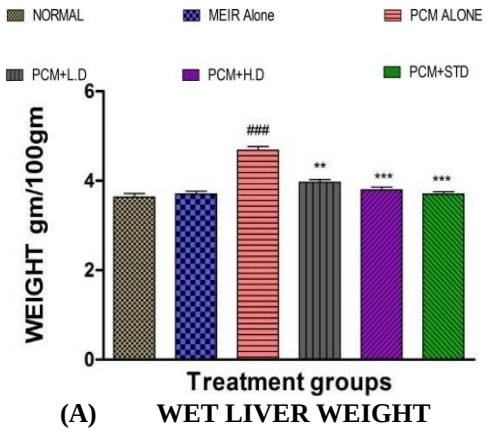
Group	Treatment	Wet liver weight(gm/100gm)	Wet liver volume(ml/100gm)
A	Normal	3.6±0.084	3.5±0.095
B	Paracetamol	4.6±0.083***a	4.67±0.092***a
C	MEIR alone	3.7±0.068***b	3.83±0.15***b
D	*MEIR* (LD)+Paracetamol	3.9±0.055** b	4.07±0.069** b
E	*MEIR* (HD)+Paracetamol	3.8±0.057*** b	3.91±0.070*** b
F	*Silymarin*	3.7±0.057***b	3.82±0.097***b

Table 2: Effect of *MEIR* on SGOT, SGPT, T.bilirubin, D.bilirubin and T.Protien levels in Paracetamol induced Hepatotoxic Rats.

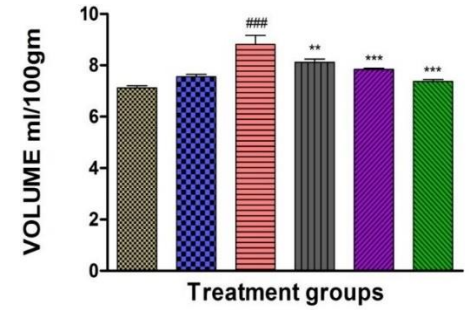
Treatment	SGOT levels (U/L)	SGPT levels (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (gm/dl)
Normal	140.5 ±5.92	93.93±2.97	0.25±0.019	0.29±0.058	9.53±0.122
Paracetamol	273.2±9.68***a	134.3±9.35*** a	0.78±0.02***a	0.78±0.007***a	3.91±0.057*** a
MEIR alone	130.5±9.67***b	93.10±6.76***b	0.35±0.016***b	0.26±0.009***b	9.42±0.14***b
***MEIR* (LD)+ Paracetamol**	240.2 ±7.87**b	100.2±8.72** b	0.64±0.049** b	0.63±0.068** b	7.86±0.259** b
***MEIR* (HD)+ Paracetamol**	174.7±9.07***b	87.15±6.30*** b	0.37±0.018*** b	0.33±0.022*** b	7.75±0.194*** b
Silymarin	161.2±3.88***b	76.23±6.20*** b	0.35±0.039***b	0.27±0.032*** b	9.13±0.036*** b

Table 3: Effect of *MEIR* on Glutathione and LPO in Paracetamol induced Hepatotoxic rats.

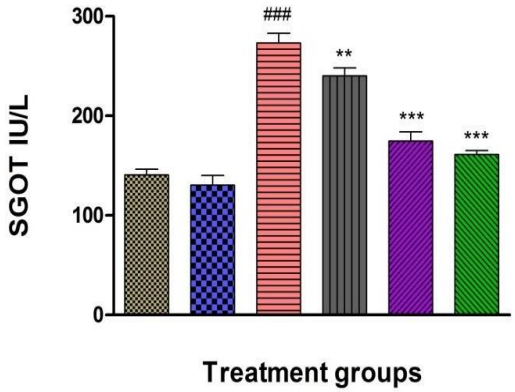
Group	Treatment	Glutathione	LPO
A	Normal	0.27±0.0007	43.75±7.089
B	Paracetamol	0.07±0.001***a	248.0±38.13*** a
C	*MEIR* alone	0.17±0.008***b	71.69±7.645***b
D	*MEIR* (LD)+Paracetamol	0.16±0.007**b	183.0±14.25** b
E	*MEIR*(HD)+Paracetamol	0.25±0.009***b	75.53±14.33*** b
F	Silymarin	0.24±0.011***b	70.97±13.24*** b



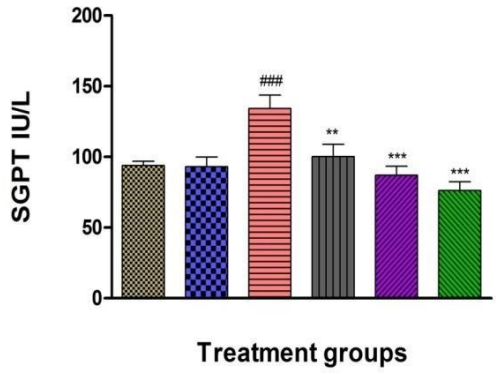
(A) WET LIVER WEIGHT



(B) WET LIVER VOLUME

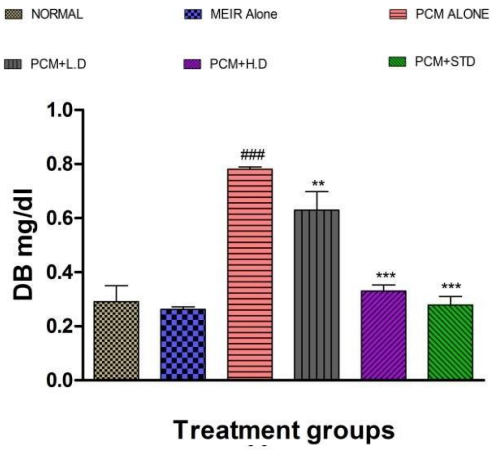


(B) SGOT



(D) SGPT

Figure 1 : Effect of *MEIR* on (A) Wet Liver Weight (B) Wet Liver Volume (C) SGOT (D) SGPT levels in Paracetamol induced Hepatotoxic rats.



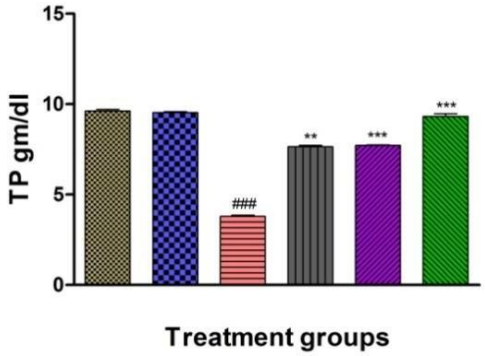
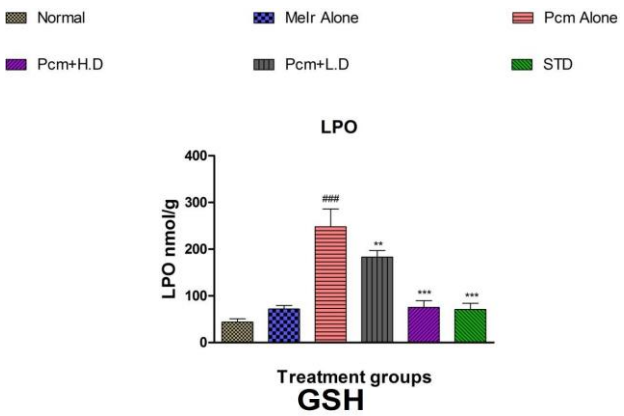


Figure 2 : Effect of *MEIR* on D.bilirubin,(DB), T.protien (TP) levels inParacetamol induced Hepatotoxic rats.



GSH

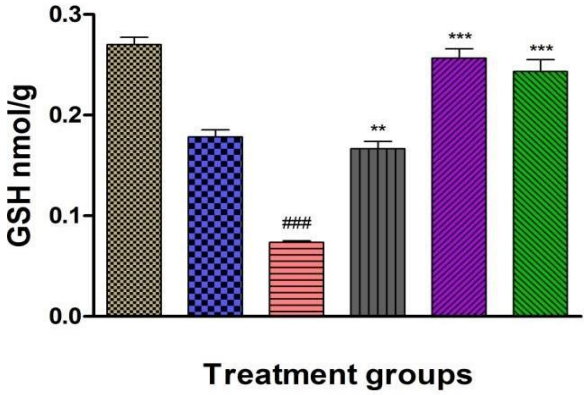
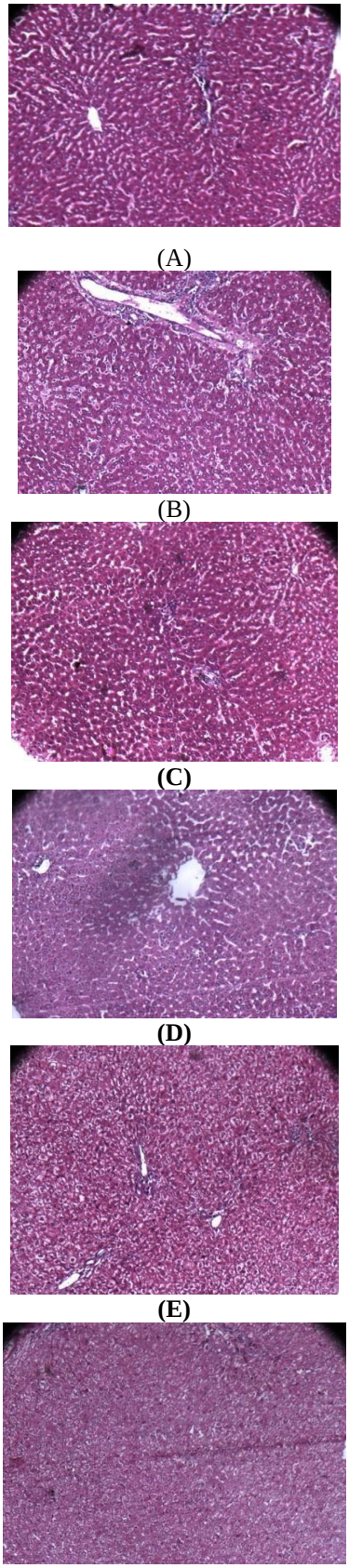


Figure 3 : Effect of *MEIR* on LPO and Glutathione in Paracetamol inducedHepatotoxic rats.



(A)

(B)

(C)

(D)

(E)

(F)

Figure 4 : Histopathology of PCM induced hepatotoxicity A) NORMAL B) MEIRALONE 400 mg/kg C) PCM ALONE 3 g/kg D) MEIR LD 200 mg/kg E) MEIRHD 400 mg/kg F) SILYMARIN 100 mg/kg

Ethyl alcohol Induced ModelPhysical parameters:

Wet liver weight and Wet liver volume:

Control group (Ethyl alcohol) treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups treated with *MEIR* 200 mg/kg showed better restoration. Silymarin 100 mg/kg and *MEIR* 400 mg/kgshowed significant restoration of wet liver weight and wet liver volume nearer to normal.

Bio chemical Parameters:

Rats treated with Control group (ethyl alcohol) developed a significant hepatic damage observed as elevated serum levels of hepato specific enzymes like SGPT, SGOT, Bilirubin and Total Protien, when compared to normal control. Pretreatment with *MEIR* 200mg/kg showed better protection. Silymarin (100 mg/kg) and *MEIR* 400 mg/kg showed good protection against paracetamol induced toxicity to liver. Significant reduction in elevated serum enzyme levels with *MEIR* treated animals compared to toxiccontrol animals.

Total bilirubin and direct bilirubin:

Elevation of total bilirubin and direct bilirubin levels after administration of control group (ethyl alcohol) indicates hepatotoxicity. Pretreatment with *MEIR* at 400 mg/kg moderately reduced levels of total bilirubin. Pretreatment with Silymarin (100 mg/kg) and *MEIR* at (200 mg/kg) significantly reduced levels of total bilirubin when compared to toxic control group indicating Hepatoprotective effect.

Total protein:

Control group treatment has considerably reduced serum total protein levels. Pretreatment with *MEIR* 400 mg/kg moderately increased the level of total protein.

Pretreatment with Silymarin (100 mg/kg) and *MEIR* 200 mg/kg had shown a significant increase in total protein level as compared with toxicant group.

Antioxidant parameters:

From the results it was found that rats treated with Ethyl alcohol group showed a marked decrease in activity of Glutathione when compared to normal control group. In the animals treated with *MEIR* at 200 mg/kg the activity of Glutathione had moderately increased when compared to toxicant group. Whereas, in animals treated with Silymarin (100 mg/kg) and *MEIR* (400 mg/kg) significant increase in the level of these enzymes was observed. In vivo lipid peroxidation study revealed that Ethyl alcohol treated group showed significant increase in Malon dialdehyde (MDA) level when compared with normal control group. *MEIR* and silymarin were able to significantly prevent this raise in MDA level.

Histopathology:

Microscopic examination of normal liver, section studied shows liver parenchyma with intact architecture. The central vein, portal triad, perivenular and periportal regions found to be normal.

Microscopic examination of ETH induced liver, Section studied shows the perivenular and periportal regions show mixed inflammatory infiltrations. Some of the hepatocytes show apoptosis.

Microscopic examination of MEIR LD, MEIR HD, and Silymarin treated animal livers section studied shows liver

parenchyma with intact architecture, showing unremarkable, mild hepatocytic changes respectively.

Table 4 : Effect of *MEIR* on Wet liver weight and Wet liver volume in Ethyl alcohol induced hepatotoxic rats.

Group	Treatment	Wet liver weight(gm/100gm)	Wet liver volume(ml/100gm)
A	Normal	2.92±0.02	2.83±0.08
B	Ethyl alcohol	4.70±0.06***a	4.79±0.06***a
C	*MEIR* alone	2.7±0.027***b	2.8±0.028***b
D	*MEIR* (LD)+ Ethyl alcohol	3.29±0.16** b	3.38±0.09** b
E	*MEIR* (HD)+ Ethyl alcohol	3.14±0.16*** b	2.94±0.05*** b
F	Silymarin	3.07±0.18***b	2.84±0.03*** b

Table 5: Effect of *MEIR* on SGOT, SGPT, T.bilirubin ,D.bilirubin and T.Protien levels in Ethyl alcohol induced Hepatotoxic Rats.

Treatment	SGOT levels (U/L)	SGPT levels (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (gm/dl)
Normal	90.46 ±5.85	56.28±8.06	0.311±0.030	0.29±0.026	9.53±0.12
Ethyl alcohol	137.0±11.28***a	115.9±9.05*** a	0.82±0.043***a	0.89±0.031***a	3.91±0.057*** a
MEIR alone	130.5±9.67***b	93.10±6.76***b	0.34±0.016***b	0.26±0.008***b	9.42±0.14***b
***MEIR* (LD)+EA**	128.2 ±9.90**b	67.3±12.00** b	0.62±0.057** b	0.65±0.082** b	7.86±0.259** b
***MEIR* (HD)+EA**	70.98±10.6***b	51.86±8.007*** b	0.44±0.029*** b	0.38±0.034*** b	7.75±0.194*** b
Silymarin	82.98±5.88***b	57.76±6.43*** b	0.38±0.014***b	0.30±0.028*** b	9.13±0.036*** b

Table 6 : Effect of *MEIR* on Glutathione and LPO in Ethyl alcohol induced hepatotoxic rats.

Group	Treatment	Glutathione	LPO
A	Normal	0.028±0.002	0.079±0.001
B	Ethyl alcohol	0.006±0.003***a	0.149±0.003*** a
C	*MEIR* alone	0.271±0.007***b	0.093±0.001***b
D	*MEIR* (LD)+Ethyl alcohol	0.019±0.003**b	0.138±0.009** b
E	*MEIR* (HD)+Ethyl alcohol	0.025±0.005***b	0.125±0.004*** b
F	Silymarin	0.027±0.001***b	0.078±0.011*** b

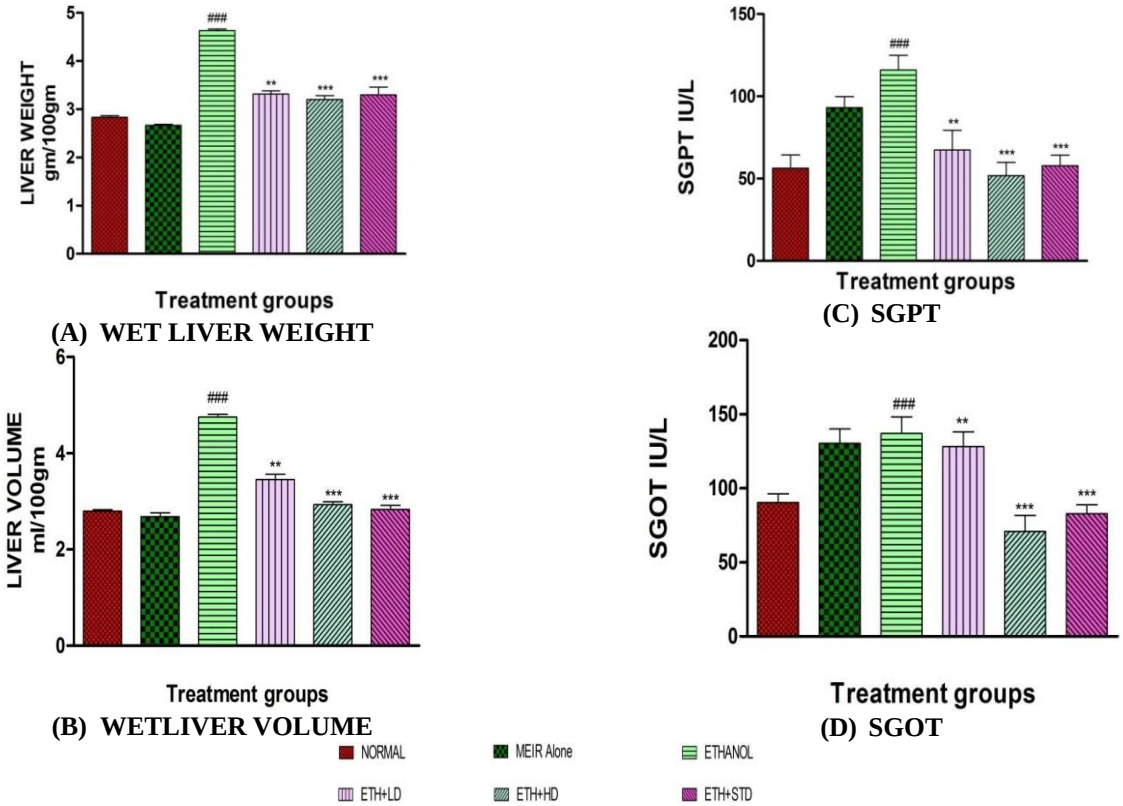


Figure 5 : Effect of *MEIR* on (A) Wet Liver Weight, (B) Wet Liver Volume, (C) SGPT,(D) SGOT levels in Ethyl alcohol induced Hepatotoxic rats.

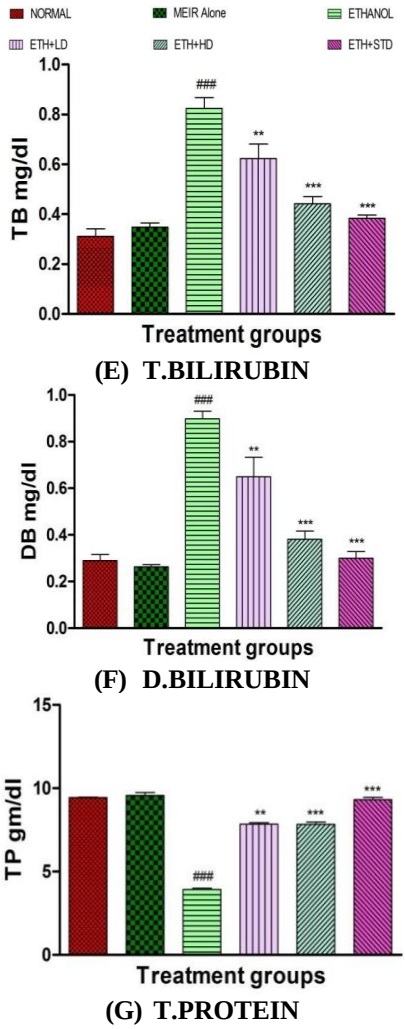


Figure 6 : Effect of *MEIR* on (E) T.bilirubin, (F) D.bilirubin (G) T.protien levels inEthyl alcohol induced hepatotoxic rats.

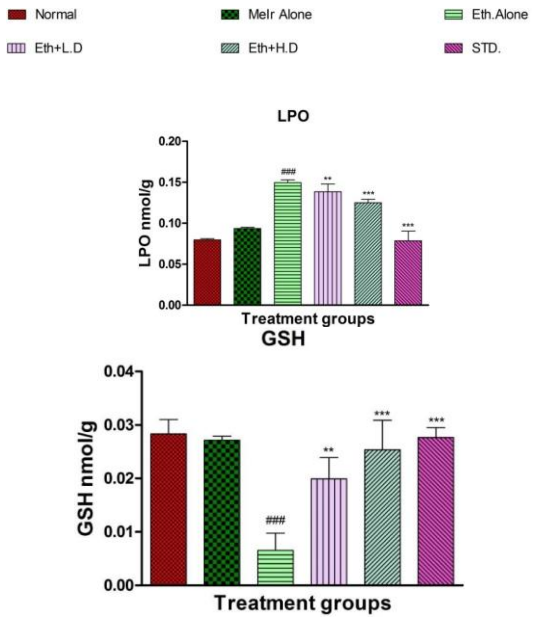


Figure 7 : Effect of *MEIR* on LPO and Glutathione levels in Ethyl alcohol inducedhepatotoxic rats.

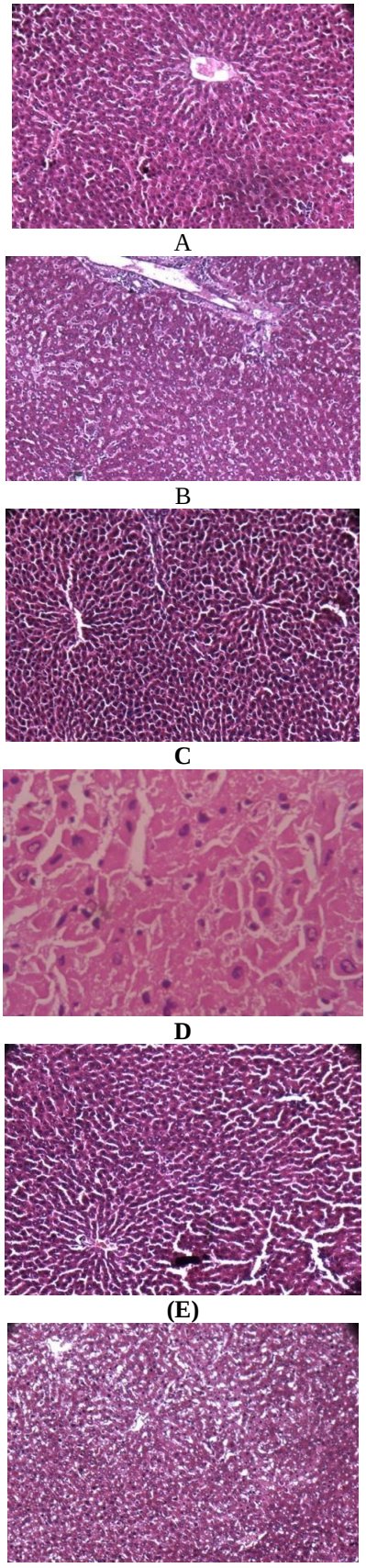


Figure 8 : Histopathology of ethyl alcohol induced hepatotoxicity (A) NORMAL (B) DRUGALONE (C) ETH ALONE 4g/kg (D) MEIR LD (E) MEIR HD (F) SILYMARIN

CONCLUSION

Hepatoprotective activity:

The hepatoprotective effect of *MEIR* was confirmed by the following parameters:

The isolated livers from the toxicant treated animals exhibited increase in their physical parameters like wet liver weight and wet liver volume. Indeed, animals treated *MEIR* showed decrease in the values of above physical parameters which is an indication of hepatoprotection.

Biochemical parameters:

In case of toxicant treated groups there will be rise in serum marker enzymes such as SGPT, SGOT, total bilirubin and decrease in the level of total protein. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function.

In the present study, treatment with *MEIR* significantly reduced the toxicant elevated levels of above mentioned serum marker enzymes and increase in the levels of total protein. Hence, at this point it is concluded that the *MEIR* possess hepatoprotective activity.

Antioxidant enzymes: In case of toxicant treated groups there will be decrease in enzyme activities such as Glutathione. Pretreatment with *MEIR* restored the activity of both these antioxidant enzymes possibly by reducing generation of free radicals and hepatocellular damage.

In the present study, pretreatment with *MEIR* significantly reduced the toxicant elevated levels of thiobarbituric acid reactive substances (TBARS) like Malondialdehyde. Hence, it was concluded that *MEIR* possesss hepatoprotective activity in the dose used.

Histopathological studies: In toxicant treated animals there will be severe histopathological disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. In the present study animals treated with *MEIR* under study exhibited minimal hepatic derangements and intact cytoarchitecture of the liver was maintained, indicating hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameters, Antioxidant parameters, and histopathological studies, it is concluded that the *MEIR* possesses hepato protective activity and thus supports the traditional application of the same under the light of modern science. The whole plant extract have been reported to exhibit chemopreventive and antioxidant activity. Under the present experimental results suggest that *MEIR* may have future clinical application after further studies.

Acknowledgement

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