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Original Article

HEPATOPROTECTIVE ACTIVITY OF *MAYTENUS EMARGINATA* AGAINST PARACETAMOL INDUCED LIVER INJURY IN MALE WISTAR RATS

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ABSTRACT

Objective: To study the hepatoprotective activity of ethanol extract of *Maytenus emarginata* against Paracetamol induced toxicity in male Wistar rats.

Methods: The liver injury was induced by Paracetamol @ 500 mg/kg B.W. orally for entire duration of study. The rats were divided in six groups. Rats of Group C1 served healthy control received no treatment, Group C2 served vehicle control received 1ml of Propylene glycol orally. C3 group rats acted as toxic control and received Paracetamol orally @ 500 mg/kg B.W. of rats. Group T1, T2 and T3 received Silymarin @ 100 mg/kg B.W., *Maytenus emarginata* extract @ 100 mg/kg B.W. and *Maytenus emarginata* extract @ 150 mg/kg B.W. orally of rats respectively along with Paracetamol @ 500 mg/kg B.W. of rats orally for 28 days.

Results: Histopathological examination and elevation of biochemical marker enzymes ALP, ALT and AST along with decreased levels of serum total protein and serum albumin confirmed the liver injury produced by Paracetamol. Treatment of rats with *Maytenus emarginata* extract showed significant reduction in levels of serum ALP, ALT and AST with elevation of serum protein and albumin near to normal in a dose dependant manner. Dose of 150 mg/kg B.W. of *Maytenus emarginata* extract showed significant effects comparable to the effects showed by standard treatment drug Silymarin @ 100 mg/kg B.W. orally.

Conclusion: *Maytenus emarginata* ethanol extract possess potent hepatoprotective effect.

Keywords: *M. emarginata,* Hepatoprotective, Paracetamol toxicity, Histopathology.

INTRODUCTION

Liver is the largest organ of great importance involved in vital body process viz. maintenance of homeostasis, metabolic substances detoxification and disposition of endogenous substances like xenobiotics, drugs, etc., most importantly, the liver is considered to be the center of metabolic transformation of drugs and other toxins entering from the gastrointestinal tract. As such the normal functioning of the liver determines the health status of an individual. Different homeostatic mechanisms get affected, if liver functions are impaired with potential serious and adverse consequences [1]. The liver has several important functions and any diffuse disease of the organ interferes with most or all of the functions to the same degree. Variations occur in acuteness and severity of damage but the effects are same and clinical manifestations vary in degrees only. The major hepatic functions like maintenance of normal blood glucose level, synthesis of proteins, formation of bile salts and pigments and detoxification of many toxic substances including photodynamic substances, when gets disordered leads to visible clinical signs [2].

Paracetamol is a widely used analgesic and antipyretic drug. It is believed that selective inhibition of the enzyme Cox-3 in the brain and spinal cord explains the effectiveness of Paracetamol in relieving pain and reducing fever. However its mechanism of action is not fully understood, but it is generally accepted that Paracetamol is a centrally acting drug. Paracetamol is available in oral, rectal and injectable forms.

N-acetyl-P-Benzoquinoneimine (NAPQ1), a metabolite of Paracetamol is highly toxic. Acute overdose of Paracetamol can cause potentially fatal liver damage and in rare individuals, a normal dose can do the same. Paracetamol is the foremost cause of acute liver failure. Hepatotoxicity is the most remarkable feature of Paracetamol overdose along with renal effects with lesser frequency [3].

Liver disorders are mainly treated with Silymarin and n-acetyl cysteine which are not cost effective and are not feasible to use in farm animal practice. In spite of tremendous strides in the field of modern medicine, at present modern medicine don't have a therapeutic agent that could cure the different liver disorders and there are hardly any drugs that stimulate liver functions. For the reason, a lot of research on medicinal plants with hepatoprotective effect is being carried out. It has been reported that about 160 phyto-constituents from 101 plants have hepatoprotective activity [4]. *Maytenus emarginata* is a shrub found in low plane and hilly areas of India, having medicinal value and is being used for the treatment of tumors, bacterial infections, snake bite and as hepatoprotectant in Ayurveda [5]. It is found to have active principles triterpene, quinine, methides, luperone, β-amirine, ducital and sitosterol [6]. Hepatoprotective value of *Maytenus emarginata* was studied by Parmar, *et al*., (2009) [7] for the first time, along with extracts of *Phyllanthus niruri, Eclipta alba, Aloe vera, Solanum indicum* and *Aegle marmelos* in CCl_4 induced hepatotoxicity. In this study our aim was to investigate the hepatoprotective effects of *Maytenus emarginata* against Paracetamol induced liver damage in rats.

MATERIAL AND METHODS

Chemicals

Silymarin was obtained from local medical store (Silybon-140, Micro Labs Ltd, H.P. INDIA). Paracetamol (API) was obtained from SNP College, Pusad. The kits for biochemical estimation were purchased from Merk Ltd, Kalyan-Badlapur Road, Ambernath (INDIA). The solvents and other chemicals were obtained from local dealers.

Animals

The experiment was carried out on 60 male Wistar rats weighing 150 ± 20 g. Rats were maintained in standard laboratory conditions of temperature (26 ± 2°C) and 60% humidity. The rats were fed on standard feed pellets with free access to clean drinking water. The experiment was conducted on approval of Institutional Animal Ethics Committee, COVAS, Udgir, Maharashtra, INDIA.

Rats were divided into six groups. Group C1, C2 and C3 represented healthy control, vehicle control and toxic control respectively. Group

T1, T2 and T3 acted as treatment groups receiving Silymarin @ 100 mg/kg body weight of rats, *M. emarginata* extract @ 100 mg/kg body weight and 150 mg/kg body weight of rats respectively.

Collection of plant material

The leaves of *Maytenus emarginata* were collected from nearby areas of Udgir city (M.S.) in the month of October-November and authenticated by Dr. Allapure, HOD, Dept. of Botany, Maharashtra Udaygiri College, Udgir, Maharashtra, INDIA.

Preparation of extract

Leaves of *Maytenus emarginata* were shade dried at room temperature and were powdered by using mechanical grinder. The alcoholic extract of *M. emarginata* was obtained with 95% alcohol using Soxhalet apparatus by extracting at 65 °C till the discoloration of alcohol in the extraction chamber. The extract was kept in air tight vials after complete evaporation and was stored in refrigerator till use.

Induction of hepatotoxicity

The Paracetamol induced hepatotoxicity model described by Parmar *et. al.,* (2010) [8] was used with slight modifications. Rats were fasted over night and hepatotoxicity was induced by administration of Paracetamol in pure form (API) in distilled water at the dose rate of 500 mg/kg body weight by gastric gavage to the rats of groups C3, T1, T2 and T3 once daily for the entire duration of study.

Silymarin and extract treatment

The rats in groups T1, T2 and T3 were treated with Silymarin @ rate of 100 mg/kg of body weight in distilled water, ethanolic extract of *M. emarginata* @ 100 mg/kg body weight of rats and *M. emarginata* extract @ 150 mg/kg body weight of rats orally in Propylene glycol using gastric gavage, respectively daily for twenty eight days period.

Biochemical studies

Blood samples were collected from orbital plexus of rats into sterilized vials for collecting serum for biochemical estimations at the end of experiment. The serum samples were used to estimate the biochemical parameters viz. ALP, ALT, AST, Total Protein and Albumin. After collecting blood the rats were sacrificed, liver was removed immediately and the liver weights were measured and fixed in 10% Neutral buffered formalin for histopathological studies.

Histopathological observations

The pieces of liver from rats of each group were collected in 10% neutral buffered formalin for proper fixation. These tissue samples were embedded in paraffin and processed as per standard procedures. These tissue samples were sectioned at 3 – 5 µ thickness and were stained with Mayer's haematoxylin and eosin for histopathological examination [9].

Statistical analysis

Numerical data obtained from this study was expressed as mean value ± standard error and were analyzed statistically by CRD and analysis of variance by using Graph Pad Prism Software. A probability of less than 5% ($p<0.05$) was considered significant.

RESULTS AND DISCUSSION

Present study was aimed to investigate the protective effect of *Maytenus emarginata* extract against Paracetamol induced liver injury. Paracetamol induced hepatic injury is commonly used model for the screening of hepatoprotective preparations [10] and extent of hepatic damage is assessed by the level of release of cytoplasmic alkaline phosphatase and transaminase in the circulation. It is well documented that Paracetamol is metabolized to reactive metabolite N-acetyl-P-Benzoquinoneimine which covalently bind to the sulphydryl groups of proteins resulting in cell necrosis and leakage of cellular enzymes in the circulation [11]. The toxic dose of Paracetamol is highly variable, in adults, a single dose of 10 grams or chronic ingestion of dose of 4 g/day could induce Paracetamol hepatotoxicity [12]. Liver disorders remains as one of the major health problem. However till date allopathic medical practice don't have satisfactory liver protective drug. Herbal preparation play important role in the management of liver disorders in folk medicinal systems. At the same time surprisingly don't have readily available plant preparation to treat the severe liver disorders and promote regeneration of hepatocytes [13][14].

Table 1: Effect of *Maytenus emarginata* extract against Paracetamol toxicity on mean Liver and Body weights.

Parameters	Normal control	Vehicle control	Toxic control	Standard	MEE 100 mg/kg	MEE 100 mg/kg
Body weight gain (%)	13.89^{a} ±2.01	14.07^{a} ±1.96	1.66^{c} ±0.60	12.41^{ab} ±1.13	9.21^{b} ±0.48	13.56^{a} ±1.32
Relative Liver Weight (%)	3.03 +0.07	2.97 +0.50	3.88^{a} +0.12	3.18^{b} +0.06	3.27 +0.09	3.11^{b} +0.06

Table 2: Effect of *Maytenus emarginata* extract against Paracetamol toxicity on Biochemical parameters

Parameters	Normal control	Vehicle control	Toxic control	Standard	MEE 100 mg/kg	MEE 100 mg/kg
TSP (gm /dl)	7.64^{a} ±0.31	7.31^{a} ±0.25	4.86^{c} ±0.20	7.05^{a} ±0.29	5.99^{b} ±0.29	7.19^{a} ±0.15
Albumin (gm /dl)	4.64^{ab} ±0.22	4.49^{ab} ±0.13	3.53^{c} ±0.17	4.52^{ab} ±0.12	4.27^{b}±0.13	4.63^{a} ±0.12
ALP (U/L)	43.61^{c}±2.08	47.26^{c}±3.47	143.1^{a} ±12.30	57.77^{bc} ±3.03	61.70^{b}±2.66	53.60^{bc} ±1.83
AST (U/L)	21.71^{b}±0.82	22.90^{b}±1.06	132.7^{a} ±14.16	27.63^{b} ±1.68	32.98^{b}±1.68	25.83^{b}±1.00
ALT (U/L)	26.70^{b}±1.44	26.70^{b} ±1.41	124.4^{a} ±9.35	32.80^{b} ±2.59	36.43^{b} ±1.82	29.33^{b}±1.76

*Values are expressed as mean ± SEM (n=6) using one way ANOVA followed by CRD. Means bearing different superscripts within rows differ significantly from each other. TSP= Total serum protein, ALP= Alkaline phosphatase, AST= Aspartate aminotransferase, ALT= Alanine transaminase MEE = *Maytenus emarginata* extract.

In present study, table 1. depicts the percent weight gain of the rats. Rats treated with Paracetamol showed significant ($p<0.05$) reduction in percent weight gain while *Maytenus emarginata* extract found to enhance the weight gain reduced by the Paracetamol toxicity in the rats treated with 150 mg/kg extract of the body weight which were comparable to the Silymarin treated group rats. These findings were in accordance with previous reports which states significant improvement in weight gain in Paracetamol intoxicated rats treated with various plant extract treatment [15][16]. Liver weight of the rats treated with the Paracetamol found to be increased compared to healthy group rats. Liver weight of rats of treatment groups T1, T2 and T3 found to be near to normal with highest effect in the T3 group rats treated with higher dose of *M. emarginata* extract (table 1). Earlier reports by various workers showed significant reduction in the increased relative liver weight of rats in Paracetamol induced toxicity using different plant extracts [17-20]. Liver of experimental rats of C3 group showed patches of necrosis, congestion and petechial haemorrhages and were hypertrophied. While no observable changes were seen in the rats of treatment groups treated with Silymarin and *M. emarginata.* Boyed

el al., (1966) [21] found the spotted liver in Paracetamol toxicity in male Wistar rats which supports the findings of this study.

Fig. 1a and Fig. 1b shows the liver sections from C1 and C2 groups respectively, which shows the microphotograph of normal hepatic architecture. Liver sections of rats of C3 group (Fig. 1c) revealed diffuse cellular swelling and ballooning degeneration of hepatocytes, the cytoplasm of hepatocytes was cloudy and number of hepatocytes were anucleated. Central hepatic vein showed congestion with few mononuclear inflammatory cells in the lumen. Nuclei of the hepatocytes were enlarged, karyolysed and fragmented indicating degenerative changes. The hepatic architecture was disturbed with derangement of hepatic cords. Whereas liver sections of T1 group (Fig. 1d) did not reveal histopathological changes except some degree of degenerative changes in the section. Liver sections of T2 (Fig. 1e) and T3 (Fig. 1f) group revealed the hepatic architecture near to normal. The nuclei of the hepatic cells were intact and prominent, no pyknotic or defragmentation of hepatic nuclei was observed. The hepatocytes did not revealed ballooning degeneration and no cloudy appearance was observed in the cytoplasm. Necrotic changes were not evident. Focal areas of mild

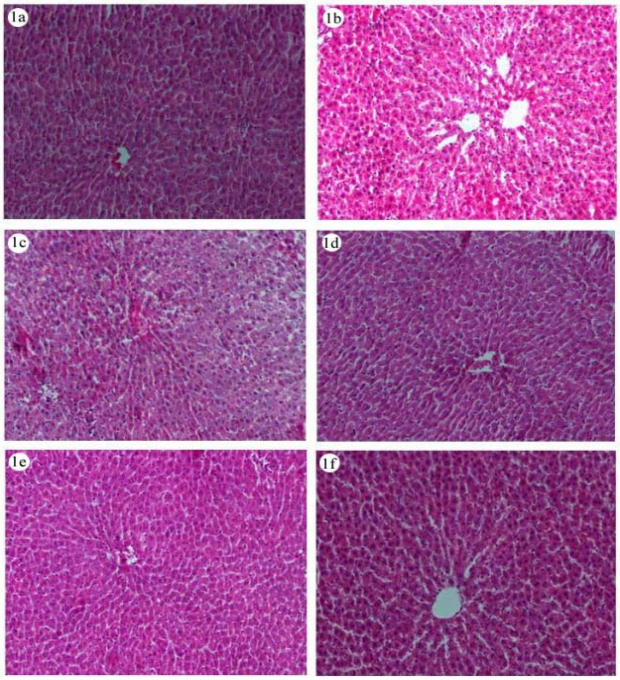

Fig. 1: Hepatoprotective effect of *Maytenus emarginata* extract a) Healthy Control b) Vehicle Control c) Toxic Control- Section Showing loss of histoarchitecture, necrosis and infiltration of inflammatory cells in hepatic vein d) Standard treatment- section showing near to normal architecture with congestion of central hepatic vein e) MEE (100 mg/kg) treatment- Section showing near to normal architecture with inflammatory cells in hepatic vein f) MEE (150 mg/kg) Treatment- Section showing maintained histoarchitecture with prominent hepatocytes (H&E X200).

degeneration were present in the liver sections of T2 and T3 treatment groups. It showed protective effect of *M. emarginata* extract against the Paracetamol induced hepatotoxicity. These findings are in accordance with previous work on various extracts against Paracetamol toxicity, which suggests potent effect of extract as hepatoprotectant against Paracetamol toxicity [22-23].

Elevation of hepatic enzymes is the evidence of Paracetamol induced liver injury, which is due to the leakage of cellular enzymes ALP, ALT and AST into the plasma. When the plasma membrane of hepatocyte is damaged, various enzymes which are normally present in cytosol are released into the blood stream. Estimation of these enzymes level in the serum has been considered as a useful quantitative marker to assess the severity of liver damage. The effects of the administration of the extract of *M. emarginata* and Paracetamol on biochemical parameters are presented in Table 2. Biochemical analysis of serum showed a significant increase $p<0.05$ in the levels of AST, ALT and ALP in rats treated with Paracetamol, which was seen near to be normal in the rats treated with *M. emarginata* extract treated rats in a dose dependant manner. Total serum protein and serum albumin levels of Paracetamol treated rats were reduced significantly $p<0.05$ while those treated with *M. emarginata* extract showed these values near to normal and dose of 150 mg/kg body weight appeared to be most effective. Earlier researchers reported decrease in elevated levels of serum ALP, ALT and AST in Paracetamol induced hepatotoxic rats on treatment with extracts of various plants [24-29][14]. Histopathological examination and these earlier reports by various research workers support the biochemical findings of present study proving the hepatoprotective activity of *M. emarginata* extract against Paracetamol induced hepatotoxicity in a dose dependant manner with maximum protective effects @ 150mg/kg body weight of rats.

CONCLUSION

In present investigation it was found that ethanolic extract of *M. emarginata* brought all the parameters affected by Paracetamol toxicity near to normal. Thus the extract of *M. emarginata* has hepatoprotective effect which minimizes the hepatotoxicity induced by Paracetamol, thereby suggesting its use as a potent hepato protective agent.

CONFLICT OF INTERESTS

Declared None

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