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HEPATOPROTECTIVE EFFECT OF *CHELIDONIUM MAJUS.L* EXTRACT AGAINST ANTITUBERCULAR DRUGS INDUCED HEPATIC DAMAGE IN WISTAR RATS.

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ABSTRACT

The present study was designed to evaluate the hepatoprotective effect of Chelidonium majus L. (Papaveraceae) in Isoniazid and Rifamipicin induced toxicity in wistar rats Material and methods: Four groups, each of six experimental Wistar rats were employed. The control (Group 1) received vehicle treatment, Group II received Rifampicin (RIF) (100 mg/kg, p.o.) and co administered with Isoniazid (INH) (100 mg/kg, p.o) in sterile water. Group III received drug Chelidonium (500mg/kg/p.o/day) and Group IV drug Chelidonium (500mg/kg/p.o/day) along with INH-RIF combination at a dose of (100mg/kg/p.o) of each continued for 21 days. After which rats were sacrificed, blood and liver were taken for biochemical studies, respectively. Liver biomarkers such as alanine aminotransferase (ALT) and aspartate amino transferanse (AST) and Total bilirubin (TB) were elevated on anti-tubercular drugs administration. The treatment of ethanolic extract of C.majus at a dose of 500 mg/kg/p.o with anti-tubercular drugs have significantly reduced liver biomarker enzymes. Antioxidant parameters such as SOD, CAT, GPx and were suppressed and an increase in malondialdehyde (MDA) level were observed due to anti-tubercular drugs administration but restored the liver biomarkers and antioxidant levels in the treatment of ethanolic extract of C.majus. The result of the present study was indicated that C.majus showed a protective effect on hepatotoxicity induced by anti-tubercular drugs. Abbreviations: Alanine Aminotransferase (ALT),Aspartate Amino Transferanse (AST) Total Bilirubin (TB), Chelidonium majus.L (C.majus), superoxide dismutase (SOD), Catalyse (CAT), glutathione peroxidase (GPx) and malodialdehyde (MDA),Isoniazid(INH), and Rifampicin(RIF).

KEYWORDS: Hepatoprotective, Chelidonium, Isoniazid, and Rifampicin.

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INTRODUCTION

Drug induced hepatotoxicity is a serious adverse drug reaction of anti-tubercular drugs[1]. Toxicity due to anti-tubercular drugs on the liver is mediated mainly through oxidative stress and free radical damage to hepatocytes[2]. Hepatotoxic agents can cause very serious damages to liver functions. Numerous medical plants and their formulations are being used to treat various liver disorders as ethno medical practices in traditional system of medicine. Conventional drugs used in the treatment of liver disease are often inadequate. It is therefore a search for supplementation/ of alternative drugs for the treatment of hepatic damage caused by anti-tubercular drugs. Chelidonium majus L. commonly known as swallow-wort, rock poppy or greater celandine belongs to Family-Papaveraceae. This plant is distributed across the globe viz. Europe, Asia, North America and in northwest Africa[3]. Chelidonium majus has been used in folk medicine as diuretic, choleretic and hipnotic[4]. C.majus is used for the treatment of liver diseases as an infusion in anatolia[4]. It is also widely used in Chinese traditional medicine and homeopathy. Earlier study revealed it had hepatoprotective effect on carbon tetra chloride induced injury[5,] .

MATERIALS AND METHODS

Collection of plant material

The dried whole medicinal plant was being collected from, Mr. Netai pal (Thirty years experience in Herbal trading) Herbal trading co. Ltd. College street. Kolkata, West Bengal, India .The plants were dried under complete shade and were made into powder using a blender. The powder (250g) was extracted with 2500ml of 95% ethanol under constant shaking using a gyratory shaker for 48 hours and then filtered. The residue was re-extracted once with again 2000ml of ethanol and the combined extracts were concentrated under vacuum and dried using a rotary flash evaporator.

Animal studies

The experiment were carried on male Wistar rats (150-250 g) at *Meenakshi Medical College* and Research Institute, *Kanchipuram* with due ethical committee clearance (765/03/ca/CPCESA) and caged in a healthy environment (22±1.C, 12 h day and night cycle) were fed with pellet and water ad libitum.

Isoniazid and rifampicin induced hepatotoxicity [5]

Rifampicin and Isoniazid solution were prepared separately in sterile distilled water. Rats were treated with Isoniazid (100 mg/kg, orally) and co-administered with rifampicin (100 mg/kg, orally) for 21 days In order to study the effect of ethanolic extract of chelidonium in rats, a dose of 500 mg/kg/p.o was selected for the present study.

Experiment protocol

Wistar albino rats (male) were divided into four groups comprising six animals in each group. All the groups were treated as described below for 21days respectively.

Group I: Control group received 10ml/kg sterile distilled water.

Group II: Hepatotoxic control received INH+RIF (100mg/kg/p.o+100mg/kg/p.o).

Group III: Animals were given Chelidonium extract (500 mg/kg/p.o)

Group IV: Animals were given both INH+RIF (100mg/kg/p.o+100mg/kg/p.o) + Chelidonium extract (500 mg/kg/p.o)

All the treatments were given orally in sterile distilled water (10 ml/kg) by means of an orogastric cannula for 21 days. At the end of the treatment, blood samples of all animals were collected by a retro- orbital puncture in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the assay of liver marker enzymes. The liver was dissected out and washed with saline under ice cold conditions for the estimation of antioxidant parameters. The estimation of marker liver enzymes such as Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were done by Reitman and Frankel method[6]

and Total Bilirubin content by Grofs method[7]. The antioxidant activity was measured from the levels of various enzymes in the liver tissue, such as catalyse (CAT)) Sinha KA method[8], superoxide dismutase Kakkar P[9], glutathione peroxidase and malodialdehyde Rotruck[10].

Statistical analysis

All data were statistically analyzed by SPSS Windows Version 14 .0 .The student's t-test was performed to compare the data from the treated groups to their respective control groups of rats. Difference below p<0.05 implied significance.

RESULTS

Effect of Chelidonium majus on AST, ALT and Total Bilirubin levels.

Animals treated with anti-tubercular drugs (toxic control) showed a significantly elevated levels (P<0.05) of serum alanine amino transferase (ALT), aspartate amino transferase (AST) and total bilirubin levels when compared to control group. Supplementation of the Chelidonium extract at a dose of 500 mg/kg/p.o significantly reversed the elevated levels of marker enzymes and total bilirubin when compared to toxic control. The results of serum AST, ALT and total bilirubin levels are expressed in table 1

Table 1
Effect of Chelidonium majus extract against INH, RIF on liver marker enzymes and total bilirubin

Parameter	Group1 (Control)	Group II (INH+ R100mg/Kg/p.o) .	Group III Cheli extract 500mg/kg/p.o	Group IV Cheli500mg/kg/p.o+IN+RIF100mg/Kg/p.o) .
ALT IU/L	18±0.67	54±0.89*a	19±1.1*b	23±0.7*b
AST IU/L	42±0.4	234±1*a	46±.4*b	86±.47*b
Total Bilirubin (mg/dl)	0.56±0.019	7.45±0.034*a	0.55±.0170*b	0.63±.040*b

All values are expressed as Mean ± SEM (n=6).
Values are expressed as Mean ± SEM.
Values were found out by using ONE WAY ANOVA followed by student t test.
**** (a) values were significantly different from normal control at p<0.05.***
**** (b) Values were significantly different from toxic control at p<0.05.***

Invivo Antioxidant parameters

In the present study, antioxidant parameters were assessed in the liver homogenate. Oral administration of anti-tubercular drugs (toxic control) significantly (P<0.05) decreased SOD, CAT, GPx, and significantly (P<0.05) increased MDA level when compared to control group. Supplementation of Chelidonium extract at a dose 500mg/kg/p.o along with anti-tubercular drugs showed significant increase in the enzymatic levels and significantly decreased MDA levels in when compared to toxic control. The results are presented in table 2

.

Table 2
Effect of ethanolic of chelidonium extract on antioxidant parameters in rats

Parameter	Group1 (Control)	Group II (INH+RIF 100mg/Kg/p.o)	Group III Chelidonium extract 500mg/kg/p.o	Group IV Chelidonium 500mg/kg/p.o+INH+RIF+ RIF 100mg/Kg/p.o)
CAT IU/mg	4953 ± 13	3955 ± 13*a	5000 ± 14*b	4990 ± 1.3*b
SOD IU/ mg	2.115± 0.044	0.466 ± 0.021*a	2.178± 0.003*b	2.231 ± 0.004*b
GPXIU/mg	1.001± 0.0003	0.207 ± .0005*a	0.016 ± 0 .0009*b	0.86 ± 0.002*b
MDA nmol/ mg	10.116± 0.008	23.5 ± 0.34*a	9.053 ± 0.012*b	11.05 ± 0.002*b

All values are expressed as Mean ± SEM (n=6).
Values are expressed as Mean ± SEM.
Values were found out by using ONE WAY ANOVA followed by student t test.
**** (a) values were significantly different from normal control at p<0.05.***
**** (b) Values were significantly different from toxic control at p<0.05.***

DISCUSSION

The liver is a vital organ which is capable of detoxifying toxic substances. Hepatotoxic chemicals cause injury. The injury of liver is manifestated by the elevation of marker enzymes such as AST &ALT. Administrations of anti-tubercular drugs for 21 days result in hepatic injury and were confirmed by elevated levels of serum marker enzymes such as serum alanine amino transferase and aspartate amino transferase levels. During hepatic injury, these enzymes leak out from liver into the blood circulation due to liver tissue damage. The treatment of chelidonium extract at a dose of 500mg/kg/p.o reversed the levels of these liver marker enzymes in serum. This can be explained as a result of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by anti-tubercular drugs. Total bilirubin, is a byproduct of the breakdown of red blood cells in the liver. Level of bilirubin is a good indicator of liver function. High levels of bilirubin are indicative of damage to the liver and bile duct[11.] Treatment with ethanolic extract of chelidonium reduced the total bilirubin levels in INH + RIF induced hepatic injury, indicating its protective effect over liver and improvement in its functional efficiency. An earlier study demonstrated hepatoprotective activity of Chelidonium majus in carbon tetrachloride induced toxicity in rats. In this study treatment with C. majus considerably reduced the number of necrotic cells and decreased the activities of transaminases and bilirubin. The results of the present study is consistent with the earlier study SOD, CAT and GPx constitute a mutually supportive team of antioxidant enzymes which provide a defense system against reactive oxygen species [12] (ROS). In the present study, SOD, CAT and GPx activity were decreased significantly in toxic control animals due to an excessive formation of reactive oxygen species such as superoxide anion and H2O2. The treatment of chelidonium extract at a dose of 500mg/kg/p.o effectively prevented the decrease in SOD, CAT and GPx activities suggesting the antioxidant potential of this herb. This proposal is supported by an in vitro study by FRAP assay[13] on C.majus revealed it possesses strong antioxidant activity. The present study showed a marked increase in the concentration of MDA in toxic control animals which indicated an enhanced lipid peroxidation. Treatment with *chelidonium* extract at a dose of 500mg/kg/p.o drastically reduced the anti-tubercular drugs induced elevation of MDA level, suggesting that *chelidonium* extract inhibited the hepatic lipid per oxidation. This report was in unison with an earlier study in which the LPO activity was reduced significantly after treatment with *C. majus* extract against p-DAB induced

hepatocarcinogenesis[14]. In conclusion the treatment with *C. majus* at a dose of 500mg*/kg* reversed the anti-tubercular drugs induced hepatotoxic activity by reducing the elevated liver marker enzymes and antioxidant parameters. Therefore it is suggested that hepatoprotective[15] activity of *C. majus* against anti-tubercular drugs induced hepatotoxicity might be due to its property of reducing oxidative stress.

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