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Original Article

Evaluation of hepatoprotective activity of stem extracts of *Cuscuta reflexa* (roxb) on thioacetamide induced liver damage in rats

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ABSTRACT

Cuscuta Reflexa (Convolvulaceae) is a plant with a variety of ethnic medicinal uses along with antioxidant activity. Hence it was planned to evaluate the hepatoprotective activity with alcoholic (AESCR) and aqueous (AQESCR) extracts of stem of *C. reflexa*. Hepatoprotective activity of both the extracts was studied against thioacetamide induced hepatotoxicity in rats. Functional (thiopentone induced sleeping time), biochemical parameters Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum direct bilirubin (BILD), Serum total bilirubin (BILT), Serum albumin (ALB), Serum total proteins (PRO) and histopathological changes of livers were assessed in control/toxicant/standard/ and extract treated animals with thioacetamide induced hepatotoxic models in rats. In LD_{50} studies for AESCR and AQESCR up to the maximum dose level of 2000 g/kg dose no mortality was observed in any of the animals, indicating the practically nontoxic. When compared to toxicant control groups both the extracts have significantly reduced the thioacetamide induced elevated levels of serum ALT, AST, ALP, BILT, BILD and elevated the levels of ALB and PRO. The histopathological changes (steatosis), necrosis etc. were partly or fully prevented in animals treated with the two extracts. AESCR and AQESCR showed a significant hepatoprotective effect against thioacetamide induced hepatic damage. The medium and high doses of AESCR and AQESCR (200 and 400 mg/kg) treated groups showed better hepatoprotective activity when compared to standard drug silymarin (25 mg/kg p.o.) treated group.

KEY WORDS: *Cuscuta reflexa*, stem extracts, thioacetamide, silymarin, hepatoprotective activity

INTRODUCTION: *Cuscuta reflexa* is a leafless, delicate yellow coloured total stem parasite, belonging to the plant family Convolvulaceae. The tiny white flowers appear in bunches. The fruits are pea shaped and seeds are black in colour [1]. It is found throughout India. The plant is acrid, bitter, astringent to the bowels, aphrodisiac, alternative, tonic and useful in diseases of the eye and of the heart, in biliousness, and in "kapha;. The herb has a bitter sharp taste; used as expectorant, carminative, tonic, anthelmintic, diuretic, blood purifier and lessens inflammation. It is also useful in jaundice, pain in the muscles and joints, headache, paralysis and also in lumbago. It was reported that decoction prepared with stem is useful in constipation, flatulence, liver complaints and bilious affections. The seeds have a bitter bad taste, sedative, emmenagogue, diuretic; useful in diseases of the liver and the spleen, quartan fever, chronic fevers, griping, hiccough, purify the blood and cleanse the bowels; the infusion is given in opthalmia, the decoction in biliousness as a purgative (Unani). The plant is purgative, it is used externally against itch and internally to protract fevers. The stems are especially useful in bilious disorders [1,2,3]. Various published journals and books have revealed that plant based drugs are showing promising hepatoprotective activity and presently except silybon (Micro Labs, Bengaluru) no other allopathic medication is available for the treatment of liver disorders. Some of the plants reported for their hepatoprotective activity are *Andrographis paniculata* [4], *Calotropis procer*[5] , *Fumaria indica* [6], *Luffa acutangula* [7], *Boerhavia diffuse* [8] *etc.* From the literature it was found that *C. reflexa* has also been traditionally indicated for treatment of hepatic disorders. Hence stem extracts of this plant was select for the study of

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hepatoprotective activity in thioacetamide induced hepatotoxic rats.

MATERIALS AND METHODS

Plant material: Stem of *C. reflexa* collected in the month of May and were identified by a botanist Prof. V. Hemanth Kumar, V.L. College of Pharmacy, Raichur and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals: Thioacetamide and Silymarin are gift samples from Shah Scientific, Mumbai, India and Micro Labs- Bangalore respectivily. Thiopental sodium was purchased from Neon Laboratories Ltd., Mumbai, India. The following biochemical kits SGPT, SGOT, ALP, BILT, BILD, ALB and PRO were purchased from Erba Diagnostics Mannheim GmbH, Germany.

Animals: Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25g were procured from National Centre for Laboratory Animal sciences, C/0 Sri.Venkateswara Enterprises, Bengaluru for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry condition. i.e.

Room temperature	-	26 ± 2^0 C
Relative humidity	-	45-55%
Light/ dark cycle	-	12:12 h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur (Karnataka). CPCSEA registration number was 557/02/c/CPCSEA and all the procedures were followed as per rules and regulations.

Preparation of extracts:

Preparation of alcoholic extract: The stem powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated [9].

Preparation of aqueous extract: About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50ºC [9]. These two extracts were stored in airtight containers in a refrigerator below 10ºC. The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies: The acute toxicity of *C.reflexa* was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts of *C. reflexa* and observed for the mortality upto 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. From the LD_{50} doses 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively[10].

Thioacetamide induced hepatotoxicity model [11]**:** Wistar rats weighing between 150-200 g were divided into 9 groups of 6 rats in each. Group A was administered with vehicle for 7 days and served as normal control, group B with thioacetamide (100 mg/kg, s.c.), group C with silymarin (25 mg/kg, p.o) which was serve as standard. Animals in group D, E, F were treated with three different doses (low, medium and high) respectively AESCR and group G, H, I will be treated with three different doses (low, medium and high) of AQESCR for 7 days. Animals of groups B, C, D, E, F, G, H and I were intoxicated with thioacetamide (100 mg/kg s.c) 48 h before last dose of AESCR and AQESCR administration.

Assessment of hepatoprotective activity:

On the 8th day after recording thiopentone induced sleeping time in all animals, all groups were anaesthetized and blood was collected from the retro-orbital puncture. Serum was separated after coagulating at 37°C for 30 min and centrifuging at 2000 rpm for 15 min, and estimated for alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), serum bilirubin (SBLN), serum total protein (PRO) and serum albumin (ALB). The hepatoprotective activity was confirmed through histopathological studies on liver of rats. After collection of blood for biochemical estimation, the rats were sacrificed and the livers were carefully

dissected out, cleaned of extraneous tissue, and fixed in 10% formalin for 24 h. Then the paraffin sections were prepared (automatic tissue processor, Autotechnique) and cut into sections of 5 µm thickness, using a rotary microtome. The sections were stained with Haematoxylin-Eosin dye and studied for histopathological changes [12].

Stastical analysis: All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). P< 0.05*, 0.01** and 0.001*** were considered as statistically significant.

RESULT

In the present study the effect of the AESCR and AQESCR on normal liver functions, was found to be non-toxic in nature. Thioacetamide intoxication in normal rats elevated the levels of ALT, AST, ALP, BILD, BILT and decreased the levels of ALB and PRO significantly, indicating acute centrilobular necrosis. The rats treated with AESCR and AQESCR showed a significant reduction in the biochemical parameters elevated by Thioacetamide (Table.1). Histopathological examination of liver sections of control group (Fig 1) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver sections of the rats intoxicated with thioacetamide (Fig 2), there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal haemorrhages and dilation. The liver sections of the rats treated with silymarin and intoxicated with thioacetamide (Fig 3) and rats treated with AESCR and AQESCR (low, medium and high doses) and intoxicated with thioacetamide (Fig 4-9) showed less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The intensity of centrilobular necrosis was less.

DISCUSSION

Liver diseases are among the most serious ailments and may are classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (fibrosis of liver). They are mainly caused by toxic chemicals, alcohol or drugs like paracetamol, anti-tubercular, anticancer agents etc. Most of the hepatotoxic chemicals damage liver cells either by lipid peroxidation or by other oxidative stress mechanisms induced cellular damage. Liver is susceptible to injury from drugs and other chemical substances, 75% of blood coming to liver arrives directly from gastrointestinal organs and spleen via portal vein which brings xenobiotics and drugs in concentrated form. Several mechanisms are responsible for inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria and intracellular organelle that produce energy and their dysfunction releases excessive amount of oxidants, which in turn injure hepatic cells. Activation of some enzymes in the Cytochrome P-450 system such as CYP-2E1 also leads to oxidative stress and injury to hepatocytes, bile duct cells causing accumulation of bile acid inside liver and which in turn promotes further liver damage. Liver plays an important role in regulation of physiological processes, involved in several vital functions such as metabolism, secretion and storage and also detoxifies a variety of drugs and xenobiotics and bile secreted by the liver has an important role in digestion. Since much of the thioacetamide (TA) transformation mechanism and toxicity studies were conducted largely prior to the advent of the discovery of CYP450 isozymes and information on specific isozymes involved in the bioactivation of TA has remained completely blurred and uninvestigated [13]. Thioacetamide originally used as a fungicide, is a potent hepatotoxicant bioactivated by CYP450 and/or Flavin-containing Monooxygenase (FMO) systems to sulfine (sulfoxide) and sulfene (sulfone) metabolites. The hepatotoxic effects of TA are expressed only after metabolism to thioacetamide S-oxide that further undergoes metabolic conversion to an unidentified metabolite, probably the reactive unstable thioacetamide sulfone. Thioacetamide sulfoxide, a relatively stable intermediate of TA is the penultimate reactive metabolite obligatory for the hepatotoxic effects.

A single large dose of TA 100 mg/kg was reported with degenerative changes in liver at 6-8 h. By 24 h this dose in rats leads to centrizonal necrosis and at 24-30 h the effect was maximal. The prenecrotic changes include loss of glycogen by 6 h and acidophilic degeneration of cells in the central zone by 8 h. By 9^{th} h this is accompanied by the dilatation of sinusoids to form pathways between the central veins of contiguous lobules. Hepatocytes of central zone contain a few lipid droplets and repair begins at 37 h and the liver returned to normal architecture by 7 days.

In chronic drug induced hepatotoxicity models, administration of thiopentone sodium results with an increased duration of sleeping time, as liver is the primary site for the metabolism of xenobiotics like barbiturates and functional damage to liver requires longer time to inactivate thiopentone resulting with an increased duration of action of this drug. Pretreatment with AESCR and AQESCR

have decreased the thiopentone induced sleeping time as compared to toxic control indicating their protection of liver function against drug induced toxicity in rats. Chronic administration of drugs (thioacetamide, ranitidine and paracetamol) to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells. Pretreatment with AESCR and AQESCR decreased the levels of ALT, AST, ALP, BILD, BITD levels and increased PRO and ALB levels, an indication for the hepatoprotective activity of these extracts against drug induced hepatotoxicity. The elevated levels of these parameters were significantly reduced by the treatment of *C. reflexa* stem extracts. It can be concluded from this investigation that stem of *C. reflexa* possess hepatoprotective activity.

CONCLUSION

The preliminary phytochemical analysis of the AESCR and AQESCR *revealed* the presence of carbohydrates, sterols, flavonoids, glycosides, fixed oils fats, saponins and alkaloids. From the studies it can be concluded that AESCR and AQESCR showed a significant hepatoprotective effect against thioacetamide induced hepatic damage as depicted by its protective activity on functional, physical, biochemical and histological changes in liver. The medium and higher doses of AESCR and AQESCR (200 and 400 mg/kg) treated groups showed better hepatoprotective activity when compared to standard drug silymarin (25 mg/kg p.o) treated group. It is found that the AQESCR is more potent than AESCR and that is confirmed by the functional and biochemical parameters followed by comparison of histological changes in liver.

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CONFLICT OF INTEREST: There is no conflict of interest.

Table 1. HEPATOPROTECTIVE EFFECT OF DIFFERENT EXTRACTS OF *C. REFLEXA* ON THIOACETAMIDE (TA) INDUCED HEPATOTOXICITY IN RATS.

Groups	Treatment mg/kg	TST min	ALT U/L	AST U/L	ALP U/L	BILD U/L	BILT U/L	ALB mg/dl	PRO mg/dl
Normal (vehicle)	10ml/kg p.o.	68.00 ±1.48	42.07 ±0.71	107.43 ± 3.30	109.14 ± 2.62	0.19 ± 0.01	0.23 ± 0.06	4.38 ± 0.15	13.94 ±0.44
Toxicant (TA)	100mg/kg s.c.	135.66 ±3.45	128.60 ±4.21	220.38 ±7.31	212.03 ±3.55	2.30 ± 0.06	2.76 ± 0.07	3.33 ± 0.05	6.62 ± 0.09
Standard (silymarin)	25 mg/kg p.o.	88.50 ±1.82**	43.69 ±4.12**	109.69 ±5.19**	115.85 ±2.68**	0.23 ± 0.02**	0.31 ±0.01**	4.22 ± 0.04**	11.70 ±0.12**
AESCR	100	124.66 ±1.50^{ns}	118.73 ±1.39^{ns}	207.31 ±2.37^{ns}	201.03 ± 3.09^{ns}	2.20 ± 0.02^{ns}	2.57 ± 0.08^{ns}	3.51 ± 0.04^{ns}	6.87 ±0.07^{ns}
AESCR	200	119.83 ±0.95**	85.11 ±3.23**	159.93 ±1.24**	166.71 ± 2.41**	1.43 ± 0.03^{ns}	1.73 ± 0.02**	3.76 ±0.04**	8.64 ± 0.11*
AESCR	400	96.50 ±0.96**	71.72 ±1.21**	138.45 ±3.18**	147.91 ±1.07**	0.69 ± 0.02**	0.86 ±0.04**	4.06 ± 0.06**	11.03 ± 0.13*
AQESCR	100	123.50 ±1.67*	112.88 ±4.60*	204.95 ±1.76 ^{ns}	199.85 ±0.95*	2.16 ± 0.05^{ns}	2.52 ± 0.07 ^{ns}	3.60 ±0.07 ^{ns}	7.21 ± 0.11 ^{ns}
AQESC	200	119.33 ±0.71**	84.83 ±1.94**	170.76 ±5.58**	164.55 ±1.59**	1.38 ± 0.03**	1.60 ± 0.09**	3.96 ± 0.06**	8.48 ±0.13**
AQESCR	400	96.00 ±0.73**	62.59 ±0.70**	142.50 ±6.61**	132.60 ±5.32**	0.72 ± 0.04**	0.80 ± 0.05**	4.20 ±0.14**	11.24 ± 0.19**

n = 6, Significant at P< 0.05*, 0.01** and 0.001***, ns = not significant
AESCR- alcoholic extract of stem *of C. reflexa*, AQESCR- aqueous extract of stem of *C. reflexa*
TST – Thiopental sodium sleeping time.
TA- Thioacetamide

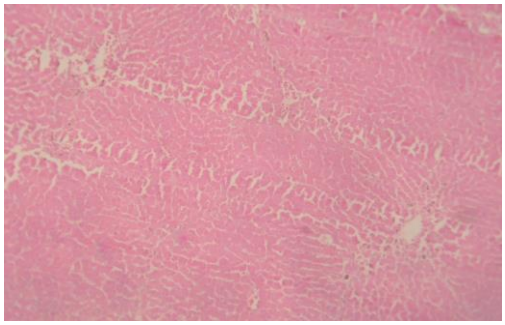

Fig 1. Histology of normal hepatic tissue

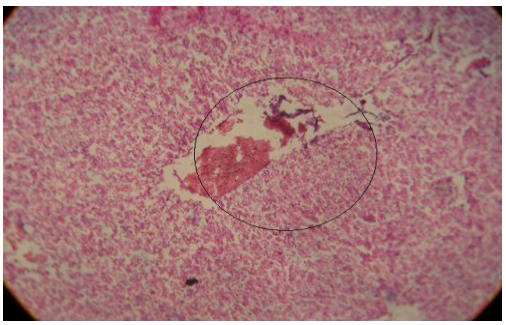

Fig 2. Thioacetamide induced damage in hepatic tissue

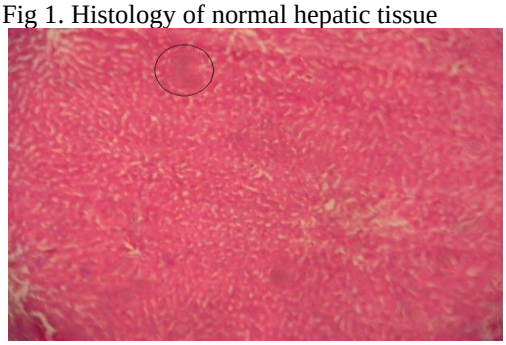

Fig 3. Effect of Silymarin on thioacetamide induced hepatic damage

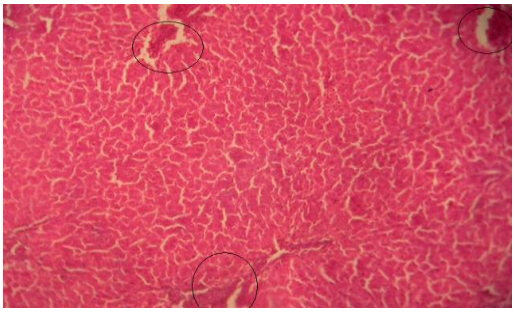

Fig 4. Effect of AESCR (Low) dose on thioacetamide induced hepatic damage

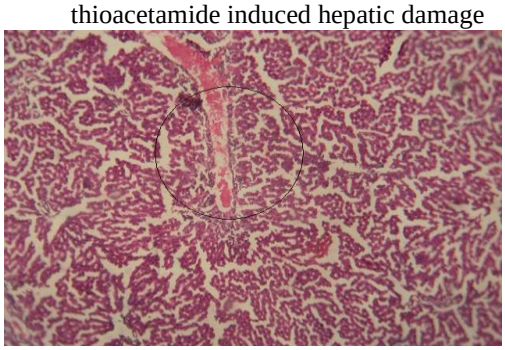

Fig 5. Effect of AESCR (Med) dose on thioacetamide induced hepatic damage

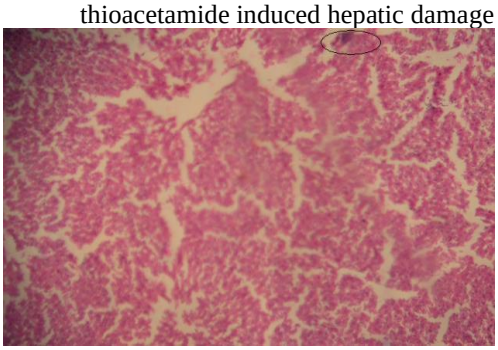

Fig 6. Effect of AESCR (High) dose on thioacetamide induced hepatic damage

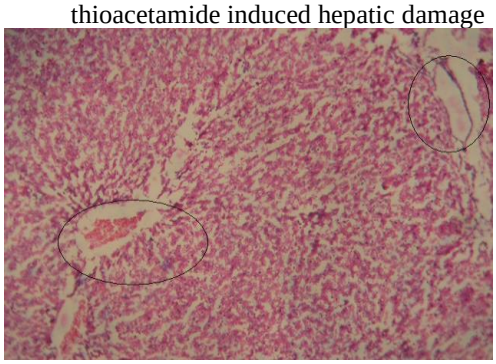

Fig 7. Effect of AQESCR (Low) dose on thioacetamide induced hepatic damage

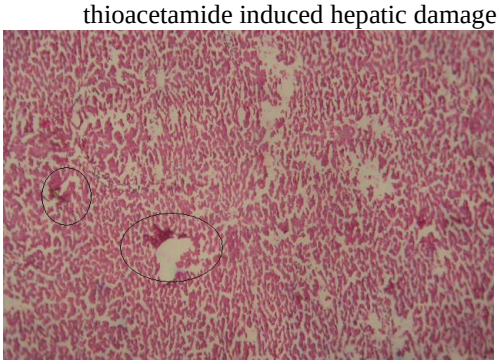

Fig 8. Effect of AQESCR (Med) dose on thioacetamide induced hepatic damage

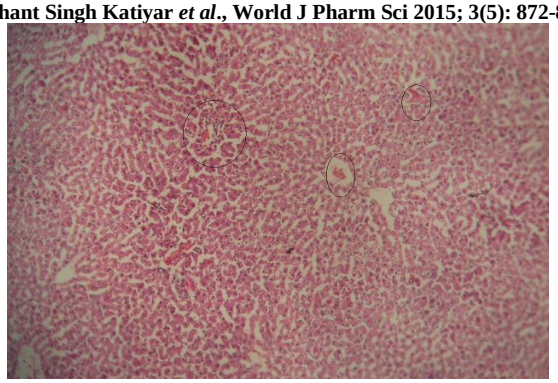

Fig 9. Effect of AQESCR (High) dose on thioacetamide induced hepatic damage

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