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ORIGINAL ARTICLE

HEPATOPROTECTIVE ACTIVITY ON *INDIGOFERA LONGIRACEMOSA* (LEGUMINOSAE)

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ABSTRACT

An ethnomedicinal plant, *Indigofera longiracemosa* Baill., (Leguminosae) was selected and tested for hepatoprotective activity. The leaf material was collected, shade-dried and subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method. The extracts were then collected and used for hepatoprotective activity in experimental animals compared with standard and control groups. The results showed that ethanol leaf extracts showed decreased in liver weight, serum biochemical marker enzymes such as AST, ALT, ALP, total bilirubin and total protein content and significant decrease in the level of enzymes such as SOD, CAT, GPx, GST. Thus the present study revealed the prevention of CCl_4-induced hepatic damage and leaf extracts would need to be explored for new drug by characterizing the active ingredient(s) and elucidating its mechanism(s) of action.

Keywords: India, *Indigofera longiracemosa*, hepatoprotective, liver damage, drug discovery

1.INDRODUCTION

The genus *Indigofera* L., of family Leguminosae comprises of about 700 species in the world, of which 50 species have been reported from India (Santapau and Henry, 1983) while 32 species and 2 subspecies were reported from Tamil Nadu state (Nair and Henry, 1983). The species *Indigofera longiracemosa* Baill., is an ethnomedicinal plant used traditionally for treating various diseases. Ethnomedicinally, Kani tribals of Tirunelveli hills, southern western ghats used leaves for the treatment of skin diseases and roots for snake bite. Chemical constituents such as 3-Isopropyl-9a-methyl-1,2,4a,9a-tetrahydroxanthene and rel-(3S, 5R, *6S*, 8R, 8aR, 12aR)-8-acetoxy-6-butyl-3-isothiocyanatodehydropyrido(2,l) quinoline were isolated from stem (Thangdurai et al., 2001a, b) and various classes of compounds such as amino acids, carbohydrates, flavonoids, gum, oil & resins, proteins, phenolic groups, saponins, steroids, tannins and terpenoids were reported from leaves (Premalatha et al., 2014). Pharmacologically, antimicrobial activity of the petroleum ether, chloroform and ethanolic leaf extract was also reported (Premalatha et al., 2014).

After scrutiny of published literature, so far only little work has been done on this selected plant. The active principles of many drugs found in plants are secondary metabolites. Hence the basic phytochemical investigation on the extracts for their main phytocompounds is very vital. In order to evaluate the ethnomedicinal information, the present study dealt with the hepatoprotective activity of ethanol extract of leaves to develop plant based drug against liver damage.

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2. MATERIALS AND METHODS

Plant Material and preparation of the Extracts

The leaves of *I. longiracemosa* were collected from Tirunelveli hills of southern western ghats, Tamil Nadu. The collected plant material was botanically identified and confirmed by the third author (ACT). The herbarium specimens were preserved and deposited at Bio-Science Research Foundation, Pondicherry (Voucher no. ACT77).
The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo* and stored at 4˚C. Ethanol extract was used for hepatoprotective activity.

Experimental animals

Animals used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India.

They were housed in polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark/10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water ad libitum. The animals were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted over night before the experimental schedule, but have free access for water *ad libitum*.

Hepatoprotective activity

The hepatoprotective of the ethanol extract of *I. longiracemosa* leaves were studied against CCl_4 induced hepatotoxicity in rat model following the method of Lee et al., (2010). The rats were segregated into5 groups of six animals each. The experiment was designed as follows:

Group I : Untreated control rats.
Group II : CCl_4 (2 ml/Kg p.o) treated rats.
Group III : Silymarin (50 mg/Kg p.o) + CCL_4 (2 ml/Kg p.o) treated rats
Group IV : Ethanolic extract (200 mg/kg p.o) + CCl4 (2 ml / kg p.o) treated rats.
Group V : Ethanol extract of (400 mg/Kg p.o) + CCl4 (2 ml /Kg p.o) treated rats.

Group-I served as control and received normal water daily for 14 days. Group-II rats received olive oil (2 ml /kg p.o.) for 14 days. Group-III received silymarin (standard hepatoprotective herbal formulation from *Silibum marianum*) every day at the rate of 50 mg/ kg p.o. for 14 days. To determine the hepatoprotective dose response of ethanolic extract daily doses at concentrations 200 and 400 mg/kg p.o. were administered for 14 days to Group IV- VI respectively. On day 14, six hours after the last dose of standard drug and test sample, CCl4 (1:1 v/v in olive oil) was administered at the dose of 2ml/ kg bw p.o. to group II-VI. Twenty four hours after the dose of CCl4 administration, blood samples were collected from all groups by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min and the serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters. After collection of blood samples, the rats were sacrificed and their liver excised, rinsed in ice cold normal saline followed by 0.15 M Tris HCl (pH 7.4), blotted dry and weighed.

Assessment of liver function

The biochemical parameters which were known to be altered by CCl_4 were measured as markers for evaluating hepatoprotective activity. In serum, total protein, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase and total bilirubin were analyzed using commercially available test diagnostic kits and their manual. In addition, various Biochemical parameters such as Protein by Lowry et al., (1957), Superoxide Dismutase by Das *et al*., (2000), Catalase by Sinha (1972), Glutathione Peroxidase by Ellman (1959), Glutathione S Transferase activity by Habig *et al*., (1974) and LPO by Niehius and Samuelsson (1968) were also estimated.

Statistical Analysis

The results were expressed as mean standard error mean (SEM). Statistical analysis was carried out by analysis of variance (ANOVA) followed by Dunnet's test. $p<0.01$ and $p<0.05$ were considered as indicative of significance, as compared to the control group. All calculations were performed using: SPSS (version 11.0; Chicago, IL, USA).

3. RESULTS

The results of liver weight observed were given in the Table 1. Regarding the liver weight, high value was recorded as 7.66 ± 0.72 in CCl_4-treated animals than in control animals while the extracts resulted significant reduction from 7.24 ± 0.17 at 200 mg to 6.12 ± 0.22 at 400 mg compared to those of CCl_4-treated rats. In the present study, the CCl_4 treated animals exhibited a significant increase in the serum biochemical marker enzymes such as AST, ALT, ALP, total bilirubin and total protein content compared to control group (Table 2). Ethanol extracts significantly reduced the effect of CCl_4 i.e.,AST reduced to 132.27 ± 0.52 at 200 mg and 89.07 ± 0.49 at 400 mg compared to control group. Similarly, ALT reduced to 125.37 ± 0.39 at 200 mg and to 78.32 ± 0.62 at 400 mg, ALP reduced to 129.02 ± 0.11at 200 mg and 77.32 ± 0.17 at 400 mg, total bilirubin reduced to 8.19 ± 0.28 at 200 mg and 6.01 ± 0.27 at 400 mg while total protein content was increased to 6.92 ± 0.23 at 200 mg and 7.86 ± 0.43 at 400 mg respectively. The effects of ethanol extract of *I. longiracemosa* on Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-S-transferase (GST), Lipid peroxidation (LPO) were given in the Table 3. In the present study, the CCl_4 treated animals exhibited a significant decrease in the level of enzymes such as SOD, CAT, GPx, GST and significant increase in the LPO compared to control group. Ethanol extracts significantly decreased the effect of CCl_4 i.e.,SOD reduced to 0.19 ± 0.022 at 400 mg and 0.28 ± 0.033 at 200 mg compared to control group. Similarly, CAT reduced to 20.45 ± 0.16 at 400 mg to 23.53 ± 0.25 at 200 mg, GPx reduced to 66.34 ± 0.65 at 400 mg and to 76.35 ± 0.39 at 200 mg, GST reduced 78.59 ± 0.37 at 400 mg and 95.93 ± 0.72 at 200 mg while LPO increased to 18.39 ± 0.69 at 200 mg and 12.26 ± 0.33 at 400 mg respectively.

Table 1. Effect of ethanolic extract of *I. longiracemosa* on liver weight.

Groups	Liver weight (g)
Control	5.43 ± 0.48
Induced	7.66 ± 0.72
Standard	5.88 ± 0.48
200 mg	7.24 ± 0.17
400 mg	6.12 ± 0.22

Values are expressed as mean ± SD (n=6).

4. DISCUSSION

The present study demonstrates the hepatoprotective effects of ethanol extract of leaves against CCl4-induced liver injury in rats. The liver is one of the vital organs in our body responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Numerous studies noted that CCl_4 is widely used to induce liver damage

Table 2. Effect of ethanolic extract of *I. longiracemosa* on serum biochemical markers.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Total Bilirubin (mg/dl)
Control	64.60 ± 0.73	55.19 ± 0.68	62.22 ± 0.68	8.60 ± 0.21	4.21 ± 0.39
Induced	177.41 ± 0.43	161.79 ± 0.82	184.56 ± 0.68	5.70 ± 0.08	10.41 ± 0.42
Standard	71.48 ± 0.68	61.69 ± 0.63	69.75 ± 1.05	8.44 ± 0.06	5.10 ± 0.39
200 mg	132.27 ± 0.52	125.37 ± 0.39	129.02 ± 0.11	6.92 ± 0.23	8.19 ± 0.28
400 mg	89.07 ± 0.49	78.32 ± 0.62	77.32 ± 0.17	7.86 ± 0.43	6.01 ± 0.27

Values are expressed as mean ± SD for six animals.

Table 3. Effect of ethanolic extract of *I. longiracemosa* on certain antioxidant markers against CCl4 induced hepatotoxicity.

Groups	SOD	CAT	GPx	GST	LPO
Control	0.30 ± 0.006	25.05 ± 0.66	89.54 ± 0.98	107.08 ± 0.58	9.07 ± 0.23
Induced	0.21 ± 0.005	16.52 ± 0.37	58.30 ± 1.60	51.37 ± 0.38	23.86 ± 0.27
Standard	0.26 ± 0.096	23.59 ± 0.35	85.58 ± 0.27	103.68 ± 0.45	11.88 ± 0.24
200 mg	0.28 ± 0.033	23.53 ± 0.25	76.35 ± 0.39	95.93 ± 0.72	18.39 ± 0.69
400 mg	0.19 ± 0.022	20.45 ± 0.16	66.34 ± 0.65	78.59 ± 0.37	12.26 ± 0.33

Values are expressed as mean ± SD for six ;animals; SOD – Superoxide dismutase (Units/min/mg protein)
CAT – Catalase (μ moles of H2O2 consumed/min/mg protein)

because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis (Bahceioglu et al., 1990; Aleynik et al., 1997; Halliwell and Gutteridge, 1998; Ashok et al., 2001; Weber et al., 2003; Fang et al., 2008). CCl_4 induced the significant increase in liver weight, may due to the blocking of secretion of hepatic triglycerides into the plasma (Yoko et al., 2005). To prove this, in the present study, the ethanol extract of *I. longiracemosa* and standard drug significantly prevented the increase in liver weight. Increased levels of ALT, AST and ALP in serum of the CCl_4-treated animals indicate liver damage as these enzymes leak out from liver into the blood at the instance of tissue damage, which is always associated with hepatonecrosis (Naik and Panda, 2008; Ree and Spector, 1961). With the treatment of leaf extract, the levels of these marker enzymes were near normal substantially reduced, indicating protection against liver damage. ALP activity is related to the functioning of hepatocytes. Suppression of increased ALP activity suggests the stability of biliary dysfunction in rat liver during chronic hepatic injury with CCl_4. Decreases in total protein and albumin levels induced by CCl_4 is a further indication of liver damage (Navarro and Senior, 2006). In the present study, the leaf extract has increased the levels of serum total protein towards the respective normal values. Stimulation of protein synthesis assumes significance as it has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells (Rip et al., 1985; Tadeusz et al., 2001). CCl_4 not only initiates lipid peroxidation (LPO) but also reduces tissue CAT, GPx, GST and SOD activities, and this depletion may result from oxidative modification of these proteins (Augustyniak et al., 2005). Our results showed that administration of leaf extract effectively protected against the loss of these antioxidant activities despite CCl_4 administration and it is well known to serve diverse biological functions, including protection of cells from oxidative damage by ROS and free radicals (Nakamura et al., 2001; Gabele et al., 2009). The activity may be attributed due to the presence of various phychemicals in leaves as previously reported by Premalatha et al., (2014). Phytochemicals have also been shown to stimulate synthesis of anti-oxidant enzymes and detoxification systems at the transcriptional level, through antioxidant response elements (Masella et al., 2005) and to increase α-glutamylcysteine synthesis (Kim et al., 2007). Phytoconstituents like the flavonoids (Baek et al., 1996), triterpenoids (Xiong et al., 2003), saponins (Tran et al., 2001) and alkaloids (Vijayan et al., 2003) are known to possess hepatoprotective activity. The presence of flavanoids in the ethanol extract may be responsible for its hepatoprotective activity. In conclusion, the results of this study revealed that the prevention of CCl_4-induced hepatic damage can be attained through *I. longiracemosa*. However, the protective, curative and liver protection qualities of *I. longiracemosa* need to be explored for new drug by characterizing the active ingredient(s) and elucidating its mechanism(s) of action.

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