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Hepatoprotective effect of *Helicanthus elastica*

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Abstract

Search for medicinal plants to treat liver disorders is an important research topic on herbs. Acute toxicity study is a prerequisite for safety and dose fixation for further pharmacological actions. In the present study, aqueous and 95% ethanolic extract of whole plant of *Helicanthus elastica* were subjected to acute oral toxicity. The aqueous and ethanolic extract revealed no observable changes in the rats up to the dose level of 2,000 mg /kg body weight. The extracts were then screened for paracetamol-induced hepatic injury at dose levels of 200 and 400 mg/kg body weight (1/10 and 1/5 LD_{50} based on toxicity study). The aqueous extract of whole plant of *H. elastica* was found to produce significant ($p<0.05$) reversal of the paracetamol-induced changes in the measured biochemical and histopathological parameters at lower dose of 200 mg/kg which was found to be better than ethanol extract at the same dose level.

Introduction

Mistletoes, the hemiparasites of Loranthaceae are medicinally important (Bussing, 2004). Many of these types of plants have not been studied scientifically though references are found in texts of Ayurveda and other folklore medicines. Six Indian mistletoes, two belonging to the genus *Loranthus* and four belonging to the genus *Viscum* are considered to be medicinal. *Helicanthus elastica* (Desr.) Danser syn. *Loranthus elasticus* Desr. is a less studied common Indian mistletoe claimed to be anti-abortifacient, useful in vesical calculi and kidney affections (Kirtikar and Bsu, 1935). The plant is reported to have diuretic (Aleykutty et al., 1991; Jadhav et al., 2010), anti-tumour (Mary et al., 1994), antimicrobial (Sunil Kumar et al., 2014a), anti-oxidant (Sunil Kumar et al., 2014b) activities. It is a source of many nutritional elements (Sunil Kumar et al., 2014c) and is found to contain friedelin, epifriedelinol, β-amyrin, β-sitosterol, ethyl gallate, gallic acid and β-sitosterol-3-β-D-glucopyranoside as major constituents (Sunil Kumar et al., 2015).

Acute toxicity study, as per Organization for Economic Co-operation and Development guidelines (OECD) 423, is a stepwise procedure to fix a minimal number of animals, which allows acceptable data based scientific conclusion about the toxicity potential of the test drug. The method uses defined doses and the results allow a substance to be ranked and classified according to the globally harmonized system for the classification of chemical which cause acute toxicity.

Hepatotoxicity from the drugs and chemicals is the commonest form of iatrogenic disease and it is among the most prevalent serious health problems (Schiodt et al., 1997). Only symptomatic relief is achieved through corticosteroids or immunosuppressive agents without any modification in the course of disease.

On the other hand, herbal drugs play a major role in the

management of various liver disorders most of which speed up the natural healing processes of the liver (Negi et al., 2008).

In the present study, aqueous and 95% ethanolic extract of whole plant of *H. elastica* growing on mango trees were subjected to acute toxicity study and hepatoprotective activity. The extracts were screened against paracetamol-induced hepatic injury at dose levels of 200 and 400 mg/kg body weight (1/10 and 1/5 LD_{50} based on toxicity study).

Materials and Methods

Preparation of test extracts

Authenticated fresh plants of *H. elastica* growing on *Mangifera indica* were collected during flowering in the month of August, 2010 from Kasaragod District of Kerala. Voucher specimen of the plant (No. 00637) was deposited at the Pharmacognosy Department of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai. Shade dried whole plant material was extracted with ethanol (95%) and double distilled water by cold percolation. The ethanolic extract was concentrated over a water bath and aqueous extract was dried in freeze drier to obtain coffee brown residue. The yield was 19 and 12% w/w for ethanolic and aqueous extracts respectively.

Animals

The animals were obtained from the animal facility attached to the Pharmacology Laboratory of Central Research Institute for Siddha. The experiments were carried out after obtaining permission from institutional animal ethics committee (Approval number 79/PHARMA/SCRI/2010). Animals were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature (22 ± 2°C) and humidity (50 to 60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and water given *ad libitum*.

Acute toxicity (Video Clip)

The acute oral toxicity study of ethanolic and aqueous extracts were carried out using Swiss albino mice of female sex. The mice were fasted overnight and provided with water *ad libitum* and divided into 4 groups of three animals each. The ethanol and the aqueous extracts were suspended in 1% sodium carboxyl methyl cellulose (Sanjeev et al., 2013). The starting dose level of aqueous and ethanolic extracts were 2,000 mg/kg body weight p.o. as most of the crude extracts possess LD_{50} value more than 2,000 mg/kg. After oral administration, the mice were observed on an hourly basis for 24 hours to assess the general behavior, any signs of toxicity and mortality. They were further observed for 14 days for toxic symptoms and mortality. Mice were weighed before starting and ending acute toxicity and any changes in skin fur, eyes, mucous membranes, central nervous system and behavioral pattern were observed. Tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and any specific severe sign were also noted.

Hepatoprotective activity

Forty two Wistar strain albino rats of both sexes weighing between 180 to 200 g were used for hepatoprotective activity evaluation. Rats selected for the study were randomly divided into 7 groups of either sex comprising six rats in each group. Ethanolic extract, aqueous extract, paracetamol and silymarin were suspended with 1% sodium carboxyl methyl cellulose. The test extracts and vehicle to control were administered according to the body weight of the rats by oral route with the help of gastric catheter of suitable size sleeved to a syringe nozzle.

Rats of either sex were grouped randomly into groups of six rats each, male and female rats were housed separately to prevent possibility of pregnancy. Group I – Normal control rats received 1% sodium carboxyl methyl cellulose for 7 days (10 mL/kg body weight). Group II – Toxicant control rats received vehicle (1% sodium carboxyl methyl cellulose) for the first 5 day and on 5th day hepatic injury was induced by the administration of paracetamol 2 g/kg body weight through oral route. Group III - Positive control (reference standard) rats were treated with silymarin (40 mg/kg) for the first 5 days and on 5th day hepatic injury were induced by the administration of paracetamol 2 g/kg body weight through oral route. Group IV – Rats were treated with 200 mg/kg body weight of ethanolic extract of *H. elastica* for the first 5 days and on 5th day hepatic injury was induced by the administration of paracetamol 2 g/kg body weight through oral route. Group V - Rats were treated with 400 mg/kg body weight of ethanolic extract of *H. elastica* for the first 5 days and on 5th day hepatic injury was induced by the administration of paracetamol 2 g/kg body weight through oral route. Group VI - Rats were treated with 200 mg/kg body weight of aqueous extract of *H. elastica* for the first 5 days and on 5th day hepatic injury was induced by the administration of paracetamol 2 g/kg body weight through oral route. Group VII - Rats were treated with 400 mg/kg body weight of aqueous extract of *H. elastica* administered for the first 5 days and on 5th day hepatic injury was induced by the administration of paracetamol 2 g/kg body weight through oral route.

On 7th day blood was collected from all rats before sacrifice under light ether anesthesia by retro-orbital puncture and allowed to coagulate for 30 min at 37°C and centrifuged to obtain serum. Clear serum was used for estimation of SGOT, SGPT, alkaline phosphatase activity, serum total protein and total bilirubin levels.

Immediately after the sacrifice, liver and kidney were dissected out. Wet weight was recorded and part of the tissue was fixed in 10% formalin and processed for histopathological study as described above. The liver and kidney sections from different groups were examined under trinocular research microscope at different magnifications to record the magnitude of liver injury. The sections from paracetamol control group were compared with the sections of paracetamol administered reference standard or test extract pre-treated groups.

Estimation of biochemical parameters

Estimations were done using semi-auto-analyser Bayer RA-50. Requisite quantity of serum was fed to the analyzer, which was automatically drawn in to the instrument for estimating different parameters. Methodology mentioned in the kit (SIEMENS Autopak) for the respective estmation along with literature mentioning the basis of the methods on which test procedures have been evolved was followed for the biochemical estimations.

SGOT, SGPT and ALP was estimated by UV kinetic IFCC method (Bergmeyer et al., 1986), total bilirubin was estimated by Jendrassik and Grof method (Garber, 1981), and serum total protein content was estimated by Biuret method (Lubran, 1978).

Statistical analysis

Data were expressed as mean ± SEM. The statistical analysis was carried out using one way ANOVA with Tukey multiple comparison tests as post hoc test. For this purpose Graph Pad Instat version 3 was used.

Results

Acute toxicity study

No mortality was observed with 2,000 mg/kg oral dose for both aqueous and ethanol extracts. Further no discernible behavioral changes could be observed. There was no effect on body weight, food consumption and fecal output.

Biochemical observation of animals grouped under hepatoprotective study

Administration of paracetamol in the dose of 2 g/kg orally produced marked elevation in all the biochemical parameters studied except serum total protein content which was found to be decreased. The elevation observed was 203% in SGOT activity; 119% in SGPT activity; 219% in ALPase activity; 229% in serum total bilirubin level, however, total protein content was found to be decreased by 48%. All these changes were found to be statistically significant in comparison to the normal control group. Administration of silymarin was found to reverse the paracetamol-induced changes to almost normal level. The observed reversal was found to be statistically significant with respect to all the parameters ($p<0.01$). Administration of ethanol extract of *H. elastica* at both of the dose levels was found to antagonize the paracetamol-induced elevation in SGOT and SGPT activity however, it was not dose dependent. In contrast to the effect observed with silymarin the elevated level of ALPase was not reversed by administration of the ethanol extract of *H. elastica* instead a mild non-significant further elevation at 200 mg/kg dose and moderate but statistically significant elevation with 400 mg/kg was observed in comparison to paracetamol control group. The paracetamol induced decrease in serum total protein content was found to be reversed by both the doses of ethanol extract of *H. elastica.* Significant reversal of paracetamol-induced elevation in serum total bilirubin level ($p<0.05$) was observed with 200 mg/kg dose of ethanol extract of *H. elastica.* Though reversal was observed with 400 mg/kg dose it did not reach statistically significant level.

In aqueous extract treated groups also reversal of many of the paracetamol-induced biochemical parameter alterations was observed. However, there was some difference in the changes observed. Aqueous extract at both the dose level significantly lowered paracetamol induced elevation in SGPT activity ($p<0.05$ Vs paracetamol control). Though lowering of the toxicant induced elevation in SGOT activity was also observed the reversal was found to be statistically non-significant. The alkaline phosphatase activity was found to be apparently elevated in comparison to paracetamol control with both the doses of the aqueous extract, however, the increase was found to be statistically non-significant. The toxicant induced lowering of serum total protein was found to be significantly reversed at both the dose level of aqueous extract ($p<0.05$). Similarly, reversal of toxicant induced elevation in serum total bilirubin was also observed. However, the reversal was found to be statistically significant ($p<0.05$) only with respect to the lowering observed with lower dose. The reversal observed with aqueous extract was not dose dependent with respect to all the parameters in which reversal of the toxicant induced changes were observed (Table I).

Histopathological examination of liver sections

Microscopic examination of the liver sections from control group rats showed normal cytoarchitecture (Figure 1A). The liver sections from paracetamol control group exhibited features of extensive liver damage. The changes observed were appearance of hemorrhagic areas, fatty degeneration of centrilobular region, hepatocellular necrosis at multiple loci, congestion and inflammatory changes in the form of cell infiltration (Figure 1B). The liver sections obtained from sylimarin pre-treated and paracetamol dosed animals showed only few degenerative changes. Few spots of micro-

Table I

Effect of *Helicanthus elastica* extracts on different biochemical parameters

Groups	SGOT [U/L]	SGPT [U/L]	ALP [U/L]	TP [g/100 mL]	SB [U/L]
Control	62.8 ± 4.1	42.3 ± 2.8	75.3 ± 4.9	6.2 ± 0.3	0.3 ± 0.1
Paracetamol 2 g/kg	190.5 ± 7.5[a]	92.7 ± 4.7[a]	240.7 ± 4.0[a]	3.2 ± 0.7[a]	1.0 ± 0.1[a]
Sylimarin 40 + Paracetamol 2 g/kg	63.3 ± 1.5[b]	45.3 ± 2.7[b]	75.0 ± 1.4[b]	7.2 ± 0.3[b]	0.4 ± 0.1[b]
HEAl 200 + Paracetamol 2 g/kg	98.8 ± 14.9[b]	60.5 ± 3.8[b]	285.7 ± 20.1	5.5 ± 0.4[b]	0.4 ± 0.1[b]
HEAl 400 + Paracetamol 2 g/kg	123.0 ± 23.6[b]	63.2 ± 9.3[b]	403.2 ± 50.5[b]	6.7 ± 0.5[b]	0.8 ± 0.2
HEAq 200 + Paracetamol 2 g/kg	155.3 ± 11.0	44.6 ± 06.7[b]	296.8 ± 18.2	6.5 ± 0.4[b]	0.6 ± 0.1[b]
HEAq 400 + Paracetamol 2 g/kg	157.8 ± 20.4	49.8 ± 09.6[b]	304.2 ± 28.4	5.7 ± 0.3[b]	0.7 ± 0.1

Data are mean ± SEM; [a]p<0.05, when compared with normal control, [b]p<0.05, when compared with paracetamol control (Tuckey-Kramer test); HEAq, Aqueous extract of *H. elastica;* HEAl, Ethanolic extract of *H. elastica;* ALP, Alkaline phosphatase; SB, Serum bilirubin; SGOT, Serum glutamate oxaloacetate transaminase; SGPT, Serum glutamate pyruvate transaminase; TP, Total protein

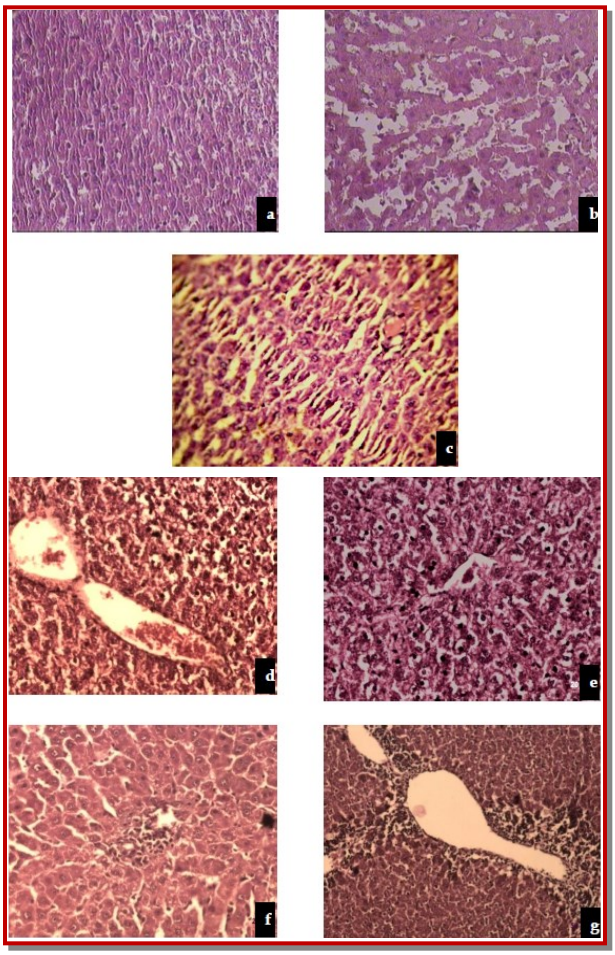

Figure 1: Histopathological study of liver in paracetamol-induced hepatotoxicity in rats (a) Normal control; (b) Paracetamol 2,000 mg/kg treated; (c) Silymarin 100 mg/kg treated; (d) Paracetamol 2,000 mg/kg and HEAl 200 mg/ kg; (e) Paracetamol 2,000 mg/kg and HEAl 400 mg/ kg (new); (f) Paracetamol 2,000 mg/kg and HEAq 200 mg/ kg(both are of same); (g) Paracetamol 2,000 mg/kg and HEAq 400 mg/ kg

fatty changes were observed. Necrosis was not present; cell infiltration was also much less (Figure 1C). Microscopic examination of sections from 200 mg/kg dose ethanolic extract pre-treated and paracetamol administered group showed almost normal cytoarchitecture – only few loci of mild cell infiltration and cell depletion could be observed. Fatty changes were almost absent (Figure 1D). The histological architecture of liver sections of the rats treated with ethanolic extract 400 and paracetamol dosed showed moderate changes in degeneration of hepatocytes and central vein and sinus congestion and centrolobular necrosis was completely prevented. Mild cell infiltration and few spots of fatty degeneration were observed (Figure 1E). Microscopic examination of sections from 200 mg/kg dose of aqueous extract pre-treated and paracetamol administered group showed almost normal cytoarchitecture, only few loci of mild cell infiltration could be observed. Fatty changes were absent (Figure 1F). The histological architecture of liver sections of the rats treated with aqueous extract 400 and paracetamol dosed showed severe inflammatory changes in the form of focal cell infiltration around central vein, however, fatty changes and necrosis were not observed (Figure 1G).

Discussion

Acute oral toxicity of the aqueous and ethanolic extract of *H. elastica* as per OECD guideline number 423 revealed no observable changes in the animals under investigation up to the dose level of 2,000 mg /kg body weight. Since no mortality was observed at the dose level tested, 200 and 400 mg /kg body weight were fixed as therapeutic dose for further pharmacological activity evaluation.

In the present study administration of toxic dose of

paracetamol lead to marked liver injury. Liver sections from this group exhibited extensive degenerative changes. Further there was significant elevation of bio-markers like SGOT, SGPT, ALPase activity, serum bilirubin and significant decrease in serum total protein. All these paracetamol induced changes were reversed or significantly attenuated by pretreatment of the rats with silymarin. This shows that the test protocol employed is sensitive enough for assessing the hepatoprotective activity in putative hepatoprotective agents (Eoin et al., 2007).

The ethanolic extract of whole plant of *H. elastica* was found to produce significant reversal of the paracetamol induced changes in the measured bio-chemical and histopathological parameters. However, surprisingly the reversal was not dose dependent. At lower dose level there was significant reversal of paracetamol induced histopathological changes in the liver and reversal of SGPT, SGOT, SB elevation and SP decrease. ALPase elevation was though reversed with the pre-treatment with silymarin the ethanolic extract did not affect it at lower dose level and further elevation was observed at higher dose level. The exact reason for this observation is not known, it does not seems to be indicative of status of functional integrity of liver. At higher dose level the reversal of liver histopathology was moderate corroborating the changes observed in bio-chemical parameters. In fact at the higher dose level paracetamol induced change in total bilirubin was not reversed to significant extent. Even the reversal observed in SGPT and SGOT elevation was moderate and less in comparison to the reversal observed with lower dose. This indicates that the ethanol extract at higher dose level has only moderate hepatoprotective activity.

Analysis of the data obtained with aqueous extract of whole plant of *H. elastica* showed results similar to the ethanolic extract. The liver sections from the lower dose aqueous extract administered group exhibited almost normal cytoarchitecture. Only a mild cell infiltration was observed. The observed effect was found to be even better than that observed with lower dose of ethanol extract. Again surprisingly at higher dose level the hepatoprotection was only moderate. At higher dose marked peri-vascular cell infiltration was observed. The exact reason for not observing the dose dependent action is not known. It can be suggested that the extract contains many active principles, some of which may have mutually antagonistic effects because of this at higher dose level the effect of hepatoprotective active principle may get reduced. It is also possible that there may be pharmacokinetic reasons for the observed decrease in the magnitude of hepatoprotection at higher dose level which require further elaborate studies.

Phytochemical analysis of the extracts revealed presence of significant amount of polyphenols in them (Sunil, 2011). Polyphenol content has been held responsible for the observed hepatoprotective activity of many plants (Tirtha et al., 2007; Sangeetha and Krishnakumari 2010; Desai et al., 2012). This effect has been attributed to the strong anti-oxidant activity of polyphenol fractions (Kaviarasan et al., 2007). It is possible that the same mechanism of action may be responsible for the observed hepatoprotection. Based on the observation from anti-oxidant study it can be suggested that at least part of the observed activity is due to anti-oxidant activity reported for it (Sunil Kumar et al., 2014b).

It has been reported that when rats are treated with N-acetyl-p-aminophenol, they develop inflammatory response in the liver (Bauer et al., 2000). This inflammatory reaction is supposed to be due to the release of chemotactic factors from the hepatocytes. It is possible that the tested extracts may produce their protective effect by neutralizing or down grading this inflammatory response by modulating the formation of inflammatory cytokines.

Paracetamol therapy causes depletion of the anti-oxidant enzymes, including SOD, CAT and GSH-Px. It is also showed a depletion of GSH-Px (glutathione peroxidase) activity in paracetamol toxicity (Guven et al., 2008). GSH-Px is an important enzyme for protection against GSH depletion in tissues. Paracetamol may produce hepatotoxicity by direct effect also. Possibly, it may be through either protein damage or by the accumulation of reactive oxygen species and pro-inflammatory cytokines after the initial injury. It has also been observed by the same authors that nitric oxide level dramatically increased following paracetamol administration. It can be assumed that the oxidative injury due to that toxicant would be attenuated due to the anti-oxidant activity of the polyphenols present in the test extracts.

The observed hepatoprotection is probably through preventing or down grading generation of damaging free radical cascades, oxidant radical release, and through its attenuation of the consequent pro-inflammatory processes.

Conclusion

The ethanolic extract of whole plant of *H. elastica* was found to produce significant reversal of the para-cetamol-induced changes in the measured biochemical and histopathological parameters at lower dose of 200 mg/kg.

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