Research Article

In vivo safety evaluation and choleretic activity of ethanolic extract of *Pergularia daemia* (Forsk.)

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Abstract

Objective: *Pergularia daemia* (Forsk.) of Asclepiadaceae family is well known for its hepatorenal protective ability, however very less data is available for its safety evaluation and choleretic activity. The present study was performed to investigate no-observed-adverse-effect level (NOAEL) and choleretic activity of *P. daemia* leaves extract. **Materials and Methods:** *P. daemia* leaves extract was orally administered at 100, 200, 400 and 800 mg/kg dose to female wistar rats for seven days. Blood was collected and serum biochemical parameters were performed after 24 h of last treatment. Evaluation of choleretic activity was done by intraduodenal administration of extract at 400 mg/kg followed by bile collection. **Results:** There were no significant change in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, albumin, urea, uric acid, creatinine, triglycerides and cholesterol. Slight increase in bile volume and bile solid contents was found in tested and dihydrocholic acid (DHC) treated standard group compared to control. **Conclusion:** *P. deamia* leaves extract is safe for *in vivo* studies in rats (NOAEL 800 mg/kg) and it also possess choleretic potential.

***Keywords*:** *Pergularia daemia*, No-observed-adverse-effect level, cholerectic activity

Introduction

The benefits of plants on human health are often ascribed to presence of various polyphenols and secondary metabolites. Phytochemicals have potential to scavenge free radicals responsible for oxidative stress induced diseases in biological system (Jahromi et al., 2017). Therefore search for potent herbal therapeutic agent for treatment of oxidative stress induced diseases is current research interest. *Pergularia daemia* Forsk. (*P. daemia*) commonly known as "*Utranajutuka*" in hindi, belongs to Asclepiadaceae family predominantly found at roadsides in India. This plant contains phytochemical compounds including alkaloids, flavonoids, terpenoids, tannins, steroids, proteins and carbohydrate (Karthishwaran et al., 2010). Leaves of *P. daemia* are reported to possess large amount of flavonoids such as qurecetin, naringenin and taxifolin responsible for its excellent therapeutic activity (Ananth et al., 2016). In traditional medicine, *P. daemia* is prescribed as remedy for rheumatic swelling (Karthishwaran et al., 2010), diabetes (Wahi et al., 2002), bacterial diseases (Senthilkumar et al., 2005) and liver disorders (Suresh kumar and Mishra, 2006). The plant is also reported to have potential anti-inflammatory, anti-rheumatic and anti-pyretic activity (Minirth et al., 2005).

Bile is secreted by hepatocytes and released into duodenum to facilitate lipid absorption in intestine. Furthermore it also contributes in excretion of waste substances such as metals, drugs, breakdown product of haemoglobin and excess cholesterol. Thus biliary system is elimination pathway for toxic substances and plays a major role in liver purification (Bevalot et al., 2016). Choleretics are substances which increase the secretion of bile volume as well as bile solids. Plants are reported to possess choleretic potential that stimulate hepatocytes to increase output of bile (Wang et al., 2016).

Risk assessment of plant extracts prior to their medicinal use is an inevitable part of drug validation and standardization.

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Although, *P. daemia* extract is popularly used traditional medicine against various diseases, however systematic toxicity study of the plant extract is lacking. Therefore, present study was carried out to investigate no-observed-adverse-effect-level (NOAEL) and choleretic activity of *P. daemia* leaves extract.

Materials and Methods

Collection and extraction of plant materials

The whole plant was collected from Bilaspur district, Chhattisgarh in India. The plant was identified by an eminent botanist and voucher specimen No. GG/C/APO/102 was deposited in the herbarium of Department of Botany, Guru Ghasidas University. Fresh leaves were separated from plant and washed in distilled water to remove dust. Then it was dried in shade at room temperature. Dried leaves were subsequently sieved separately to obtain a fine powder. Then 70% ethanolic extract of fine powder was prepared using accelerated solvent extractor. Extract was dried at room temperature and stored in refrigerator at 4°C for future use in toxicity analysis. Aqueous suspension of leaves extract was prepared at 100, 200, 400 and 800 mg/ kg dose for evaluation of NOAEL and choleretic activity.

In vivo safety evaluation

Animals and chemicals

Female wistar rats (10–12 weeks old; 180 ± 20 g) were bought from Defense Research and Development Establishment, Gwalior, India. All animal procedures were approved by institutional animal ethics committee (994/Ere/GO/06/CPCSEA). Rats were housed in chemical contamination free and thermally controlled (25 ± 2°C) room. Relative humidity of 60-70% and 12 h dark–light cycle was maintained. Standard pelleted diet (Pranav Agro., Pune) was fed to rats with free access to tap water. Animals were acclimatized to laboratory environment for ten days before the beginning of experiments. Pure and analytical grade chemicals acquired from standard chemical suppliers were used in present study.

No-observed-adverse-effect-level (NOAEL)

For estimation of NOAEL activity rats were randomly stratified to 5 groups of 6 animals in each. Group 1 served as control, Group II-V were administered with *P. daemia leaves* extract at 100; 200; 400 and 800 mg/kg dose respectively for seven days. Normal saline was gavaged to control group. All the animals were weighed and partially anaesthetized with ether after 24 h of last treatment. Blood samples were collected by puncturing retro-orbital venous plexus. Blood was kept for 30 min at room temperature and then clot was smoothly separated from the wall of the test tubes with support of thin disinfected needle. The test tubes were centrifuged for 20 minutes at 2000 rpm and serum was isolated and stored at -20°C (Riley, 1960). Various parameters of liver function tests i.e. alanine aminotransferase, aspartate aminotransferase alkaline phosphatase, bilirubin, triglycerides, cholesterol and kidney function tests i.e. urea, uric acid and creatinine were performed using standard commercial kits (Erba diagnostics Mannheim Gmb H Mallaustr, Germany) according to the manufacturer's instructions.

Choleretic activity

Female rats were anesthetized followed by overnight fasting with intraperitoneal injection of (25%) urethane at a dose of 6 ml/kg body weight. Bile duct was surgically exposed and cannulated with PE-10 tubing for collecting the bile secretion. The body temperature of anesthetized rats was maintained at 37°C with a heat lamp. Bile was collected for successive one hour period in eppendorf. At the end of first hour, intraduodenal administration of physiological saline in control, dihydrocholic acid (DHC) (50 mg/kg) in positive control and test plant extract (400 mg/kg) in experimental group was done. Bile was collected hourly for 4 h following administration of plant extract and standard. Bile was evaporated to dryness and weight of bile solids residue was recorded (Klaassen and Plaa, 1969).

Statistical analysis

Results were stated as the mean ± SE of six animals in each group. For statistical analysis the Student's t-test was used to compare the mean values of different parameters obtained in various groups at ($P \leq 0.05$) (Snedecor and Cochran, 1994). Data were subjected to statistical analysis through one- way analysis of variance (ANOVA) at 5% level of significance.

Results

No-observed-adverse-effect-level

Ethanolic extract of *P. daemia* did not show significant alteration in liver specific enzymes i.e., ALT, AST, ALP as compared to control group. Total bilirubin content did not show significant deviation from its normal value (Table 1). Concentration of triglycerides and cholesterol were near to control in animals treated with *P. daemia* at 100; 200; 400 and 800 mg/kg. There were no significant ($p \leq 0.05$) difference in serum urea, uric acid and creatinine concentrations in all the groups treated with *P. daemia* as compared to control (Tables 2).

Choleretic activity

Bile flow rate for 1 h is slightly higher than 2-5 h duration in control and treated groups (Table 3). Slight increase in bile flow and bile solid after treatment of *P. daemia* leaves extract (400 mg/kg dose) was found during 2-5 h period as compared to control group. Less amount of bile volume and bile solid was found in *P. daemia* treated group as compared to DHC treated positive control.

Table 1. Serum biochemical values of rats treated with *P. daemia* leaves extract for a week

Concentration of extract (mg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
Control	28.4±2.23	73.4±5.23	433±34.8	0.23±0.01
100	27.3±2.32	75.4±5.14	416±33.8	0.21±0.01
200	28.5±2.29	72.3±5.02	429±33.6	0.24±0.01
400	25.1±2.19	74.7±5.24	461±36.5	0.21±0.02
800	27.1±2.26	83.1±5.70	527±32.7	0.30±0.03
ANOVA	0.44	0.78	2.00	2.22

Data are expressed as mean ± SE; n=6; Significant *P* value Therapy vs control $^{*}P$0.05; Significant ANOVA at 5%. ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= alkaline phosphatase

Table 2. Serum biochemical values of rats treated with *P. daemia* leaves extract for a week

Concentration of extract (mg)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	27.3±2.19	10.6±0.71	20.3±1.16	1.87±0.12	0.64±0.04
100	28.6±2.26	11.6±0.81	19.3±1.16	2.05±0.14	0.65±0.04
200	28.5±2.37	11.4±0.81	19.2±1.29	2.08±0.13	0.66±0.04
400	26.5±2.22	11.2±0.73	18.2±1.35	1.84±0.12	0.71±0.05
800	26.5±2.29	11.9±0.88	18.6±1.26	2.12±0.12	0.63±0.04
ANOVA	0.24	0.45	0.49	1.15	1.14

Data are expressed as mean ± SE; n=6; Significant *P* value Therapy vs control $^{*}P \leq 0.05$; Significant ANOVA at 5%.

Table 3. Effect of *P. daemia* leaves extract on choleretic activity of rat

Treatment	Bile flow (ml)			Bile solid (mg)		
	1 h	2-5 h	Ratio	1 h	2-5 h	Ratio
Control	0.64±0.04	1.67±0.10	1:2.60	19.7±1.75	50.0±3.50	1:2.53
DHC (50 mg/kg)	0.55±0.03	2.09±0.17	1:3.80	18.1±1.37	58.2±4.87	1:3.21
P. daemia (400 mg/kg)	0.61±0.03	1.75±0.10	1:2.86	20.0±1.52	51.0±3.34	1:2.55
ANOVA	1.68	3.43		0.51	1.52	

Data are expressed as mean ± SE; n=6; Significant *P* value Therapy vs control $^{*}P \leq 0.05$; Significant ANOVA at 5%. DHC= Dihydrocholic acid (standard).

Discussion

Toxicity evaluations of plant extracts are obligatory to establish optimum safe dose of novel drugs. *P. daemia* has been traditionally used for many years against various diseases by folk people. However, potential therapeutic efficacy of plant requires careful toxicological evaluation so that the drug may reach to marketplace. In present study rats were orally administered with aqueous suspension of *P. daemia* leaves extract showed no mortality and toxic effects.

The NOAEL is the highest experimental dose lacking any signs of adverse effects. It includes acute, sub chronic or chronic exposure of an organism to a substance/drug at which no significant alteration in behavioural or clinical parameters occurs as compared to control (Engelhard and Dorato, 2005). Alteration in serum enzyme is a specific biomarker of liver toxicity. The major liver function tests includes AST, ALT and ALP. Aminotransferases are released from damaged hepatocytes into blood during toxic insult (Ozer et al., 2008). The ALP present in bile duct of epithelial cells transports metabolites across cell membrane (Eric Sulava et al., 2017). Administration of all the four doses of *P. daemia*

i.e. 100, 200, 400 and 800 mg/kg did not showed disturbed level of AST, ALT and ALP as compared to control. Therefore leaves extract of the plant has no toxic effect on plasma membrane of hepatocytes. Bilirubin is an orange yellow bile pigment formed during normal breakdown of RBCs and excreted in bile. Serum bilirubin level showed no significant alteration in bilirubin content, which suggested liver is breaking down and properly eliminating waste via bile and maintained bilirubin content in blood. Disturbance in the concentration of major lipids like triglycerides and cholesterol can give useful information on lipid metabolism in the body (Bhadauria et al., 2008). Treatment of *P. daemia* extract showed insignificant alteration in the level of triglycerides and cholesterol at 100, 200, 400 and 800 mg/kg doses, therefore the extract has no adverse effect on metabolic processes of liver. High level of serum urea indicates disturbances in protein metabolism during liver toxicity. Uric acid is breakdown product of purines, known to inhibit release of nitric oxide within renal vasculature which leads to decreased renal blood flow and glomerular filtration rate. Urea, uric acid and creatinine are significant marker of kidney function tests (Nazmi et al., 2016). The results of kidney function tests showed that the *P. daemia* extract at all the doses caused no significant alteration in serum urea, uric acid and creatinine. The nephroprotective effect of the plant is well documented in previous studies (Karthishwaran and Mirunalini, 2010).

Bile acids are end product of cholesterol utilization and bile flow is a complex process of secretion and excretion of waste substances. Active transport of bile salts and other osmotically active substances induces an osmotic gradient which in turn stimulates flow of water and electrolytes (Boyer, 2013). The volume of bile flow may be stimulated by choleretic compounds found in plants (Saenz Rodriguez et al., 2002). Intra duodenal administration of *P. daemia* leaves extract at 400 mg/kg induced a slight increase in bile volume and bile solid contents, clearly indicated its choleretic action. Flavonoids are major phytochemical found in aerial parts of *P. daemia* responsible for its hepatoprotective activity (Suresh kumar and Mishra, 2008). It may be possible that the polyphenolic constituents of the plant extract may be responsible for increasing cholephilic compounds into bile canaliculi (Gebhardt and Uberham, 1998). Present study is in corroboration of findings of Garcia et al. (1990), who reported choleretic action of aqueous extract of *Helichrysum* species due to presence of flavonoid and phenolic contents. Increased bile secretion by administration of plant extracts of *Cynara scolymu* (Saenz Rodriguez et al., 2002) and *Polygonum bistorta* (Mittal et al., 2012) also supports the findings of present study.

In summary, the results of serum biochemical endpoints evaluated in the present study showed NOAEL of *P. daemia* leaves extract is 800 mg/ kg body weight in rats. The results also confirmed the choleretic potential of *P. daemia* leaves extract at 400 mg/kg dose. The high bile solid content support the hepatoprotective potential of the plant extract.

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Conflict of interest statement

The authors declare that they have no competing interest.

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