International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology, 3 (1): 96-100, 2007
ISSN 1811-7775

Preliminary Toxicity and Phytochemical Studies of Aqueous Bark Extract of *Helicteres isora* L.

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Abstract: The present study was designed to determine the preliminary oral toxicity profile of the aqueous extract of bark of *Helicteres isora* L. (HIL) in rats and its active chemical constituents by way of phytochemistry. The acute oral toxicity study was conducted using limit dose test of up and down procedure according to the OECD/OCDE Test Guidelines on Acute Oral Toxicity (AOT425statPgm, version: 1.0) at a limit dose of 2000 mg/kg/p.o. Repeat dose oral toxicity studies were conducted by daily oral dosing of 500 mg kg^{-1} b.w of HIL dissolved in 1 mL of 0.9% saline and 1 mL of 0.9% saline to rats in the test and control groups, respectively, for 28 days. On day 29, blood samples for bioassays were collected by cardiac puncture under chloroform anesthesia. The phytochemical analysis was conducted using standard procedures. The LD_{50} estimate of the extract was calculated to be greater than 2000 mg/kg/p.o. The extract caused a significant ($p<0.05$) decrease in weight gain, differential eosinophil count and increase in serum creatinine but did not affect the organ weights, other serum electrolytes (Na^+, K^+, HCO_3), liver enzymes and other hematological indices in test rats. Its phytochemical analysis showed it contains saponins, flavonoids, alkaloids, tannins, phlobatannins, glycosides, reducing sugars and anthraquinones. These results show that the aqueous extract of *Helicteres isora* is relatively safe toxicologically when administered orally. Thus, its use in folkloric medicine as an oral antidiabetic is relatively safe when used over the tested period.

Key words: *Helicteres isora*, rats, oral toxicity, phytochemical analysis

INTRODUCTION

The bark of *Helicteres isora* Linn. (Sterculiaceae) has been used in the indigenous systems of medicine in India for the treatment of diabetes mellitus since time immemorial. The plant is a shrub or small tree available in forests throughout the Central and Western India. The roots and the bark are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery (Kirtikar and Basu, 1995; Narayan Das Prajapathi *et al*., 2003). The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary and useful in griping of bowels, flatulence of children (Chopra *et al*., 1956) and antispasmodic effect (Pohocha and Grampurohit, 2001). From the roots, Cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity (Bean *et al*., 1985). The aqueous extract of the bark showed significant hypoglycemic effect (Kumar *et al*., 2006a) and lowering effect in hepatic enzymes (Kumar *et al*., 2006b).

This study, therefore, was designed to evaluate its toxicity in rats and to identify the active principles in the extract by way of phytochemistry, as toxicity may result from drug overdose or the active principles contained in the plant.

MATERIALS AND METHODS

Collection and processing of plant material: The bark of *Helicteres isora* L. was collected from Solakkadu, Kollimalai, Namakkal District, Tamil Nadu, India and authenticated by Fr. K.M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher Herbarium specimens have been deposited in the (collection number 23644, 27406) Herbarium for future references.

The dried bark of *Helicteres isora* L. was ground in to fine powder with auto-mix blender. Then the fine powder was suspended in equal amount of water and stirred intermittently and left overnight. The macerated

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pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 185 g kg^{-1} of powdered bark) served as aqueous extract of *Helicteres isora* L. for experimentation.

Animals: Male Wistar albino rats (weighing 160-200 g) were procured from the Animal house, Bharathidasan University, Tiruchirapalli under st and ard environmental conditions (12 h light/dark cycles at 25-28 C, 60-80% relative humidity). They were fed with a standard diet (Hindustan Lever, India) and water ad libitum and allowed to acclimatize for 14 days before the procedure. All studies were conducted in accordance with the National Institute of Health Guide (1985). This study was conducted in Department of Biochemistry, Selvamm Arts and Science College, Namakkal, Tamilnadu, India during 2004-2005.

Acute oral toxicity study: Acute toxicity study was carried out *in vivo*. All solutions were prepared using 2 mL of 0.9% Saline solution and administered per oral using gastric tube. The Acute oral toxicity study was conducted using the limit dose test of up and down procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425statPgm, version 1.0 (Acute Oral Toxicity (OECD Test Guideline 425) (AOT), 2001), at a limit dose of 2000 mg/kg b.w/p.o and default of Sigma at 0.5.

A total of five rats were systematically selected out of a population of 40 rats by systematic randomization techniques. The rats were fasted of rat chow overnight prior to dosing on each occasion. A rat was picked at a time, weighed and dosed with equivalent 2000 mg/kg b.w of the crude extract dissolved in 1 mL of 0.9% saline used as the vehicle. Feeding was done using gastric feeding tube.

Each animal was observed each time for the first 5 min after loading for signs of regurgitation and then kept in a metabolic cage. Each was watched for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 48 h for the short-term outcome and the remaining 12 days for the long-term possible lethal outcome which in this case was death. Behavioural manifestations of acute oral toxicity were also noted. All observations were systematically recorded with individual records being maintained for each rat.

Repeat dose oral toxicity study: The experimental rats consisting of a total of 20 rats were systematically randomized and divided into two groups of 10 rats namely the control and test groups. At the start of the experiment, the rats were weighed and their weights recorded.

The aqueous extract of bark of HIL was administered on daily basis to the tested group by gastric feeding at the dose of 500 mg kg^{-1} of body weight dissolved in 1 mL of 0.9% normal saline. They were fed daily for a total of 28 days. Drug administration was terminated on the 28th day, after which the rats were fasted for 24 h. The control group which consisted of 10 rats was fed with 1 mL of the vehicle only, under the sample experimental conditions and shamhandling. On the 29th day, the rat in each group was weighed, anesthetized with chloroform and blood samples for biochemical and hematological analyses were collected.

Collection of blood for bioassay: Blood samples were collected by cardiac puncture under chloroform anesthesia. Whole blood for FBC and Hb determination was collected into bottles containing the anticoagulant, sodium citrate while samples for LFT and electrolytes, urea and creatinine were collected into plain sample bottles. The PCV was determined by the micro-hematocrit method while total and differential leucocytes count, platelets count were made from blood smears stained with Giemsa (Schlam *et al*., 1975) The hemoglobin concentration, Hb, was determined by cyanomethemoglobin method using the Beckman Model Spectrophotometer (Drabkin and Austin, 1932). The plasma activities of liver enzyme, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alamine aminotransferase (ALT) were estimated by King and Armstrong (1988) method. Sodium and potassium were estimated by flame photometer while urea and creatinine were determined by Autoanalyser using reagent kit obtained from Boehringer, Mannheim, Germany. The total protein was estimated by Lowry *et al.* (1951) method while that of albumin was determined by Reinhold (1980). The total bilirubin and the conjugated bilirubin were determined by Jendrassik-Grof method (Spencer and Price, 1977). The serum cholesterol was measured using diagnostic kits, Boehringer Mannheim, Germany.

Phytochemical analysis: The presence of saponins, tannins, anthraquinones, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars and phlobatannins were detected by simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).

Statistical analysis: Results were analyzed using matched paired t-test on SYSTAT 10.2 software programmer (Acute Oral Toxicity (OECD test Guideline 425) (AOT), 2001) and were expressed as Mean±SD.

RESULTS

Acute toxicity study: There were no deaths of rats administered 2000 mg kg^{-1} b.w of bark extract within short and long-term outcome of the limit dose test of up

Table 1: Sequence and results of limit dose test of HIL in rats

Test sequence	Animal ID	Dose (mg kg^{-1})	Short-term result (48 h)	Long-term result (12 days)
1	01	2000	Survival	Survival
2	02	2000	Survival	Survival
3	03	2000	Survival	Survival
4	04	2000	Survival	Survival
5	05	2000	Survival	Survival

Table 2: Effect of HIL on weight gain in rats

Dose (mg kg^{-1})	No. of rats	Mean initial weight on day 0 (g)	Mean final weight on day 28 (g)	Mean weight changes (g)
0	10	174.2±9.0	209.2±33.4	35.0±28.2
500	10	172.4±8.7	197.4±7.9*	25.0±20.0*

*Values are expressed as Mean±S.D of 10 rats. *Values significant at $p<0.05$

Table 3: Effect of HIL on the organ weights

Dose (mg kg^{-1})	No. of animals	Mean weight of liver (g)	Mean weight of kidneys (g)	Mean weight of lungs (g)	Mean weight of heart (g)
0	10	7.9±0.6	1.7±0.1	1.6±0.3	0.8±0.1
500	10	7.5±0.3	1.5±0.1	1.5±0.0	0.7±0.0

Table 4: Effect of HIL on PCV, Hb, MCHC and platelet counts

Dose (mg kg^{-1})	No. of rats	PCV (%)	Hb (g dL^{-1})	MCHC (%)	Platelets count (× 10^3 mm^3)
0	10	36.8±0.5	12.2±0.2	33.5±0.4	217.8±13.8
500	10	36.6±0.6	12.3±0.3	32.5±0.3	179.2±10.6

Table 5: Effect of HIL on total and differential white blood cells

Dose (mg kg^{-1})	No. of rats	Total WBC (× 10^9 L^{-1})	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)
0	10	2.2±0.4	31.8±5.8	6.6±1.8	62.8±5.2	0.5±0.2
500	10	1.9±0.3	32.2±3.7	4.8±1.7	64.3±4.7	0.3±0.1

*Values are expressed as Mean±SD of 10 rats. *Values significant at $p<0.05$

Table 6: Effect of HIL on serum electrolytes, urea and creatinine in rats

Dose (mg kg^{-1})	No. of rats	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})	HCO_3^- (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})
0	10	148.8±3.9	5.6±0.4	27.5±1.4	6.2±1.3	0.5±0.1
500	10	151.2±4.0	5.5±0.4	25.2±1.2	6.0±0.1	0.9±0.1

*Values are expressed as Mean±SD of 10 rats. *Values significant at $p<0.05$

Table 7: Effect of HIL on serum proteins, cholesterol and bilirubin in rats

Dose (mg kg^{-1})	No. of rats	Total proteins (g dL^{-1})	Albumin (g dL^{-1})	Cholesterol (mmol L^{-1})	Total bilimbin (× mol L^{-1})	Conjugated bilimbin (× mol L^{-1})
0	10	92.8±2.5	24.8±1.2	1.5±0.1	1.2±0.1	0.3±0.1
500	10	104.6±4.8	25.6±1.1	1.6±0.3	1.1±0.2	0.2±0.1

and down procedure (Table 1). However, the observed behavioural signs of toxicity include irritation, restlessness, tachypnoea, anorexia, bilateral narrowing of the eyelids and abnormal posture (which was characterized by tugging of the head in-between the hind-limbs). The LD_{50} was calculated to be greater than 2000 mg kg^{-1} b.w/p.o (Table 1).

Repeat dose toxicity study

Effect of HIL on weight gain of rats: HIL administered to rats for 4 weeks of this study decreased the weight gain of the animals significantly ($p<0.05$) when compared to control (Table 2).

Effects of extract on the weight of body organs: HIL administration did not cause a significant difference in the organ weights of rats in both control and test groups (Table 3).

Hematological responses of rats to HIL: The extract caused no statistically significant ($p>0.05$) difference on the hematological parameters being investigated at the tested doses (Table 4) However, there was a remarkable decrease in the differential eosinophils count in the treated group when compared to control (Table 5).

Biochemical responses of rats to HIL

Effect of HIL on plasma electrolytes, urea and creatinine: The extract elevated the plasma creatinine significantly ($p<0.05$) while no significant increase occurred in other plasma electrolytes and urea ($p>0.05$) (Table 6).

Effect of HIL on plasma proteins, cholesterol, bilirubin and liver enzymes: The extract caused no significant changes in the plasma proteins, cholesterol and bilirubin in rats in both groups (Table 7). So also, was for its effect on the circulating liver enzymes (Table 8).

Table 8: Effect of HIL on the liver enzymes in rats

Dose (mg kg^{-1})	No. of rats	Alkaline phosphatase	ALT (IU L^{-1})	AST
0	10	52.2±3.6	8.5±2.7	18.4±2.5
500	10	45.2±2.4	8.6±1.4	22.8±2.4

Table 9: Chemical constituents of aqueous extract of bark of *Helicteres isora*

Tests	Result
Saponins	
Benedict's test	++
Emulsion test	++
Frothing test	++
Tannins	
Bromine water test	+
ferric chloride test	+
Phlobatannins	++
Alkaloids	
Draggendoff's test	+++
Mayer's test	++
Wagner's test	+++
Flavonoids	
Lead acetate test	+++
Ferric chloride test	+++
Sodium chloride test	+++
Cardiac glycosides	
Keller-Kelliani test	+++
Salkowski's test	++
Legal's test	+++
Reducing sugars	
Hexose sugar	++
Ketosugar	++
Pentosugar	++
Monosaccharide	++
Anthraquinones	
Free anthraquinones	+
Bound anthraquinones	++
Anthocyanides	+

-: Not detected; +: Present in low concentration; ++: Present in moderate concentration; +++: Present in high concentration

Phytochemical analysis: Phytochemical analysis of the HIL showed it contains saponins, flavonoids, alkaloids, tannins, phlobatannins, cardiac glycosides, reducing sugars and anthraquinones (Table 9).

DISCUSSION

Acute toxicity study of aqueous extract of bark of *Helicteres isora* showed that no mortality of rats occurred, at a limit dose of 2000 mg kg^{-1} b.w/p.o. This is an indication of the extract has low acute toxicity when administered per oral. According to Clarke and Clarke (1977), substances with LD_{50} of 1000 mg kg^{-1} b.w/p.o are regarded as being safe or of low toxicity. The high LD_{50} obtained is an indication that the extract could be administered with a high degree of safety where the absorption might be incomplete due to inherent factors impeding absorption along the gastrointestinal tract (Abatan and Arowolo, 1989).

The absence of change in body weight after 28 days treatment with the bark extract revealed that there is no major negative impact on the general metabolic status of the animals. Though the therapeutic index of the extract was not calculated in the present study, the oral LD_{50} value may highlight the safety nature of the bark extract (Dennis, 1984).

Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are hemoglobin, packed cell volume, white blood cell count, platelets count (Schlam *et al*., 1975). The normal range of these parameters can be altered by the ingestion of some toxic plants (Abatan and Arowolo, 1989; Ajagbonna *et al*., 1999). These blood indices were all measured in this present study after 28 days of oral administration without any significant alterations from the control values (except for eosinopenia), still corroborating the wide safety margin of the extract. Administration of the aqueous bark extract for 28 days in this study did not affect most of the biochemical parameters except for creatinine which was significantly elevated. This discrepancy may be explained by the fact that variation may sometimes occur in bioactive compounds of the different parts of the same plant and even in same plant parts found in different environment (Elujoba, 1989). The increase in the serum level of creatinine may be a reflection of an increased catabolic state in the rats resulting from prolonged reduced appetite as evidenced in the behavioural manifestation of acute oral toxicity or could be a manifestation of some degree of renal insult (Alexis Vidal *et al*., 2003). However, these assertions need to be clarified. The lack of significant alterations of liver enzymes is also remarkable.

Some parts of plants, such as bark or fruits, can contain alkaloids, tannins or other compounds that have toxic effects in various animal species (Segura *et al*., 1990). It is known that many toxic plants compounds accumulate in the liver where they are detoxified (Clarke and Clarke, 1977). A study of liver function tests may therefore prove useful in assessing especially the toxic effects of medicinal plants on the liver. These tests involve mainly determination of AST and ALT (Tilkian, 1979) and any marked necrosis of the liver cells lead to a significant rise of these enzymes in the blood serum. The lack of this effect on these liver enzymes shows that the extract is non-toxic on the hepatocytes.

The toxicity observed in these studies could have resulted from various active organic constituents like saponins, tannins, alkaloids, flavonoids and glycosides as shown by the result of phytochemistry. Although it is known that variations may sometimes occur in bioactive compounds of the same plant found in different environments (Elujoba, 1989), but, this was not the case. The apparent lack of clinical signs of acute toxicities in

human when administered the extract orally as an antidiabetic, may be a reflection of the oral route of administration, low dose administration as well as short duration of exposure. The extract, in folkloric medicine is administered two to three times per day for 1-2 weeks as an antidiabetic remedy. However, these studies only serve as a template for future research into plant extract's toxicity profile.

REFERENCES

Abatan, M.O. and R.O. Arowolo, 1989. Toxicity of *Eugenia uniflora* to rats. Nig. J. Anim. Prod., 16: 16-19.

Acute Oral Toxicity (OECD Test Guideline 425) (AOT), 2001. Statistical Programme (AOT425StatPgm), Version1. 0. http://www.oecd.org/oecd/pages/home/display general/0, 3380, EN-document-524-nodirectorate-no-24-6775-8, FF.htmL.

Ajagbonna, O.P., K.I. Onifade and U. Suleiman, 1999. Hematological and biochemical changes in rats given extract of *C. procera*. Sokoto J. Vet. Sci., 1: 3-12.

Alexis Vidal, Adyary Fallarero, Blanca R. Peña, Maria E. Medina, Bienvenido Gra, Felicia Rivera, Yamilet Gutierrez and Pia M. Vuorela, 2003. Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. J. Ethnopharmacol., 89: 295-300.

Bean, M.F., M. Antoun, D. Abramson, C.J. Chang, J.L. Mc Laughlin and J.M. Cassady, 1985. Cucurbitacin B and Isocucurbitacin B Cytotoxic components of *H. isora*. J. Nat. Prod., 48: 500-503.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi, India. 1st Edn., pp: 131.

Clarke, E.G.C. and M.L. Clarke, 1977. Veterinary toxicology. Cassel and collier macmillan publishers, London, pp: 268-277.

Dennis, V.P., 1984. Mammalian Metabolism of Xenobiotic Chemicals. Kacew, S. and M.J. Reasor (Eds.), Toxicology and Newborn, pp: 1-3.

Drabkin, D.L. and J.M. Austin, 1932. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit-blood. J. Biol. Chem., 98: 719-733.

Elujoba, A., 1989. Chemical and biological analysis of Nigerian *Cassia* species for laxative activity. J. Pharma. Biomed. Anal., 712: 1457-1685.

King, K.J. and A.L. Armstrong, 1988. Calcium, Phosphorus and Phosphatase. In: Practical Clinical Biochemistry, 4th Edn., Varley, H. (Ed.), CBS Publishers, New Delhi, pp: 457-461.

Kirtikar, K.R. and B.D. Basu, 1995. Indian medicinal plants. Vol. 1, International book distributors, Dehradun, India, pp: 371-372.

Kumar, G., A.G. Murugesan and M. Rajasekara Pandian, 2006a. Effect of *Helicteres isora* bark extract on blood glucose and hepatic enzymes in experimental diabetes. Pharmazie, 61: 353-355.

Kumar, G., G. Sharmila Banu, A.G. Murugesan and M. Rajasekara Pandian, 2006b. Hypoglycaemic effect of *Helicteres isora* barks extract in rats. J. Ethnopharmacol., 107: 304-307.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.L. Randall, 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 264-275.

Narayan Das Prajapathi, S.S. Purohit, Arun K. Sharmi and T. Kumar, 2003. A hand book of Medicinal Plants: A complete source book. Agrobios (India), Shyam Printing Press, Jodhpur, pp: 265.

National Institute of Health Guide for the Care and Use of Laboratory Animals, 1985. DHEW Publication (NIH), revised, Office of Science and Health Reports, DRR/NIH, Bethesda, USA.

Pohocha, N. and N.D. Grampurohit, 2001. Antispasmodic activity of the fruits of *Helicteres Isora* Linn. Phytother. Res., 15: 49-52.

Reinhold, J., 1980. Determination of Serum Total Protein, Albumin and Globulin Fractions by the Biuret Method. In: Practical Clinical Biochemistry, 5th Edn., Vol. I. Varley, H., A.H. Gowen Lock and M. Bell (Eds.), William Heinemann Medical Book Limited, London, pp: 545-547.

Schlam, O.W., N.C. Jain and E.J. Caroll, 1975. Veterinary Haematology, 3rd Edn., Lea and Tebiger Publishers, Philadelphia, pp: 207-209.

Segura, J.J., L.H. Morales-Ramos, J. Verde-Star and D. Guerra, 1990. Growth inhibition of *E. histolytica* and *E. invadens* produced by pomegranate root (*Punica granatum* L.). Archivos de Investigaciones Médicas, 21: 235-239.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, pp: 150.

Spencer, K. and C.P. Price, 1977. Chemical analysis of bilirubin in biological fluids. Ann. Clin. Biochem., 14: 105-115.

Tilkian, S.M., 1979. Clinical Implications of Laboratory Tests. The C.V. Mosby Company, Missouri, pp: 11-17.

Trease, G.E. and W.C. Evans, 1989. A Textbook of Pharmacognosy, 13th Edn. Bailliere Tindall Ltd., London.