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## RESEARCH ARTICLE

# EXPLORING THE ANTI-DIABETIC POTENCY & ACUTE TOXICITY OF AQUEOUS LEAF EXTRACTS OF *HYGROPHILA RINGENS* (L.) R.BR EX (STEUD) IN EXPERIMENTAL MICE

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**ABSTRACT**

The present study reports the effect of leaf extracts of *Hygrophila ringens* (L.) on the blood sugar and body weight changes in alloxan induced albino mice and acute toxicity. The results showed that no mortality and abnormal behaviour was observed even after administration of a dosage of 2000 mg/Kg of body weight in the treated mice. A comparison of blood sugar levels & weight changes between treated & untreated mice indicated distinct changes after 21 days. The preliminary studies also suggest that the aqueous leaf extract of *H. ringens* (L.) contained safe principles and thus *Hygrophila ringens* (L.) could be a more useful plant possessing medicinal properties.



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## INTRODUCTION

A variety of herbal preparations have been mentioned in Ayurveda and other indigenous system of medicine for the treatment of diabetes mellitus. Some of the commonly studied plants such as *Momordica charantia, Allium cepa, Allium sativum, Ficus bengalensis, Eugenia jambolina* L. Skeels*, Azadirachta indica* A. Juss.*, Coccinia indica* (L.)*, Curcuma longa* (Linn.)*,* and *Ocimum sanctum* Linn. *Aborma augusta, Azadirachta indica, Coccinia indica, Pterocarpus marsupia* etc. are widely used in indigenous medicine and many of its products are known to possess a wide array of medicinal properties (Jarald *et al*. 2008). A new coumestan, tephcalostan has been isolated from the whole plant of *Tephrosia calophylla* BEDD. Together with two known flavonoids, 7-O-methylglabranin (2) and kaempferol 3-O-beta-D-glucopyranoside (Kishore *et al*., 2003). Leaves are used as hepatoprotective and also exhibit diuretic activity (Sangameswaran *et al*., 2007). Despite the considerable progress in the treatment of diabetes by oral hypoglycaemic agents and synthetic drugs such as sulfonylureas, metformin, etc. the search for safe and effective herbal drugs will help in understanding the critical role of plant products for treatment of diabetic complications. The present study is to assess the effects of leaf extracts of *Hygrophila ringens* (L.) (Tubiflorae) that can be ascertained through experiments for their safety and efficacy in experimental animal mice.

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## MATERIALS AND METHODS

### Study design

The test system consisted of ten Swiss albino mice and were 9 to 10 weeks old, nulliparous and non-pregnant, bred and reared at Intox Pvt Ltd., Pune. The study was performed at a limit dose of 2000 mg/Kg body weight as the test article has a relatively low acute toxicity hazard. After dosing, the mice were observed for incidence of mortality and signs of intoxication for 14 days. The mice were housed in different groups (five per cage) in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk. The animals were accommodated in air-conditioned rooms with 10-15 air changes per hour, temperature between 19°C to 25°C relative humidity 30 to 70% and artificial fluorescent illumination cycle set to 12 hours light and 12 hours dark. The animals were provided with Nutrilab brand extruded pelleted mouse feed manufactured by M/s Tetragon Chemie Pvt Ltd, Bangalore at ad libitum, during the acclimation and observation period. The mice were fasted 3 to 4 hours prior to treatment. Food was offered two hours after dosing. Potable water passed through online aquaguard water filter was provided ad libitum in glass bottles with stainless steel slipper tubes.

**Dose formulation and administration of test article**

Formulation of the test article was done freshly, prior to dosing. The test article, *Hygrophila ringens* (L.) R. Br ex (Steud) water extract was suspended in distilled water to obtain final concentration of 200 mg/L. The extract was administered by oral gavage to each mouse as a single dose using a suitably graduated syringe and a stainless steel 18G incubation needle. The dose volume was recorded just before dosing to give a constant dosage volume of 10 mg/Kg body weight.

**Determination of acute toxicity**

The toxicity study was carried out with thirty-five (35) male and female Swiss albino mice weighing 20 – 25 g at Intox Pvt Ltd, Pune. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. After starving the animals' overnight, the control group received 0.3 ml of normal saline orally. Each treated group received orally the aqueous extract of the leaf suspension prepared by dispersing 8 g of the extract with 5 ml normal saline, thoroughly mixed and the volume made up to 10 ml with normal saline, in different doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 h and then for each hour for the next 24 h and at 6 hourly interval for the next 48 h after administering of the extract to observe any death or changes in general behaviour and other physiological activities (Shah *et al*., 1997; Charger *et al.* 2005).

**Screening of anti-diabetic potency of extracts of *Hygrophila ringens* (l.) r. br ex (steud) on alloxan induced diabetic mice**

Swiss Albino Mice weighing 200-320 gm of body weight were acclimatized to laboratory conditions for 24 to 48 hours. They were segregated into four categories and 18 groups (6 animals/ group) and were labelled as group I to IV respectively. Fasting blood sugar was determined after depriving food for 16 hours, with free access to drinking water. From the following day, animals were rendered diabetic by injecting alloxan monohydrate dissolved in sterile normal saline at a dose of 150-mg/kg body weight intraperitoneally. The rats were provided with 5% glucose solution for the next 24 hours to prevent hypoglycaemia. After 96 hours, the animals from all the groups were subjected to blood sugar levels determination using endpoint colorimetric method. The blood samples were collected from tail vein. Animals showing fasting blood sugar level more than 350 mg/dl were selected for the study and started treatment from same day. In case of the diabetic control group, the hyperglycaemia condition was maintained for 7 days after which none of the animals survived. The extracts' treatment was carried out for a period of 21 days. During this period, animals in all groups had free access to standard diet and water. Body weights and blood glucose levels were estimated on 1st, 7th, 14$^{th}$ and 21st day of the treatment with plant extract.

## RESULTS

**Alloxan induced antidiabetic studies**

The extracts of various plants have been shown to produce hypoglycaemia in normal and experimental diabetic animals. In the current study, crude extracts of aerial parts (leaves) of *Hygrophila ringens* (L.) R.Br ex (Steud) was evaluated for anti-diabetic effects in mice for 21 days, as they are known to possess a range of chemical constituents. We believe that these constituents may be involved in a wide array of medicinal properties including hypoglycaemic effects. In order to confirm our hypothesis, we have selected Swiss albino mice to check about the anti-diabetic effects of the aqueous extract of the leaves of *Hygrophila ringens* (L.) R.Br ex (Steud). When the Swiss mice were subjected to alloxan induced hyperglycaemic conditions, it was observed from the data that the control mice extract exhibited significant changes in the blood sugar levels.

**Blood sugar and body weight changes**

Table-1 showed the effects of aqueous leaf extract on blood sugar levels and body weight changes at different time intervals. The experiments were carried out for 21 days and the blood sugar levels were monitored in replicates for each group. The animals were divided into four groups. The first group was control. The second group was diabetic control (alloxan treated) and the other two groups were administered with 200 mg/Kg and 400 mg/Kg of the leaf extract. The values represent the average value of blood sugar levels after the administration of leaf extract in mice.. The data illustrates that the average blood sugar levels in control mice was found to vary from 90.30 ± 1.97 mg/dl to 91.26 ± 1.40 mg/dl throughout the period of study. In alloxan induced mice, the average values fluctuated from 96.66 ± 3.64 mg/dl on day zero to 611.06 ± 4.37 mg/dl for seven days. Thereafter, all the animals showed morality. In case of animals subjected to a dosage of 200 mg/Kg, the average values in the blood sugar levels varied from 96.36 ± 1.01 mg/dl to a maximum of 579.36 ± 11.73 mg/dl. The values indicate that the blood sugar levels increased from 96.36 ± mg/dl on day zero to 579.36 ± 11.73 mg/dl on day 1 and subsequently the values decreased from day 7 onwards up to 21 days. There was a remarkable difference in the blood sugar values during the experimental period. As the dosage of the extract was increased to 400 mg/l, the blood sugar levels in the mice showed corresponding changes throughout the period of study. It is evident from the data that on day zero, the blood sugar value was 97.36 ± 2.21 mg/dl. The dramatic changes in blood sugar levels varied from 552. 0 ± 30.12 mg/dl on day 1 to 148.23 ± 3.66 mg/dl on 21$^{st}$ day of the experiment. These results suggest that the effect of leaf extracts have a definite influence in alloxan induced diabetic mice.

Table–1 also indicates the average body weight changes observed for 21 days in control, alloxan induced and after leaf extract injected Swiss albino mice. In control mice, the average body weight was found to vary from 238.53 ± 3.22 gm/Kg to 245.3 ± 1.0 gm/Kg throughout the period of study. In alloxan-induced mice, the average values fluctuated from 220.6 ± 1.06 gm/Kg on day zero to 242.23 ± 2.67 mgKg after seven days of treatment. In case of animals subjected to 200 mg/Kg dosage, the minimum average values in the body weight was found to be 233.64 ± 1.78 gm/Kg and it has a maximum value of 245.43 ± 0.21 gm/Kg. The values indicate that the body weight changes decreased from day zero to 14 days and subsequently there was a negligible increase in the values on the terminal day of the experiment. As the dosage of the extract was increased to 400 mg/l, the body weight changes in the mice showed no significant changes throughout the experimental

period. It is observed from our findings that administration of the alloxan induced hyperglycaemic conditions has not resulted in marginal or no weight changes in the mice. In contrast, when the extracts were administered to mice subjected to hypoglycaemic conditions noticeable changes in body weight was observed.

## Biochemcial parameters

In addition to the anti-diabetic effects, the animals were also monitored for biochemical parameters in alloxan induced and leaf extracts treated mice separately. The parameters that were considered for the study include Plasma urea, Plasma creatinine, Plasma triglyceride and Plasma cholesterol. It is observed from Table- 2 that the average changes in control mice of different parameters after 21 days of treatment were found to be 35.23%, 53.33%, 76.49 mg/dl and 84.70 mg/dl respectively. In the alloxan induced mice the experiment was terminated after 7 days. The average values for the above parameters were found to be 142.89%, 1.80%, 181.94 mg/dl and 166.38 mg/dl respectively.

When the animals were administered 200 mg/Kg of leaf aqueous extract of *Hygrophila ringens* (L.) R.Br ex (Steud), it was observed that the values of different biochemical parameters were drastically reduced after 21 days of treatment. The corresponding values for these parameters were found to be 43.45%, 0.68%, 88.73 mg/dl and 89.26 mg/dl. Similarly, as the dosage was increased from 200 mg/Kg to 400 mg/Kg, the changes were more prominent. It is clear from the data that the plasma urea was found to be 33.87% and for plasma creatinine it was 0.60%. The levels of plasma triglyceride and plasma cholesterol were found to be 82.31 mg/dl and 85.31 mg/dl respectively.

## Toxicity

In acute oral toxicity study, the observations were based on mortality and clinical signs (Tables–3, 4, 5 and 6). On the day of dosing (2000 mg/Kg of body weight), all the animals were observed for mortality and signs of intoxication at 10 minutes, 30 minutes, 1, 2, and 4 hours following dosing.

**Table 1. Illustrates the data showing the blood sugar levels (mg/dl) and body weights following treatment with leaf extract of *H. ringens* (L) in albino mice at different time intervals**

| Treatment | Blood sugar level (mg/dl) / body weight (gm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 day | | 1 day | | 7 day | | 14 day | | 21 day | |
| Control | 90.83 ±1.77 | 238.53 ±3.22 | 90.40 ±1.93 | 239.83 ±3.19 | 90.73 ±1.33 | 242.26 ±2.63 | 90.30 ±1.97 | 243.83 ±1.93 | 91.26 ±1.40 | 245.30 ±1.00 |
| Diabetic control | 96.66 ±3.64 | 242.23 ±2.67 | 545.46 ±21.58 | 235.2 ±4.51 | 611.06 ±4.37 | 220.66 ±1.06 | - | - | - | - |
| Extract (200 mg/kg) | 96.36 ±1.01 | 245.43 ±0.21 | 579.36 ±11.73 | 240.96 ±1.07 | 514.66 ±8.06 | 238.13 ±2.47 | 185.00 ±8.94 | 233.64 ±1.78 | 66.46 ±3.84 | 235.83 ±3.34 |
| Extract (400 mg/kg) | 97.36 ±2.21 | 255.76 ±2.801 | 552 ±30.12 | 247.7 ±4.83 | 546.06 ±57.26 | 247.56 ±3.41 | 177.63 ±7.51 | 247.0 ± 4.08 | 148.23 ±3.66 | 246.06 ± 3.86 |

- = animal died

**Table 2. Changes in biochemical parameters after 21 days of treatment**

| Treatment | Plasma Urea (mg %) | Plasma creatinine (mg%) | Plasma triglyceride (mg/dl) | Plasma cholesterol (mg/dl) |
|---|---|---|---|---|
| Control | 35.23 | 0.54 | 76.68 | 84.69 |
| | 32.40 | 0.52 | 78.25 | 83.52 |
| | 38.22 | 0.54 | 74.54 | 85.91 |
| Diabetic control* | 139.25 | 1.82 | 183.64 | 165.85 |
| | 145.53 | 1.78 | 180.23 | 166.91 |
| | - | - | - | - |
| Extract (200 mg/kg) | 45.21 | 0.68 | 88.82 | 89.23 |
| | 41.51 | 0.66 | 88.21 | 89.63 |
| | 43.62 | 0.69 | 89.17 | 88.92 |
| Extract (400 mg/kg) | 32.63 | 0.60 | 82.36 | 85.40 |
| | 33.55 | 0.60 | 81.62 | 84.21 |
| | 35.24 | 0.60 | 82.97 | 86.32 |

*The measurements were taken on 7th day; All the values represent samples taken in three replicates

**Table 3. Depicts the incidence of clinical signs and mortality data in Albino Mice (Males) after treatment with leaf extracts of *Hygrophila ringens* (L)**

| Observation | Results |
|---|---|
| Mortality at the end of the 14 days observation period | No mortality observed. |
| Abnormal clinical signs on the day of treatment and during the 14 day observation period. | No abnormal clinical signs were observed in the treated female mice at the dose of 2000 mg/kg body weight |
| Necropsy findings at termination on day 15. | No gross pathological alterations encountered. |

**Table 4. Illustrates the results obtained after treatment with leaf extracts of *Hygrophila ringens* (L) extracts on Albino Mice (Males) at the end of 15 days (Dosage of 2000 mg/kg)**

| No. of animals | Fate | Time of death | Necroscopy findings |
|---|---|---|---|
| 1 | TS | Day 15 | NAD |
| 2 | TS | Day15 | NAD |
| 3 | TS | Day15 | NAD |
| 4 | TS | Day15 | NAD |
| 5 | TS | Day 15 | NAD |

**NAD** – No abnormalities detected **TS** – Terminal sacrifice

**Table 5. Illustrates the results obtained after treatment with leaf extracts of *Hygrophila ringens* (L) on Albino Mice (Females) at the end of 15 days (Dosage of 2000 mg/kg)**

| No. of animals | Fate | Time of death | Necroscopy findings |
|---|---|---|---|
| 1 | TS | Day15 | NAD |
| 2 | TS | Day15 | NAD |
| 3 | TS | Day15 | NAD |
| 4 | TS | Day15 | NAD |
| 5 | TS | Day15 | NAD |

**NAD** – No abnormalities detected
**TS –** Terminal sacrifice

**Table 6. The incidence of clinical signs and mortality of *Hygrophila ringens* (L) leaf extracts administered to mice**

| Sex | Dose mg/kg | Animal No. | Incidence of Clinical Signs / Mortality Observed after Dosing on | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | | | | | | | | | | Day | | | | | | |
| | | | Min | | Hour | | | | | | | | | | | | | | |
| | | | 10 | 30 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Male | | 1 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | 2000 | 2 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 3 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 4 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 5 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Female | | 6 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 7 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 8 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 9 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| | | 10 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

N - No Clinical Signs of Toxicity

Thereafter, they were observed once in a day for 14 days. All the animals were sacrificed at the end of the observation period ($15^{th}$ day) and subjected to a complete necropsy (Table-6). Step wise administration of the *Hygrophila ringens* (L.) R.Br ex (Steud) leaf water extract to groups of five male and female mice at the dose level of 2000 mg/Kg showed that there was no incidence of mortality and no clinical signs of toxicity were observed in both the sexes. When the animals were sacrificed at the end of the experimental period, the data clearly indicted that the extract showed no signs of any adverse effects and hence no abnormalities were detected. Interestingly, the extract has not elicited any signs of intoxication throughout the observation period. The acute oral $LD_{50}$ value of *Hygrophila ringens* (L.) R.Br ex (Steud) leaf extract in Swiss albino mice, both male and female, was found to be greater than 2000 mg/Kg body weight. In addition to the toxicity effects of the leaf extract of *Hygrophila ringens* (L.) R.Br ex (Steud) behavioural aspects was also considered and the observations revealed that no gross changes in the behavioural and no sensory nervous system responses were observed in the animals. Also no adverse effects on movements were observed in male and female mice used in the experiment. The results of acute toxicity study revealed that this plant might be considered to be safe without non-toxic effects. Further studies under long term conditions would reveal a more prominent protective action of the plant extracts under in-vivo conditions.

## DISCUSSION

### Anti-diabetic studies

The plant families, including the species (sp), most studied for hypoglycaemic effects include: Leguminoseae (11 sp), Lamiaceae (7 sp), Liliaceae (8 sp), Cucurbitaceae (7 sp), Asteraceae (6 sp), Moraceae (6 sp), Rosaceae (6 sp), Euphorbiaceae (5 sp) and Araliaceae (5 sp). The most studied species are: *Citrullus colocynthis* (*Opuntia streptacantha* Lem. (Cactaceae), *Trigonella foenum greacum* L. (Leguminosea), *Momordica charantia* L. (Cucurbitaceae), *Ficus bengalensis* L. (Moraceae), *Polygala senega* L. (Polygalaceae), and *Gymnema sylvestre* R. Br. (Asclepiadaceae) (Bnouham *et al*., 2006). *Hygrophila* species are claimed to be beneficial to diabetes. It was observed from our studies that the aqueous extract of *Hygrophila ringens* (L.) R.Br ex (Steud) possesses significant anti-diabetic activity at the dose of 400 mg/kg as compared with alloxan induced diabetes mice. The bioactive principles found in *Hygrophila ringens* (L.) R.Br ex (Steud) must be responsible for the changes observed in the tested animal model. Thus, we report for the first time that the plant, *Hygrophila ringens* (L.) R.Br ex (Steud) has medicinal properties which are evident from the results obtained in our study. We can further substantiate our findings after isolation of the active fractions involved for such an activity to confirm their role in anti-diabetic effects in animal models. *Hygrophila Spinosa* (K. Schum) Heine (syn. Asteracantha longifolia Nees, Acanthaceae) was widely used in the Indian systems of medicine for the treatment of various liver ailments. The hepatoprotective activity of the aqueous extract of the stem was studied on carbon tetrachloride induced liver toxicity in rats. The activity was assessed by monitoring the various liver function tests, viz. alanine transaminase, aspartate transaminase (AST), alkaline phosphatase (ALP), total protein and total bilirubin (Usha *et al*. 2007).

The anti-diabetic activity of *Mangifera indica* L (Mango) was seen when an extract of the leaves of *M. indica* was given to rats 60 min before the glucose. The hypoglycaemic effect of the aqueous extract was compared with that of an oral dose of chlorpropamide (200 mg/kg). The hypoglycaemic action of this plant may be due to a reduction in the intestinal absorption of glucose (Aderibigebe and Emudianughe, 1999). The antihyperglycaemic effect *Cuminum cyminum* L. was studied in healthy rabbits subjected to weekly subcutaneous glucose tolerance tests after gastric administration of water, tolbutamide or a traditional preparation of the plant. The results showed that the *Cuminum cyminum* (L.) significantly decreased the area under glucose tolerance curve and the hyperglycaemic peak (Bnouham *et al*., 2006).

Oral administration of the flavonoids content (8%) of the seeds of *Cuminum nigrum* (L.) caused a significant blood glucose lowering at a dose range of 0.5 to 1.5 g/kg, both in normoglycaemic and alloxan-induced diabetic rabbits. The maximum of decrease in glycaemia was obtained within 4-8 h; the normal level of glycaemia was reached within 24 h of drug administration (Roman-Ramos *et al*., 1995). In contrast, the alkaloids isolated from *C. nigrum* (0.01%) had no significant hypoglycaemic effect in either normoglycaemic or diabetic rabbits. A high dose of 5g/kg did not produce any adverse effects in a 7-day acute toxicity study in rabbits (Ahmad *et al*., 2001). The extracts of *Tournefortia hirsutissima* Linn, decreased the hyperglycaemic peak and the area under the glucose tolerance curve in hyperglycaemic rabbits (Alarcon-Aguilar *et al*. 1997). The changes in body weight of animals administered *Hygrophila ringens* (L.) R.Br ex (Steud) leaf extracts were significant ($P<0.05$) when the animals were administered crude extracts as compared to control and the changes were more prominent for the period of 3 weeks of administration. James *et al*. (2008) observed the changes in weight of animals administered with extracts of *Ximenia Americana* L. and noticed a significant ($P<0.05$) increase in weight of the animals administered with root extracts when compared with other extracts for the period of 3 weeks of administration. No significant ($P>0.05$) difference was found on the packed cell volume (PCV) level of the animals compared with the control. The effect of the aqueous extracts of *X. Americana* L. on the haematological parameters revealed significant ($P<0.05$) higher values of all the antinutrients determined in the root extract as compared to the leaf and stem extracts (James *et al*., 2008). Intraperitoneal administration of 300 mg/kg of chloroform extract from Bignoniaceae, *Parmentiera edulis* DC to diabetic mice decreased the blood glucose levels by 43.75 %. This extract administered to normal mice reduced glycaemia by 29.61%. A stem bark decoction from *Spathodea campanulata* Buch-Harm caused a decrease in plasma levels of glucose in mice. Polar fractions of stem bark exerted prominent effects in different biological models (Bnouham *et al*., 2006). Oral administration of an extract obtained from *Convolvulus althaeoides* Linn. to normoglycaemic rats produced a persistent hypoglycaemic effect compared with Daonil (Shabana *et al*. 1990). Oral administration of *Ipomea batatas* L. (white skinned sweet potato) produced a reduction in hyperinsulinemia in Zucker fatty rats by 23%, 26%, 60% and 50%, 3, 4, 6 and 8 weeks after treatment respectively. These results were comparable to that of troglitazone, an insulin sensitizer. After 7 weeks of treatment, increase in glycaemia after glucose load was inhibited by the administration of *I. batatas*. Moreover, it normalized lipid metabolism and produced a regranulation of pancreatic islet B-cells after 8 weeks of treatment (Kusano and Abe, 2000). The boiled whole extract of *Ipomea aquatica* produced a significant decrease in glycaemia after glucose loading in healthy Wistar rats with both single (33%) and multiple (25%) doses. The optimum dose was 3.4 g/kg while the optimum activity was observed 2 h after the administration of the extract (Malalavidhane *et al*., 2000).

**Toxicity**

Plant derived compounds apart from their nutritional values have to be monitored for their toxic effects. The toxic principles of the plant extracts are to be ascertained through experiments for their safety and efficacy in experimental animal models. It was interesting to know from the data that no death was seen in the experimental group that received the leaf extracts. There were no apparent abnormal changes in the behaviour of the experimental animals. Obgonnia *et al*. (2009) studied the acute and sub-chronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl. (Verbenaceae), an important medicinal plant and noticed that low and moderate doses of the plant extracts have not produced any toxic effect in the animals but higher doasge caused changes in kidney. Ali *et al*. (2008) examined the toxicological properties of *Zingiber officinale* (Roscoe) extracts to rats and found no morphological malformations or deaths or treatment related adverse effects. In terms of toxicity, it has been reported that the aqueous extract of *Andrographis paniculata* Nees. has an LD50 > 5000 mg/kg in rat (Trivedi and Rawal, 1998), whereas the alcoholic extract has an LD50 > 1000 mg/kg in mice (Nakanishi *et al*. 1965). In addition, oral administration of andrographolide at 18 g/kg in rat appears no physical abnormality in the animal (Patarapanich *et al*. 2007).

According to Ghosh (1984) and Klassen *et al*. (1995), *S. angustifolia* (Mill) Vahl (Fam. Verbanaceae) extract can be classified as being slightly toxic, since the LD50 was found to be between 5 -15.0 g/Kg. The gram equivalent of the LD50 in an adult man would amount to 523.25 g, making *S. angustifolia* (Mill) Vahl (Fam. Verbanaceae) relatively safe. All the mice that received 20.0 g/Kg dose of the extract died within 4 h while the animal that received 6.0 g/kg dose survived beyond the 24 h of observation. The median acute toxicity value (LD50) of the extract was determined to be 8.721 g/Kg mg/ml. The EtOH extract (40%) was introduced to the rats by injection directly to stomach at range of dose from 150 to 300g/kg bw body weight) and the water extract was injected through the peritoneum at dose from 6 to 40g/kg body weight.

In conclusion, our studies conducted in Swiss Albino Mice following administration of aqueous leaf extract of *Hygrophila ringens* (L.) R.Br ex (Steud) revealed a dramatic decrease in blood sugar level in experimental mice as compared to normoglycaemic and alloxan induced mice. The results clearly indicate that leaf extracts at two different dosages have shown marked differences in blood sugar levels. Thus, the plant extract do contain anti-diabetic principles. The changes in body weight of animals (Swiss Albino Mice) administered *Hygrophila ringens* (L.) R.Br ex (Steud) leaf extracts were significant ($P<0.05$) when the animals were administered crude extracts as compared to control and the changes were more prominent for the period of 3 weeks of administration. Similarly, acute toxicity studies were carried out to confirm whether the plant contains any toxic principles. The leaf extracts have not provoked any adverse changes in mice treated with plant extracts. Since the extracts did not show any significant changes in the condition of the animals tested, it can be inferred that the extracts have not provoked any toxic effects. Studies with *Hygrophila ringens* (L.) R.Br ex (Steud) leaf extracts clearly indicated that the toxicity principles seem to be absent or lacking in *Hygrophila*. However, long term toxicity studies and microscopic changes in tissue architecture of the extracts would substantiate our findings for their safety aspects.

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