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ORIGINAL PAPER

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Acute Tocicity Studies on *Iphonia (Grantia) aucheri* and Atractyloside in Mice

Abstract: The desert plant *Iphonia aucheri* Boiss (found in the Arabian Gulf countries and Iran) has been claimed to be highly toxic to rats, camels and sheep. In the present work we have studied the acute toxic effects of oral treatment with water and methanol extracts of this plant in mice. A compound isolated from the methanol extract (termed compound A) and authentic atractyloside were also similarly tested. No mortality in mice was found twenty four hours after the administration of the water and methanol extract (2 g/Kg), compound A or the authentic atractyloside (100 and 500 mg/Kg). However, these treatments caused minor clinical or biochemical signs of toxicity, a small degree of depletion of reduced glutathione in the liver and little or no significant macro- or microscopic changes in the vital organs. These results suggest that, contrary to the previous reports, the plant is of low toxicity to animals.

Key words: mice, *Iphonia aucheri*, toxicity, atractyloside

Short title: *Iphonia aucheri* toxicity

الآثار السمية الحادة لنبات حوا الغزال العطرية (*Iphonia aucheri*) ولمادة ايتراكتالوسايد على الفئران

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المستخلص: تناولت هذه الدراسة تقويم بعض تأثيرات نبات حوا الغـــزال على الفئران إذ أن بعض الدراسات السابقة قد أشارت إلي أن هذا النبـــات بالغ السمية في الجمال و الغنم وان كمية ضئيلة نسبيا من مستخلصه تقتل الفئران في نحو دقيقة عند إعطائه بالفم.

استخدمنا في تجاربنا مستخلص من النبات مائى و آخر ميثانولى (2 مجم لكل كجم) و استعملنا مادة نقية نسبيا (مركب أ) مستخلصة من النبات بذات الطريقة التي استخلصه بها من ذكروا أن النبات بالغ السمية (100 و 500مجم لكل كجم) . كذلك قومنا التأثير السام لمادة كيميائية نقية (اتراكتلو سايد atractyloside) ذكر بعض الباحثين إنها السبب في إحداث سمية النبات.

لم تمت أي من الحيوانات المعالجة بأي من المواد المستعملة في هذه التجارب ولم يحدث في أعضائها تغير عياني او نسيجي يذكر ، بيد أن بعض الجرعات المستخدمة سببت بعض مظاهر سريريه و كيميائية ضئيلة الاثر شملت بعض النقص فى مادة القلوتاثيون فى الكبد.

تشير هذه الدراسة إلى انه (و خلافا لما هو منشور) فان نبات حوا الغزال ليس نباتا ساما فى الفئران تحت ظروف التجربة التى قمنا بها.

Introduction

Iphonia (*Grantia*) *aucheri* Boiss. (Compositae-Inuleae-Inulinae) is a plant commonly found in the Arabian Gulf desert, Iran and in northeast Africa (Anderber, 1985). As far as we are aware, there are no reports on its use in traditional herbal medicine. However, based on circumstantial evidence, a case of poisoning with the plant has been reported in camels (Wernery, *et al.* 1992). The acute toxicity of the whole plant (given orally at a dose of about 18 g/Kg) has also been studied in one sheep, and was found to cause haemorragic liver cell necrosis and death within 35 hrs (Roeder, *et al.* 1994). In mice, the plant has been claimed to be very toxic, as the administration of an aqueous extract (of an unstated concentration) at a dose of 1 ml/mouse killed the animals within 1-2 min (Roeder, *et al.* 1994).

Several classes of constituents have been isolated from the plant. These include pyrrolizidine, alkaloid isotussilagine, stigmasterol, beta-amyrin, lupeol, taraxasterol, and the acetate and palmitate of beta–amyrin, lupeol and taraxasterol (Meyer 1993). Atractyloside and carboxyatractaloside have also been isolated (Wernery, *et al.* 1997).

In view of the apparent rapid lethality of the plant in mice, and the fact that in the previous work

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in a single sheep, a very high dose of the plant was administered orally to the animal, and the fact that the toxicity was suggested to be caused by atractyloside, we thought of verifying the acute toxic effects of the plant extract (and isolated compounds therefrom) in mice using a wider range of doses.

Materials and Methods

Animals

Male albino mice (OT strain) weighing about 25-30 g, and obtained from the UAE University Laboratory Animal Facility were used. They were housed in plastic cages (5-7 mice /cage), at a room temperature of 22 ± 2°C, relative humidity of 50-60%, and with a 12-h light/dark cycles (600-1800h). They had free access to pelleted diet (Abu Dhabi Animal Feed Factory) and water.

Plant Material

The plant was collected from around the hills near Al Madam (in Dubai Emirate, UAE), identified by a plant taxonomist at the herbarium of the Desert and Marine Environment Research Center (DMERC) where a voucher specimen was deposited for reference. The whole plant was dried under the shade and coarsely powdered. The powdered plant material (500g) was successively extracted with hexane, ethyl acetate and methanol, as described (Meyer 1993).

The marc was macerated with water, and the water extract was lyophilized. The methanol extracts were also freeze-dried to give a powder, which was used in subsequent experiments.

Treatments

Four experiments were conducted as follows:

Experiment 1: In this experiment three groups of mice were used. The first group received distilled water (10 ml/Kg) by gavage. The second and third groups received aqueous extract of *I. Aucheri* at oral doses of 1 and 2 g/Kg, respectively. Rectal temperature was measured using a digital thermometer 1, 2, 4 and 24 hrs. following treatment. The mice were then weighed, stunned and rapidly decapitated. Blood was collected in heparinized tubes and centrifuged at 900 g for 10 min at 5°C to obtain plasma. The latter was used for biochemical measurements indicative of liver and kidney damage (see below). The body was opened, and the organs examined grossly for macroscopic changes. The liver was excised, washed with 0.9% NaCl, blotted and weighed. A small piece (from the same lobe each time) was placed in formol-saline for subsequent histological processing. Another piece of the liver (about 200 mg) was frozen at – 40° C pending reduced glutathione (GSH) measurement.

Experiment 2: In this experiment methanol extract of the plant was given to two groups of mice at single oral doses of 1 and 2 g/Kg. A control group received distilled water orally (10 ml/Kg). The rest of the procedure was as above.

Experiment 3: A compound isolated from the methanol extract (termed compound A) was administered to mice at doses of 100 (group 1) and 500 mg/Kg (group 2). Animals receiving distilled water (10ml/Kg) served as controls (group 3). The rest of the procedure was as above.

Experiment 4: In this experiment the authentic glycoside atractalocyte (Sigma, St. Louis, MO, USA) was given to mice by oral gavage at a single dose of 100 (group 1) or 500 mg/Kg (group 2). The rest of the procedure was as above.

Biochemical measurements

In the plasma, the concentrations of glucose, creatinine, urea, cholesterol, total protein, and the activities of aspartate aminotransferase, (AST), alanine transferase (ALT), and gamma gluturyl transferase (GGT) were measured in a clinical chemistry auto-analyzer (Cobas Fara II, Roche, Switzerland) using kits from the manufacturer. In experiment 1 the activity of 5-nucleotidase was measured in plasma using a Sigma kit (procedure No. 265-UV). GSH concentration in the liver was estimated spectrophotometrically as total acid soluble sulfhydryl groups using the method of Sedlak and Lindsay (Sedlak and Lidsay 1968).

Behavioral observations:

The general behavior (e.g. spontaneous motor activity, grooming, rearing) of the control and treated animals was evaluated visually by an observer unaware of the treatments. Food and water intake of the different groups was calculated by subtraction at the end of the experiment.

Histopathology

Fixed tissues were embedded in paraffin wax, and sections, cut at 5 μm thickness were stained with haematoxylin and eosin (H&E). A person

unaware of the treatments examined the sections.

Statistical analysis

Values reported are means ± SEM (number of mice). Differences between group means were assessed by Analysis of Variance (Williams, 1993), followed by Dunnett's test, using the computer software Statview version 5. A P value less than 0.05 was considered significant.

Results

Experiment 1

The treatment of mice with the aqueous extract at a dose of 1 or 2 g/Kg did not cause any overt behavioral change, morbidity or mortality. The aqueous extract at a dose of 1 g/Kg did not significantly affect the liver or body weight. At a higher dose (2 g/Kg), the extract significantly increased the absolute and relative liver weight (Table 1). The rectal body temperature was also not affected by the treatment.

As shown in Table 2, the concentrations of urea and AST activity were significantly increased by about 33% and 63%, respectively, 24 hrs. after treatment with mice at a dose of 2 g/Kg. The lower dose caused a significant increase of about 38% in AST activity ($P< 0.05$).

Hepatic GSH concentration was significantly reduced by about 19.2%, 33.5%, and 28.7%, 2, 6, and 24 hrs. after treatment with the extract (2 g/Kg), respectively (data not shown). The water extract significantly lowered GSH concentration by 14.5-28.7% 24 hrs. after treatment (Table 3).

The activity of 5-nucleotidase in plasma was slightly and insignificantly increased by the two doses of the extract when measured 2 and 6 hrs. after treatment (data not shown).

There were no gross or microscopic lesions of note in any of the organs inspected.

Experiment 2

About 45 min following treatment of mice with the methanol extract, the animals became less active, closed their eyes, and remained huddled together. The body temperature of treated mice decreased progressively, and was lowest 4 hrs. after the administration of the highest dose.

Twenty four hours after the treatment, mice lost about 9% and 14% of their body weight when treated with 1 or 2 g /Kg of the extract, respectively ($P< 0.05$).

As shown in Table 3 the hepatic GSH concentration of mice given the two doses of the extract were about 75.5% and 66.1% of the control value ($P< 0.05$).

The effects of the methanol extract on plasma constituents were mostly statistically insignificant except that AST activity was increased by about 10% and 17% ($P<0.05$) in mice receiving the extract at doses of 1 and 2 g /Kg, respectively.

Macroscopic inspection of the internal organs revealed hepatic congestion in the treated mice, especially those given the highest dose. Histologically, there were mild signs of fatty degeneration, congestion and necrosis in liver. No other microscopic sign was seen in other vital organs examined.

Experiment 3

No mortality or overt morbidity was observed in mice receiving 100 and 500 mg/Kg of "compound A". The rectal temperature of the treated mice remained normal, and there were no changes in plasma biochemistry. The hepatic GSH concentration was reduced by about 10% and 13% ($P>0.1$) when "compound A" was given at the lowest and highest doses, respectively (Table 3). Results of the rectal body temperature of mice treated with the two doses of "compound A" showed that two and three h after the oral administration of the compound the rectal temperature dropped by 2.4-3.3^{o} C. By 24 hrs. the rectal temperatures of all mice were normal. On autopsy, the internal organs appeared macro- and microscopically normal.

Experiment 4

No overt sign of behavioral or clinical change were seen in mice receiving the authentic compound atractalocyte (100 or 500 mg/Kg). The rectal temperatures of mice given the two doses of the compound were normal when measured 0,1, 2,4 and 24 hrs after its administration. The liver/body weight ratios in mice treated with the lowest and highest dose of the atractalocyte were 6.04±0.32% and 5.17±0.36%, respectively compared to the controls in which this ratio was 6.16±0.14%.

Table 1. Changes in body and liver weight in mice treated with *Iphonia aucheri*

	Body weight (g)	Liver weight (g)	Body/liver weight (%)
Control	30.69 ± 0.72	1.67 ± 0.17	5.69 ± 0.37
I. aucheri (1g/Kg)	27.98 ± 0.70	1.67 ± 0.04	5.82 ± 0.13
I. aucheri (2 g/Kg)	29.54 ± 0.41	1.94 ± 0.04*	6.57 ± 0.09*

Values in the table are means ±SEM (n=6).
Mice were killed 24 h after treatment with aqueous extract of the plant I. *aucheri* given orally by gavage.
* $P < 0.05$ (compared to controls).

Table 2. The concentrations of certain analytes in the plasma of mice treated with aqueous extract of *Iphonia aucheri*

	Glucose (mmol/l)	Creatinine (umol/l)	Urea (mmol/l)	Cholesterol (mmol/l)	Protein (g/dl)	AST (U/l)	ALT (U/l)	GGT (U/l)
Control	8.3 ± 0.8	23.7 ± 2.3	4.5 ± 0.5	8.1 ± 0.7	5.7 ± 0.3	72.8 ± 8.1	43.1 ± 4.2	3.0 ± 0.2
I. aucheri (1 g /kg)	8.1 ± 0.8	25.6 ± 3.5	4.8 ± 0.5	8.3 ± 0.8	6.0 ± 0.5	100.5 ± 12.5*	50.2 ± 5.1	2.7 ± 0.3
I. aucheri (2 g /kg)	8.3 ± 0.8	27.0 ± 2.9	6.0 ± 0.6*	8.9 ± 0.7	6.0 ± 0.6	118.7 ± 15.1*	45.1 ± 5.5	3.0 ± 0.3

Values in the table are means ± SEM (n=6). Mice were treated with the extract and killed 24 hrs. later.

* $P <$ than 0.05 (compared to control in the same treatment group)

Table 3. The effect of different extract of *Iphonia aucheri* on the concentration of glutathione (GSH) in mice

	GSH concentration (100% control)
Control	100.0 ± 0.0
I. aucheri, aqueous extract (1g /Kg)	85.5 ± 6.6 *
I. aucheri, aqueous extract (2g/Kg)	71.3 ± 5.4 *
I aucheri, methanol extract (1g/Kg)	75.5 ± 8.8 *
I. aucheri, methanol extract (2g/Kg)	66.1 ± 7.6 *
Compound A (100mg/Kg)	89.9 ± 11.3
Compound A (500mg/Kg)	87.6 ± 9.9
Atractalocyte (100mg/Kg)	87.5 ± 8.8
Atractalocyte (500mg/Kg)	78.4 ± 7.7*

*Values reported are means± SEM (number of observations).
* P less than 0.05 (compared to control). The control value of GSH was 0.56 mg protein/ g tissue

Discussion

Taken together, the present results suggested that, on the whole, *I. aucheri* is a relatively non-toxic plant in mice. The effects produced by the aqueous and methanol extract, at doses of 1 or 2g /Kg were indicative of a very mild hepatic damage which was reflected in a modest depletion of GSH, a rise in plasma AST activity, but little or no macro- or microscopic evidence of hepatic pathology. These results in our mice are in sharp contrast to the results of Roeder, *et al.* (1994) who reported death of mice within 1-2 min following the oral administration of 1 ml of the aqueous extract (the concentration of which was not stated). Roeder, *et al.* (1994) reported that the diterpene glycosides atractyloside and carboxyatractyloside were the presumed 'toxic principle' of the plant. In the present work we tested the toxicity of a compound (designated "compound A") that was isolated as described by Roeder, *et al.* (1994) and Meyer (1993), and also tested the authentic compound atractyloside. Both were found to be essentially non-toxic. The reasons for these discrepancies are not known. The plant material was obtained from the same geographical location as that of the previous reports (Wernery, *et al.* 1992; Roeder, *et al.* 1993; Meyer 1993; Wernery, *et al.* 1997)), and the procedures used were similar. The only difference between our work and theirs is that the plant material used by the other workers was collected in June 1989 and 1990, and ours was collected in March of 1997. It is possible but rather unlikely that a change in the climate may have affected the concentration of the presumed 'toxic' material in the plant. However, this does not explain the lack of toxicity of the authentic atractyloside in our mice. Moreover, the toxicity of high doses of atractyloside and carboxyatractyloside causes necrosis in the renal proximal tubules (Obatomi and Bach, 1998), and this was not reported by the above workers.

Wernery, *et al* (1992), based on purely circumstantial evidence, implicated *I. aucheri* as the cause of toxicity in camels. As this plant is commonly found in areas where camels abound for years, and no previous cases of toxicity of this plant in any domestic or wild animal have been claimed, and the fact that no experimental work to reproduce the presumed toxicity in animals was conducted, it may be suggested that the effect, if any, of *I. aucheri* (collected in different seasons and from different locations) in domestic and laboratory animals be re-examined and verified.

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