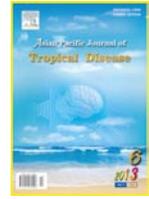


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Efficacy and toxicological evaluation of *Coccinia grandis* (Cucurbitaceae) extract in male Wistar rats

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Comments

Results of this study confirms the antidiabetic properties of *C. grandis* reported by previous investigators in other rat and human models. However, no previous studies have been carried out to evaluate in depth, the toxicological potential of this plant. Hence the toxicity study carried out using alloxan diabetic rats is of value and the results demonstrates that the plant can be used safely in the management of diabetes. Methodologies used and interpretation of results are satisfactory.

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ABSTRACT

Objective: To investigate the oral antihyperglycemic effect of aqueous leaf extract of *Coccinia grandis* (L.) (Cucurbitaceae) along with toxicological effects in alloxan induced diabetic and healthy Wistar rats.

Methods: A single graded dose of aqueous leaf extract of *Coccinia grandis* (0.25–2.00 g/kg) was administered orally to alloxan induced (150 mg/kg, *i.p.*) diabetic Wistar rats ($n=6$). In acute toxicity assessment, Wistar rats were administered the extract at the same dose range and observed for three days. Sub-chronic toxicity was evaluated by daily administration of the extract for 30 d at the dose which showed the optimum antihyperglycemic effect. Signs of toxicity, body weight of animals, consumption of food and water were monitored during the study period. The effects of the extract on biochemical (lipid parameters and activities of liver enzymes), hematological parameters (full blood count) and histopathological effects in organs were assessed at the end of the study.

Results: The optimum effective dose on glucose tolerance for *Coccinia grandis* leaf extract was found to be 0.75 g/kg in diabetic rats. The extract neither produced significant changes in consumption of food, intake of water, relative weight of organs nor affected biochemical, hematological and histopathological parameters ($P<0.05$).

Conclusions: The extract at a dose of 0.75 g/kg was found to be toxicologically safe as a potential antihyperglycemic agent in Wistar rats.

KEYWORDS

Acute and sub-chronic toxicity, Antihyperglycemic effect, *Coccinia grandis*, Hematological and biochemical parameter, Histopathology

1. Introduction

Medicinal plants have long been considered as valuable sources of medicine for treating variety of diseases and ailments. The increase in the indiscriminate use of plant extracts is further aggravated by the belief that plants are

safe simply because they are natural in origin. However, the consumption of medicinal plants as conventional medications or/and as curatives may cause adverse toxicological effects to human health^[1]. Therefore a proper scientific evaluation of medicinal plants with pertinent emphasis on toxicological paradigms is imperative while assessing the efficacy. Further,

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toxicological data from animal studies will be crucial in judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products.

Coccinia grandis (Linn.) Voigt (Cucurbitaceae) (*Coccinia grandis*) is an edible perennial climber distributed in tropical Asia, commonly found in Sri Lanka, India and Pakistan. Every part of this plant is valuable in medicine and various decoctions have been prepared in Sri Lankan indigenous system of medicine for the treatment of various skin diseases, diabetes mellitus, urinary tract infections, bronchitis, itchy skin eruptions and ulcers^[2,3]. Further, leaves extract of *C. grandis* is widely used for the treatment of diabetes mellitus by Ayurvedic physicians in Sri Lanka^[4]. The leaves extract of *C. grandis* has been evaluated for *in vivo* hepatoprotect activity^[5], antimicrobial^[6], antioxidative^[7], anti-inflammatory^[7], and antihyperglycemic activities^[8]. Among these, the antihyperglycemic effect is pronounced. However, there is limited scientific data available on the efficacy of glucose tolerance of the aqueous leaf extract of *C. grandis* or its toxicological effects (by assessment of effects on biochemical, hematological and histopathological parameters). Therefore the aim of the present study was to determine the effect of leaf extract of *C. grandis* on glucose tolerance in alloxan induced diabetic rats and also evaluate toxicological effects of the extract in male Wistar rats, identifying a toxicologically safe dose for the development of novel antidiabetic agents.

2. Materials and methods

2.1. Chemicals

D-glucose, glibenclamide and alloxan monohydrate were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Chemicals were of analytical grade and used without any purification. A UV visible spectrophotometer (Gallenkamp PLC, UK) and an automated hematological analyzer (Sysmax KH21, Japan) were used for spectrophotometric and hematological measurements respectively.

2.2. Plant material

Leaves of *C. grandis* were collected during May–June 2011 from the southern region of Sri Lanka. Botanical identity was determined by the descriptions given by Jayaweera^[2], and authenticated by Dr. A. Attanayake, Taxonomist, National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka and a voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (Attanayake/2011/ 03).

2.3. Preparation of the aqueous plant extract

The leaves were cut into small pieces, dried at 40 °C until a constant weight was reached and coarsely ground. Powdered plant material (50.00 g) was dissolved in 400.0 mL of distilled water and refluxed for 4 h. The mixture was strained through cheese-cloth and the final volume was adjusted to 50.0 mL. A single dose of 0.25, 0.50, 0.75, 1.00, 1.25, and 2.00 g/kg was

administered orally to diabetic and healthy rats in screening for the efficacy of glucose tolerance and in acute toxicity study respectively. The optimum effective dose was selected and administered orally to healthy Wistar rats in sub-chronic toxicity study.

2.4. Animals

Healthy adult male rats of Wistar strain (200±25) g body weight were used to carry out experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka at a condition of temperature (25±2) °C, relative humidity 55%–65% and (12±1) h light/dark cycle. Rats were fed with standard diet (MRI rat formulae, Sri Lanka) with free access to water before and during the experiment. The rats were randomized divided into various groups and allowed to acclimatize for a period of seven days under standard environmental conditions before the commencement of the experiment. The animals described as fasting were deprived of food for 12 h. All protocols used in this study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka guided by the CIOMS international guiding principles of biomedical research involving animals.

2.5. Development of the diabetic rat model

Alloxan monohydrate dissolved in sterile saline at a dose of 150 mg/kg was administered intraperitoneally to rats fasted for 16 h. The rats were maintained on 5% D-glucose solution for the next 24 h. Rats were allowed to stabilize for three days and blood samples were drawn from tail vein on the third day to determine the blood glucose concentration to confirm the development of diabetes mellitus. Rats with fasting blood glucose value of 9.70 mmol/L (equal to fasting serum glucose concentration of 11.0 mmol/L) or above were considered as hyperglycemic and used for experiments^[9].

2.6. Screening for efficacy and dose response in alloxan induced diabetic rats

The first and second group untreated healthy and diabetic rats ($n=6$ /group) received distilled water. Group three to eight consisted of six sub groups (a–f) of alloxan induced diabetic rats ($n=6$ /group) that received a dose of 0.25, 0.50, 0.75, 1.00, 1.25 and 2.00 g/kg of the extract of *C. grandis* respectively, orally. The ninth group ($n=6$ /group) was administered glibenclamide (0.50 mg/kg) which served as the positive control. The rats were given an oral dose of glucose (3.00 g/kg) 30 min after the administration of the plant drug. Blood samples were collected just prior to administration of extract/drug 0, 1, 2, 3 and 4 h subsequently. Blood glucose concentration was measured immediately by the glucose-oxidase method using glucose assay kit based on the Trinder reaction^[10]. The acute effect was evaluated over a 4 h period using area under the oral glucose tolerance curve^[11]. The optimum effective dose of *C. grandis* extract in diabetic rats was determined.

2.7. Acute toxicity study

Acute toxicity testing was performed for plant extracts following the Organization for Economic Cooperation and Development (OECD) guideline 425, fixed dose procedure. Six groups containing healthy male rats ($n=6/\text{group}$) received aqueous extract of *C. grandis* at doses of 0.25, 0.50, 0.75, 1.00, 1.25, and 2.00 g/kg orally while the untreated healthy control group received distilled water. Animals were observed individually after dosing once during the first 30 min, periodically during the first 24 h and thereafter for two days. Behavioral changes (restlessness, dullness, agitation) and signs of toxicity were observed.

2.8. Sub-chronic toxicity study

Rats were randomly allotted to two groups ($n=6/\text{group}$). The first group served as the untreated healthy control group received distilled water daily. The rats in the second group received the aqueous leaf of extract of *C. grandis* at the optimum effective dose (0.75 g/kg; which was determined under screening for efficacy), daily for 30 d. The body weight of each rat was assessed before the commencement of dosing, during the experimental period at weekly intervals and on the day of sacrifice. The amount of food, water consumed were measured daily from the quantity of food, water supplied and the amount remaining after 24 h.

The fasted (12 h) animals were sacrificed on the 30th day of the experiment. Blood samples were collected via cardiac puncture into clean dry centrifuge tubes and ethylenediaminetetraacetic acid (EDTA) bottles for serum biochemical and hematological analysis respectively. The heart, lung, small intestine, liver, spleen, pancreas and kidney were carefully isolated for relative organ weight assessment and fixed in buffered formalin for histopathological examination. The relative organ weight (ROW) of heart, lung, small intestine, liver, spleen, pancreas and kidney of each animal was calculated as follows.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of Wistar rat on the day of sacrifice (g)}} \times 100\%$$

2.9. Biochemical assessment

Serum concentrations of fasting glucose^[10], total cholesterol, high density lipoprotein cholesterol and triglyceride^[12] were estimated using enzyme assay kits (Prodia Int. Germany). Serum activities of ALP; alkaline phosphatase, alanine aminotransferase; alanine aminotransferase and aspartate aminotransferase; aspartate aminotransferase were estimated to assess the effects on liver using colorimetric enzyme assay

Table 1

Total area under the oral glucose tolerance curve values of test rats (mmol/L h).

Experimental group	Dose (mg/kg)						
	0	0.25	0.50	0.75	1.00	1.25	2.00
Healthy untreated	25.88±1.46						
Diabetic untreated	70.36±6.58						
<i>C. grandis</i> (0.75 g/kg)		70.11±1.75	70.19±2.12	47.96±1.94 ^a	47.32±1.48 ^a	47.16±1.36 ^a	6.74±1.39 ^a
Glibenclamide (0.50 mg/kg)	42.72±1.84 ^a						

The values are expressed as mean±SEM ($n=6/\text{group}$). ^a: Statistically different from diabetic control rats at $P<0.05$ (ANOVA followed by Dunnett's test).

kits (Stanbio, USA)^[13]. Serum concentration of total protein was determined by the method of Lowry et al^[14].

2.10. Hematological assessment

Hematological analysis was performed using a hematological analyzer. Total hemoglobin, hematocrit, total red blood corpuscles, platelet count, red cell indices including packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood corpuscles, percentage of neutrophils, lymphocytes, eosinophils and monocytes of blood samples were recorded.

2.11. Histopathological assessment

The heart, lung, small intestine, liver, spleen, pancreas and kidney were fixed in 10% formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin for light microscopic examination of histopathological changes.

2.12. Statistical analysis

Results were expressed as Mean±SEM. The dose response and toxicological data were analyzed by ANOVA followed by Dunnett multiple comparison test and two sample *t*-test using the Minitab statistical software respectively. Results were considered to be significant at $P<0.05$.

3. Results

3.1. Screening for efficacy and dose response in diabetic rats

The mean total area under the curve values of *C. grandis* extract treated diabetic rats for the six doses ranging from 0.25 to 2.00 g/kg are shown in Table 1. The extract showed a dose-dependent improvement on glucose tolerance in diabetic rats. The total area under the curve during the 4 h period was significantly increased ($P<0.05$) in alloxan induced diabetic rats as compared to healthy rats [(70.36±6.58) mmol h/L vs. (25.88±1.46) mmol h/L]. The extract of *C. grandis* showed the optimum effectiveness at the dose of 0.75 g/kg in diabetic rats (47.96±1.94 mmol h/L, improvement of 32 %). Glibenclamide treated diabetic rats demonstrated an improvement of 39% on glucose tolerance in diabetic rats. Further the *C. grandis* treated diabetic rats showed a statistically significant percentage improvement at 0.75 (optimum effective dose), 1.00, 1.25 and 2.00 g/kg ($P<0.05$). As shown in Table 2, *C. grandis* treated diabetic rats showed the highest percentage reduction in glucose concentration of 39% (3

Table 2Blood glucose concentration at the optimum effective dose of *C. grandis* extract in diabetic rats.

Treatment	Blood glucose concentration (mmol/L)				
	FBG (0 h)	1 h	2 h	3 h	4 h
Healthy untreated	4.92±0.78	8.13±0.99	6.51±0.67	5.92±1.02	5.71±0.12
Diabetic untreated	14.52±1.05	22.34±1.38	16.70±0.73	16.25±1.03	15.64±0.77
<i>C. grandis</i> (0.75 g/kg)	14.69±0.62	15.50±0.85 ^a	10.35±0.97 ^a	9.96±0.72 ^a	9.60±0.93 ^a
Glibenclamide (0.50 mg/kg)	14.13±1.16	15.00±0.60 ^a	8.60±1.06 ^a	8.14±0.60 ^a	7.83±0.63 ^a

The values are expressed as mean±SEM ($n=6$ /group). FBG: Fasting blood glucose. ^a: Statistically significant from untreated diabetic rats at $P<0.05$ (ANOVA followed by Dunnett's test).

h) at the optimum effective dose.

3.2. Acute toxicity study

There was no mortality or morbidity observed in rats through three day period following single oral administration at all selected doses of the extract of *C. grandis*. The animals did not show any changes in general appearance during the three day period. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhoea, lethargy or unusual behavior were observed.

3.3. Sub-chronic toxicity study

There was no significant difference ($P<0.05$) in body weights of animals (Figure 1), consumption of water and food between two groups (Figure 2 and Figure 3). The oral ingestion of the extract of *C. grandis* over 30 d caused no significant changes ($P<0.05$) in relative weight of the organs, *i.e.* heart, lung,

small intestine, liver, spleen, pancreas and kidney in treated rats as compared to the control rats (Figure 4). The results of biochemical and hematological analysis are shown in Table 3. There was no statistical difference in the parameters listed in plant treated rats compared to the control ($P<0.05$). In the histopathological assessment (Figure 5), very few scattered occasional lymphocytes and congested blood vessels with no edema were shown in the heart tissue of plant treated rats. Further very few peribronchial lymphocytic infiltrates, mild congestion with no edema were noted in the lung tissue of treated rats. The histopathological assessment of liver revealed few lymphocytic infiltrates around the central vein, in the portal tract and in the parenchyma in both control and plant treated rats. The histopathological examination of the tissues of kidney, small intestine, spleen and pancreas showed no changes in cellular architecture in treated rats. The histopathological study revealed no treatment-related cellular changes in vital organs in treated rats at light microscopy. Further the findings were generally consistent with the expected pattern for Wistar rats of

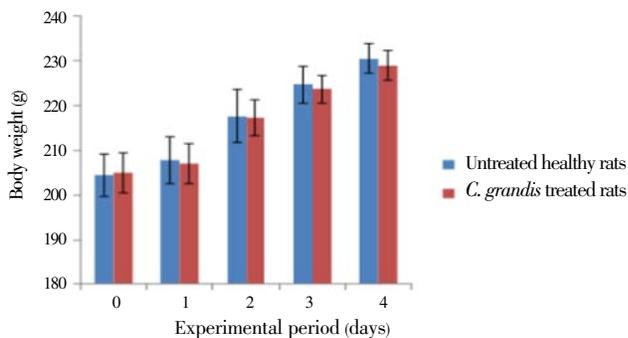


Figure 1. Effect of the *C. grandis* extract at dose of 0.75 g/kg on mean body weights of rats for 30 d. Each column represents the mean±SEM ($n=6$). The two sample t -test at $\alpha=0.05$ showed no statistical difference between mean body weight in treated rats compared to untreated rats.

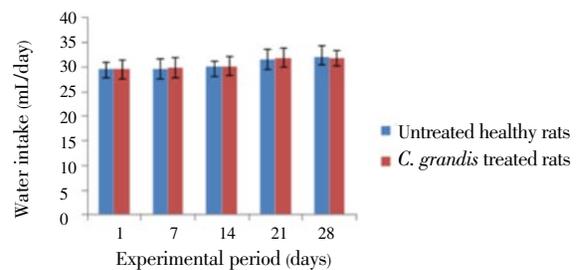


Figure 2. Effect of the *C. grandis* extract at the dose of 0.75 g/kg on consumption of water in rats for 30 d. Each column represents the mean±SEM ($n=6$). The two sample t -test at $\alpha=0.05$ showed no statistical difference between mean water intake in treated rats compared to untreated healthy rats.

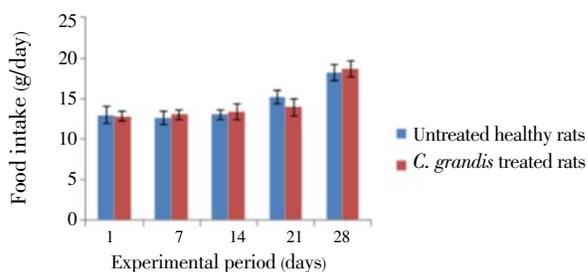


Figure 3. Effect of the *C. grandis* extract at the dose of 0.75g/kg on consumption of food in rats for 30 d. Each column represents the mean±SEM ($n=6$). The two sample t -test at $\alpha=0.05$ showed no statistical difference between mean food intake in treated rats compared to untreated healthy rats.

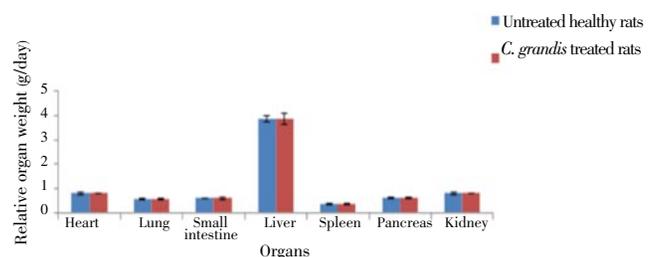


Figure 4. Effect of the *Coccinia grandis* extract at the dose of 0.75g/kg on relative weight of organs in rats for 30 d. Each column represents the mean±SEM ($n=6$). The two sample t -test at $\alpha=0.05$ showed no statistical difference between mean relative weight of organs in treated rats compared to untreated healthy rats.

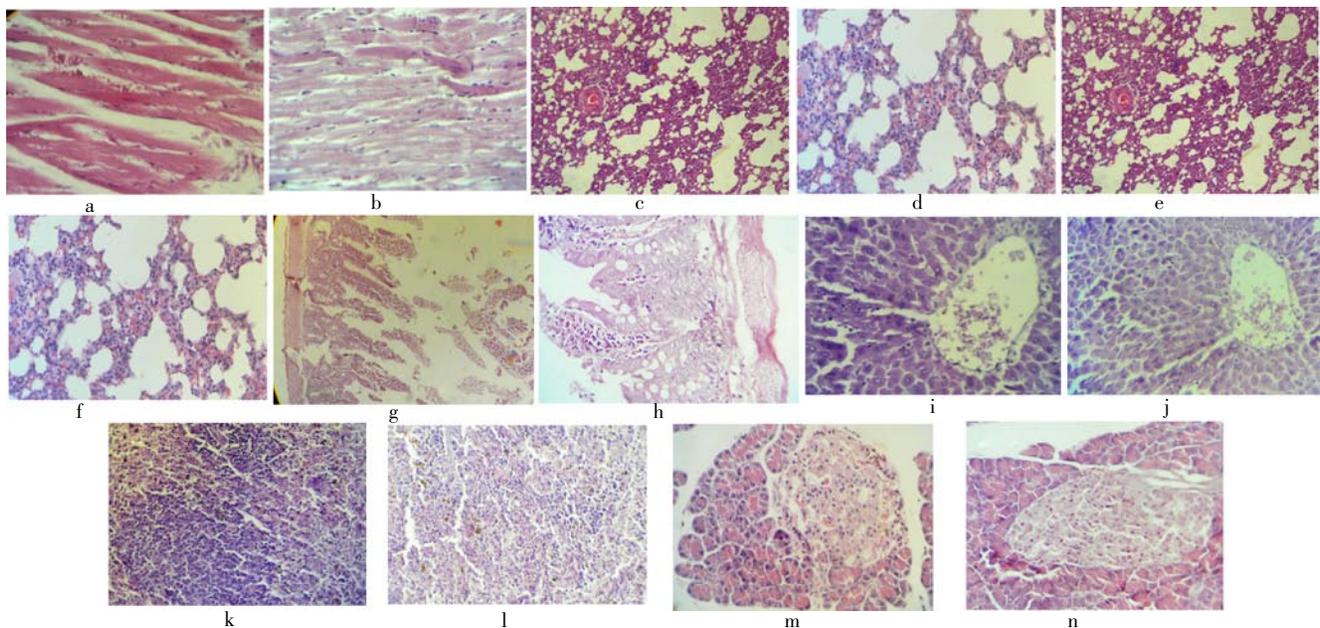


Figure 5. Photomicrographs (x 400) of heart, lung, small intestine, liver, spleen, pancreas and kidney histopathology from representative rats of untreated healthy rats ; a, c, e, g, i, k, m: *C. grandis* treated rats received (0.75g/kg); b, d, f, h, j, l, n: Control group rats.

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Table 3

Effect of the *C. grandis* extract on biochemical and hematological parameters in healthy Wistar rats.

Parameters	Untreated healthy rats	<i>C. grandis</i> treated rats
Fasting blood glucose(mmol/L)	93.66±1.74	95.23±1.09
Total cholesterol (mmol/L)	3.70±0.18	3.70±0.11
HDL cholesterol (mmol/L)	1.39±0.02	1.42±0.04
LDL cholesterol (mmol/L)	1.86±0.10	1.82±0.12
Triglyceraldehyde (mmol/L)	0.99±0.02	0.98±0.01
Alkaline phosphatase(U/L)	61.48±1.50	62.40±1.46
Alanine aminotransferase (U/L)	12.39±0.79	11.00±0.89
Aspartate aminotransferase(U/L)	44.21±1.75	42.77±2.54
Total protein (g/L)	70.54±3.70	69.85±1.90
Total hemoglobin (g/dL)	15.25±0.80	14.8±0.43
Red blood corpuscles (10 ⁶ /mm ³)	8.27±1.31	7.93±0.33
Platelet count(10 ³ /mm ³)	1096.33±93.87	945.00±61.23
Pack cell volume (%)	47.95±2.38	48.94±2.07
Mean corpuscular volume (fL)	61.45±1.28	61.76±2.47
Mean corpuscular hemoglobin (pg)	19.53±0.60	18.68±0.77
Mean corpuscular hemoglobin concentration (g/dL)	31.77±0.90	30.26±0.65
White blood corpuscles (10 ³ /mm ³)	4.82±1.60	5.68±1.09
Neutrophils (%)	42.00±2.77	40.70±3.36
Lymphocytes (%)	55.5±7.73	57.40±5.13
Eosinophils (%)	1.80±0.40	1.20±0.45
Monocytes (%)	0.70±0.00	0.70±0.00

Each value represents the mean±SEM (n=6). The two sample *t*-test at $\alpha=0.05$ showed no statistically difference between the parameters studied in treated healthy rats compared to untreated healthy rats.

4. Discussion

In the present study, the efficacy and dose response of leaf extract of *C. grandis* on glucose tolerance in diabetic rats, the acute and sub-chronic toxicological effects of the extract were investigated in Wistar rats.

Based on historical evidence, oral administration is the most convenient and commonly used route when screening for

efficacy and toxicological effects in laboratory animals[15]. The rate of absorption might be slow, but this method costs less and is painless to animals. Further, the same route was used for the administration of herbal remedies as aqueous extracts to patients by physicians since time immemorial[16].

Alloxan was frequently used to develop a diabetic rat model and also to investigate antihyperglycemic effects of plant extracts[17]. Alloxan monohydrate, a highly cytotoxic agent of pancreatic beta cells in islets, induces diabetes by destruction of the β -cells that causes reduction in insulin release[18]. This effect was demonstrated in the present study through the elevation of fasting blood glucose concentration in alloxan induced diabetic rats as compared to healthy rats.

The glucose tolerance test has been widely accepted as an initial screening tool for assessment of antihyperglycemic effects of medicinal plant extracts in animal models[19]. It is a simple test used to determine the capacity of substances to interfere with glucose homeostasis but it does not give any indication on their mechanism of action. The extent of reduction in blood glucose concentration/improvement on glucose tolerance with the treatment over a period of 4 h was evaluated using the total area under oral glucose tolerance curve. Low total area under the curve reflects high efficacy or improvement in glucose tolerance of the extract. Diabetic rats treated with glibenclamide showed a significant reduction in the total area under the curve and blood glucose concentration in oral glucose tolerance test. The blood glucose concentration in plant treated rats was increased in the first hour after ingestion of glucose load, and reduced within succeeding hours as compared to the glibenclamide. However, the reduction in blood glucose concentration in plant extract treated rats was lesser than that of glibenclamide treated rats.

Acute toxicity study was conducted with a range of six doses of 0.25–2.00 g/kg including the optimum effective dose of *C. grandis* (0.75 g/kg). The human therapeutic dose was extrapolated to compute the range of doses according to the standard guidelines[20]. The acute toxicity study indicated that treatment of *C. grandis* with selected doses was well

tolerated by all test animals, suggesting its safety for further investigations.

The dose of 0.75 g/kg was chosen for the sub-chronic toxicological evaluation because it is the optimum effective dose which shows the antihyperglycemic activity in diabetic rats. Generally, body weight gain and relative weight of organs are simple and sensitive indices of toxicity after exposure to potentially toxic substances^[21]. In the present study, the aqueous leaf extract of *C. grandis* did not significantly alter body or relative weight of organs in treated rats as compared to untreated rats, which suggested that the extract did not hinder the growth of Wistar rats. Furthermore, the determination of food intake and water consumption is important in the study of safety of a natural product for a therapeutic purpose, as proper intake of nutrients is essential to maintain the physiological state of the animal and to accomplish the proper response to plant extracts tested^[22]. In this study, the consumption of food and water were not altered, suggesting that it does not induce or suppress appetite.

The assessment of hematological parameters can be used to determine the extent of deleterious effects of foreign compounds including plant extracts on the blood constituents of an animal. The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status^[23]. Such toxicity testing is relevant for changes in the hematological system which has a higher predictive value for human toxicity, when extrapolated from animal studies. Sub-chronic exposure of Wistar rats to the leaf extract of *C. grandis* produced small and transient changes in some hematological parameters as white blood corpuscles, neutrophils and lymphocytes, however the values were statistically not significant at $P < 0.05$.

The histopathological assessment is the gold standard for evaluating treatment related pathological changes in tissues^[15]. In the present study, histopathological evaluation of repeated dose administration of plant extract indicated that the extract did not adversely affect heart, lung, small intestine, liver, pancreas and kidney of rats, thus corroborating the results of biochemical analysis. Even though there were minor alterations in the percentage of neutrophils and lymphocytes, the histopathological assessment of spleen did not exhibit any abnormalities in rats treated with the extract implying no effect of this plant on hematopoiesis and immunologic functions.

Ordinarily, liver cell damage is characterized by a rise in serum enzymes like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, etc. The absence of significant increases in alanine aminotransferase and aspartate aminotransferase observed in this study strongly suggested that the long term administration of *C. grandis* did not alter the hepatocytes and consequently the metabolism in rats. Liver controls glucose synthesis and generates free glucose from hepatic glycogen stores. The absence of any effects on fasting serum glucose concentration and all the serum lipid parameters investigated in this study suggested that lipid and carbohydrate metabolism in the animals were not altered.

The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal and cardiovascular adverse effects^[24], while certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals^[25]. However, the effects on cardiovascular, central

nervous and respiratory systems and effects on fertility, teratogenicity were not assessed during the study and these should be evaluated prior to human exposure. Furthermore, it is quite difficult to ascertain certain adverse effects in animals, such as headache, abdominal pain, dizziness and visual disturbances, which can be considered as limitations of the present study. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. The activity of aspartate aminotransferase is slightly deviated from human normal values, however the values are in accordance with the published experimental data by other authors^[26]. Studies are in progress to elucidate mechanisms of antidiabetic activity using the optimum effective and safe dose of the leaf extract of *C. grandis*.

In conclusion, the aqueous leaf extract of *C. grandis* at a dose of 0.75 g/kg possessed the optimum effectiveness in antihyperglycemic activity in alloxan induced diabetic Wistar rats. The acute toxicity study suggested that aqueous leaf extract of *C. grandis* is safe in healthy Wistar rats up to a dose of 2.00 g/kg. The oral administration of the *C. grandis* at a dose of 0.75 g/kg to rats for 30 d was not associated with adverse effects reflected in the general condition, growth, body and relative weight of organs, clinical biochemical values, hematology and did not result in histopathological abnormalities. Further the extract at a dose of 0.75 g/kg was found to be toxicologically safe as a potential antihyperglycemic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

C. grandis has been used in the traditional medical practice of Sri Lanka for many years and it has been reported to have significant antidiabetic potential. However, its toxic potential has not been investigated in depth. Since the value of any drug depends on its non-toxicity, this study will be of importance in evaluating how safe *C. grandis* containing medications are.

Research frontiers

The research findings while confirming the ability of *C. grandis* to mediate beneficial effects in diabetic rats,

demonstrates that extract of this plant is fairly safe to use at a dose equivalent to the normal human dose to treat diabetic patients.

Innovations & breakthroughs

In Sri Lankan indigenous system of medicine, *C. grandis* preparations are being used for the treatment of various skin diseases, diabetes mellitus, urinary tract infections, bronchitis, itchy skin eruptions and ulcers. The present study demonstrates that an extract of this plant is safe to use in diabetic rats.

Applications

Findings of the toxicity study indicates that *C. grandis* extract may have the potential to be used in the future for development of an antidiabetic drug with little toxic side effects, for management of human diabetic patients.

Peer review

Results of this study confirms the antidiabetic properties of *C. grandis* reported by previous investigators in other rat and human models. However, no previous studies have been carried out to evaluate in depth, the toxicological potential of this plant. Hence the toxicity study carried out using alloxan diabetic rats is of value and the results demonstrates that the plant can be used safely in the management of diabetes. Methodologies used and interpretation of results are satisfactory.

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