Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1007-1012

ISSN: 0975-4873

Research Article

Acute Oral Toxicity and Anti-inflammatory Activity of Ethanolic Extract from *Pilosocereus gounellei* (Fac Weber) in Rats

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Available Online:28th September, 2015

ABSTRACT

Pilosocereus gounellei is traditionally used by Brazil's northeastern population for treatment of prostate inflammation, skin lesions, hydrops, and kidney infections. Toxicological and pharmacological studies of *P.gounellei* are of fundamental importance towards ensuring the safety and efficacy of this plant for the community. The aim of this study was to evaluate both acute oral toxicity and anti-inflammatory activity of *P. gounellei* ethanol stem extract (Pg-EEtOH), through acute toxicity, and carrageenan induced paw edema models. The Pg-EEtOH showed an LC_{50} of 547.3 ug / mL, an LD_{50} > 2000 mg / kg; it did not cause behavioral changes, and yet caused significant decreases in the consumption of water and food. In females we observed an increase in serum urea levels, and decreased creatinine levels. In the paw edema model we observed that the dose of 25 mg / kg significantly reduced edema, and from the data we observed that despite changes in certain parameters, the Pg-EEtOH displayed low toxicity, this while having anti-inflammatory activity at a dose of 25mg / kg.

Keywords: *Pilosocereus gounellei*, acute oral toxicity; biochemic; hematology, Anti-inflammation.

INTRODUCTION

Since ancient times mankind seeks the aid medicinal plants to treat diseases at that time there was not enough information on using these herbal drugs as a cure. With the development of science gradually the use of medicinal plants to treat diseases became based on scientific studies. Medicinal plants were the main sources of treatment and prophylaxis of diseases until the advent of the chemical industry development in the 16th century, however, the decreasing effectiveness of synthetic drugs and the growing contraindications of these drugs bring back the interest in using herbal drugs[1,2].

Medicinal plants in Brazil have been used with little or no evidence of their pharmacological properties, they are often used for medicinal purposes different from those employed in ethnopharmacological use. The toxicity of medicinal plants and herbal medicines compared to allopathic medicines may seem trivial. However the adverse effects and the synergistic action of medicinal plants occur commonly[3]. Many plant species used in therapy are still obtained by wild harvest; this is due to lack of information and to difficulties in cultivation[4]. Despite the increase in plant studies, only 15 to 17% have been studied for their medicinal potential. Considering the great biodiversity of the Brazilian Northeast, this number could be much higher[5].

Pilosocereus gounellei, also known as "xique-xique" is a species from the family Cactaceae. The genre *Pilosocereus* Byles & Rowley belongs to the subfamily Cactoideae, of the Cereeae tribe. It is the largest genus with 35 species distributed from Mexico to Paraguay, yet with the greatest diversity of species in Brazil, where they occur in a number of different environments, including savannas, sandbanks (or salt marshes), and in rocky fields[6].

Although classical medicine (in a limited way) describes the medicinal potential of some cactus species, the inhabitants of Brazilian semiarid municipalities also possess this knowledge through information gathered during their continuing integration with the living environment, and they transmit it from generation to generation. The stem, root, and flowers of *P. gounellei* are popularly used to treat prostate inflammation, jaundice, hyperglycemia, and injuries. The macerated root is also used to treat inflammations in the urethra[7,8]. In the "xique-xique" pulp, coumarins, flavonoids, and traces of saponins have been detected[9,10].

The information obtained from these communities converges with the literature, but much of it differs. This suggests the need for continuing research as to the degree of toxicity, and to the anti-inflammatory effects of *Pilosocereus gounellei* in order to ensure the safety and effectiveness of its use. The present paper aims to assess acute toxicity (oral), and the anti-inflammatory activity of *Pilosocereus gounellei* ethanolic extract.

MATERIALS AND METHODS

Preparation of (Pg-EEtOH) ethanol extract from Pilosocereus gounellei (FAC Weber) Stems of Pilosocereus gounellei species were collected in Boa Vista

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- PB in November 2010. Botanical identification was made by Prof. Dr. Leonardo Felix Person (DF / CCAUFPB), and a voucher specimen was deposited in the Prof. Jaime Coelho de Morais - Center for Agricultural Sciences Herbarium, Federal University of Paraíba (CCA / UFPB) under code 15.437.

The collected material (stems) was dehydrated in an oven with circulating air at 40° C for 72 hours. The stems were then ground separately in a mechanical mill providing 2,080 g of stem powder. The powder was subjected to maceration for 72 hours at room temperature, using as 95% ethanol extraction liquid, and the process was repeated thoroughly, resulting in the respective extraction solutions which were filtered, and concentrated on a rotary evaporator, yielding 105.29 g of Pg-EEtOH.

Animals

Wistar rats (Rattus norvegicus) albino adult male rats, weighing between 180 and 250 grams, provided by Prof. vivarium Thomas George of the Biotechnology Center of the Federal University of Paraíba - UFPB were grouped in polyethylene cages containing six animals in each. They were kept under controlled temperature of 21° C +/- 1 without the use of any medications, with free access to feed (pellet type), and drinking water available in graduated polyethylene bottles with stainless steel nozzles and placed on the upper part of their metal grid cages. The research activities were developed at the Laboratory for Toxicological Testing (LABETOX), and at the Prof. Thomas George Bioterium of the Biotechnology Center of the Federal University of Paraíba - UFPB

Determination of Lethal Concentration 50% (LC50)

To perform this test, 25 mg of eggs (cysts) of Artemia salina were placed in saline water (pH 8-9, and 29° C), under artificial lighting for 24 hours for the eggs to hatch, and for the resulting release of nauplii. Then, 5 ml of the various concentrations of PG-EEtOH were placed in test tubes, and 15 nauplii were added. Each concentration was tested in triplicate and repeated in three experiments. A control group containing only solvent and larvae was also used. All were incubated for 24 hours under artificial light. After this period, the numbers of live and dead larvae were counted. The LC50 was determined according to the Probit statistical method (11,12,13) using the MicroCal Origin software. LC50 values below 1000 g / ml indicate that the examined extract has biological activity[11].

Acute toxicological test

In the acute toxicity study, animals were treated with a single dose of extract. Groups of Wistar rats, 6 males and 6 females, 6 animals per box, received 2000 mg / kg body weight orally of Pg-EEtOH. After treatment, the general effects were observed in the experimental animals presented in intervals of: 30, 60, 90, 120, 180, and 240 minutes on the first day, and once daily thereafter, (always at the same time), for the following 13 days. This was done with the objective of mapping possible behavioral changes suggestive of activity on the central nervous system (CNS), or the Autonomic Nervous System (ANS) (14). Also a group of 12 animals (6 males and 6 females) was treated with the dilutive extract vehicle, as the control group.

Daily water consumption, food intake and weight gain were observed weekly. After day 14, the surviving animals, and control animals were sacrificed, and blood samples collected for laboratory analyses of hematological and biochemical parameters, as well as organs for macroscopic examination. If gross changes were observed, histopathological examinations were performed on the organs.

Laboratory blood analysis

The sample collection was performed by tapping the brachial plexus, and blood was collected in tubes with anticoagulant ethylenediaminetetraacetic acid (EDTA) for determination of hematological parameters, in tubes with separator gel - Microtainer Becton Dickson® - which was centrifuged for 10 minutes at 3500 rpm to obtain serum for the determination of the biochemical parameters.

Hematological

Hematological analyses appear in the study of red blood (erythrocyte), white (WBC), and platelet count. The red blood cell count (HEM), determination of hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were performed. The leukocyte count was done as the global leukocyte count, and cell differentiation; sedimentation, eosinophils, lymphocytes and monocytes.

Biochemical parameters

Biochemical analyses were performed on serum samples. Dosages of glucose, urea, total cholesterol, CHDL, triglycerides, uric acid (enzymatic method), creatinine, creatine kinase, amylase, transaminases, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (using the method kinetic), total protein, albumin, and globulin (by biuret method) were performed in an automated biochemical analyzer COBAS MIRA PLUS - ROCHE.

Paw edema induced by carrageenan

The anti-inflammatory activity was analyzed according to the methodology described by Winteret al. (1962). Wistar rats received the following treatments (p.o.), vehicle (0.9% saline.), the non-steroidal anti-inflammatory indomethacin standard, and three different doses of PG-EEtOH (25, 50, and 100 mg / kg). Shortly thereafter, animal paw volume was measured at (time zero). After 60 minutes, inflammation was induced by administration of 0,1µL carrageenan (cg) at 1% in the sub-plantar region of the right hind paw of each animal. Animal paw volume was measured after the carrageenan injection (time zero), and during the 4 subsequent hours at constant intervals of 60 min with the aid of a plethysmometer.

Inhibition of the induced inflammatory process for the tested substances was estimated in terms of percent inhibition calculated according to the equation: Anti-inflammatory activity (%) = [(n-n ') / n] x 100, where n and n' indicates the mean ± standard deviation of edema volume in control and test groups at a given time (15).

Statistical analysis

For statistical analysis, the unpaired Student "t" test was used, using the Graph Pad Prism 6.0 software, and the

Table 1: Effects of oral administration of PgEEtOH at 2000 mg / kg on rats for the consumption of water and feed for 14 days.

Treatment	Water Consumption (mL)	Feed Consumption (g)
	Males	
Control	38.01 ± 5.037	25.11 ± 2.143
Treated	35.00 ± 2.87*	22.03 ± 1.941**
	Females	
Control	35.00 ± 4.349	20.01 ± 3.616
Treated	31.73 ± 1.597*	18.45 ± 2.845*

Values are expressed as mean +/- SD (n = 7); Student Test "t". * p <0.05 and ** p <0.01

Table 2: Effect of PgEEtOH oral administration at 2000 mg / kg on organs per body weight (%) in rats

Organs (g)	Males		Females	
	Control	Treated	Control	Treated
Heart	0.41±0.11	0.39±0.02	0.38±0.05	0.43±0.08
Liver	3.77±0.80	3.58±0.14	3.30±0.50	3.45±0.35
Kidneys	0.79±0.25	0.86±0.06	0.78±0.12	0.86±0.24
Spleen	0.42±0.32	0.27±0.02	0.24±0.04	0.33±0.15
Lung	0.70±0.23	0.63±0.06	0.61±0.10	0.75±0.22

Hematological parameters	Males		Females	
	Control	Treated	Control	Treated
HEM (10^6/µL)	8.44±0.29	8.02±0.72	8.10±0.30	8.10±0.22
HGB (g/dL)	12.99±0.22	12.70±1.02	12.88±0.24	12.93±0.47
HCT (%)	43.10±1.29	41.43±4.19	41.03±0.89	42.07±2.47
MCV (fL)	51.29±0.95	51.71±1.97	50.83±1.32	52.00±2.58
MCH (pg)	15.40±0.42	15.84±0.29	15.92±0.48	15.96±0.58
MCHC (g/dL)	30.16±0.61	30.70±1.24	31.38±0.34	30.81±0.87
Leucocytes (10^3/µL)	6057±2307	6186±2867	5733±1371	4771±2542
Sedimented (%)	20.00±6.27	23.86±7.38	17.50±5.75	19.14±5.36
Eosinophils (%)	0.85±0.90	0.28±0.75	1.16±1.60	1.14±1.07
Lymphocyte (%)	71.14±7.70	68.71±8.28	75.33±4.08	75.57±5.96
Monocytes (%)	7.85±2.19	7.14±3.80	6±3.22	4±2.30
Platelets (10^3/µL)	731000±88287	556143±245586	545833±214158	642571±204122

Values are expressed as mean +/- SD (n = 7); Student Test "t" test. * p <0.05

results were considered significant when presenting values of p <0.05.

RESULTS

The crude ethanol extract showed an LC_{50} of 547.3 ug / mL, in biological activity analyses (using the *Artemia salina* model), doses less than 1000 mg / mL are indicative of potentially active molecules.

In the acute toxicity evaluation, after 14 days of an administration of 2000mg / kg of PG-EEtOH, a significant reduction in the consumption of water and animal feed in both male and female rats (Table 1). Analysis of behavioral parameters showed no changes during the 14 days of evaluation.

In the analysis data of the animal organ weights calculated based on the weight of the animals compared with the respective control group (Table 2). no change was observed, With macroscopic analysis of the organs, and in the weight evaluation of the animals, there were no significant changes in the organ weights of the animals.

Index by body organ = (organ weight x 100) / body weight; a dose of 2000 mg / kg of PG-EEtOH was administered; values are expressed as mean ± standard deviation (n = 7) Test "t" Student test, and the level of significance was set at p <0.05

The analysis data of the animals' hematology parameters (Table 3). It can be seen that there were no significant changes in the evaluated hematologic parameters, when compared to the controls.

Table 3. Effect of Pg-EEtOH 2000 mg / kg oral administrations on the hematological parameters in rats

The biochemical analysis data obtained for the animals receiving a 2000 mg / kg dose of PG-EEtOH (Table 4), showed that there was a significant increase in urea levels and a significant decrease in creatinine levels in females. In male animals there were no significant changes in the parameters.

The doses of 25 and 100 mg/kg PgEtOH significantly inhibited edema formation induced by carrageenan during the first hour, and that the known so as indomethacin, a non-steroidal anti-inflammatory known (table 5). In the second, third and fourth hours, only 25mg/kg of PgEtOH continued to significantly reduce edema formation, while the other doses showed no significant reductions.

Table 4: Effect of oral administrations Pg-EEtOH 2000 mg / kg on biochemical parameters of rats.

Biochemical parameters	Males		Females	
	Control	Treated	Control	Treated
Glucose (mg / dl)	169.6±83.6	169.1±60.4	169.6±50.63	187.4±38.24
Urea (mg/dL)	44.00±12.34	49.43±5.192	40.86±5.640	56.14±14.85*
Creatinine (mg/dL)	1.614±2.817	0.4143±0.1574	0.3286±0.1113	0.1429±0.05345**
Cholesterol (mg/dL)	57.00±18.68	78.29±61.95	60.00±23.24	73.57±11.21
cHDL	22.14±3.024	23.00±3.830	27.14±5.178	28.29±3.498
Uric acid (mg/dL)	0.7714±0.5251	0.9000±0.5715	1.414±1.326	1.017±0.7333
Amylase (U/l)	1.571±0.5345	13.43±20.94	27.86±14.87	16.86±10.59
AST (U/l)	192.4±45.74	177.7±47.70	183.7±81.16	144.8±80.73
ALT (U/l)	61.14±9.317	63.00±28.75	53.00±12.57	47.14±12.90
ALP (U/l)	232.4±51.24	226.9±136.3	133.6±12.90	222.1±210.3
CK (U/l)	1201±324.0	1059±479.6	749.7±348.3	725.1±269.5
Triglycerides (mg/dL)	173.0±223.3	105.7±93.87	55.86±40.42	282.8±560.0
Total Proteins (mg/dL)	6.457±0.2149	6.229±0.4608	6.086±0.6176	6.543±0.3994
Albumin (mg/dL)	2.857±0.1902	2.586±0.3436	2.843±0.4198	3.000±0.2757
Globulin (mg/dL)	3.600±0.1826	3.643±0.3952	3.243±0.2637	3.971±0.9517

Values are expressed as mean +/- SD (N = 7); Student Test "t" test. * p <0.05, p <0.001 **

Table 5: Paw edema induced by carrageenan in rats, effects of oral administration of doses at 25, 50 and 100 mg / kg of PG-EEtOH.

Time in (hours)	Paw edema volume (mL)				
	Control	Pg-EEtOH 25 mg/kg	Pg-EEtOH 50 mg/kg	Pg-EEtOH 100 mg/kg	Indomethacin 25mg/kg
1	0.41±0.18	0.11±0.10**	0.33±0.13	0.17±0.09*	0.13±0.04**
2	0.51±0.20	0.16±0.11*	0.53±0.28	0.31±0.11	0.32±0.05
3	0.58±0.18	0.19±0.08***	0.51±0.11	0.41±0.14	0.17±0.09***
4	0.49±0.15	0.13±0.11**	0.50±0.18	0.33±0.12	0.13±0.11**

Values are expressed as mean +/- SD (N = 7); Student Test "t" test. * p <0.05, ** p <0.001 and *** p <0.0001

In the analyzing the results for percentage of edema inhibition (Table 6) demonstrated that Pg-EEtOH at 25 mg / kg reveals comparable activity to indomethacin assessed throughout time, it significantly reduced edema throughout the period evaluated. Indomethacin (a cyclooxygenase inhibitor) reduced the edema significantly, thus attesting to both its anti-inflammatory activity, and the reliability of the model used to evaluate this parameter.

DISCUSSION

Due to the increased use of medicinal plants, experimental evaluation of toxicity, and pharmacological activities of these plants is needed. Such studies are crucial to ensure their safety and effectiveness. In general, *in vivo* methods provide an early hint of toxicity in these compounds. In contrast, the application of *in vitro* methods provides important but more limited information regarding the toxicity of natural compounds. By applying *in vivo* assays one can observe real life signs of toxicity, such as pain, anxiety, allergic reactions, behavioral changes, and physical changes in the tested animals[16].

The acute oral toxicity of ethanol extract from the stems of *Pilosocereus gounellei* was determined in this study. There are no toxicological studies for this plant species. *In vivo* toxicity evaluations were performed qualitatively and quantitatively by conducting behavioral analyses and determining physiological, biochemical, and hematological parameters, as well as determining the $LC_{50,}$ using the *Artemia salina* model.

The *Artemia salina* model is considered a preliminary study for detecting the presence of biologically active substances. The test (in this case) was based on the capacity of Pg-EEtOH to become lethal to nauplii of *Artemia salina* thus demonstrating the presence of bioactive substances. Extracts of natural products that have an LC_{50} <1000 ug / mL are known to contain significantly toxic substances[16]. In this study Pg-EEtOH showed an LC_{50} of 547.3 mg / mL indicating the presence of such bioactive substances.

An acute toxicological study is a first step when initiating a toxicological analysis of a natural product. Acute oral toxicity tests in rats can be used to assess the therapeutic action of natural products for various pharmacological activities; this is based on the premise that Pharmacology is simply, "Toxicology at a lower dose"[17]. A normally toxic substance can have interesting pharmacological effects at a lower non-toxic dose. Results from animal toxicity tests are therefore crucial to definitively judge the safety of Pg-EEtOH for the potential development pharmacological products[18]. A dose of Pg-EEtOH 2000 mg / kg was administered orally in the treated group of rats of both sexes, and control groups received the vehicle alone (0.9% saline). Shortly after administration, behavioral analysis was conducted for 4 h, and then every 24 hours; no behavioral changes were observed in the

Table 6: Percentage inflammation inhibition after oral administrations of Pg-EEtOH in doses of 25, 50, and 100 mg / kg in rats

Time (hours)	% inflammation inhibition			
	Pg-EEtOH 25 mg/kg	Pg-EEtOH 50 mg/kg	Pg-EEtOH 100 mg/kg	Indomethacin 25mg/kg
1	73.17%	19.51%	58.53%	68.29%
2	68.62%	0%	39.21%%	37.25%
3	67.24%	12.06%	29.31%	70.69%
4	73.47%	0%	32.65%	73.46%

animals treated with Pg-EEtOH, and also no deaths occurred during the 14 days of evaluation. All rats gained weight during the experiment, and no significant differences between the treated and control groups were observed, although there was a decrease in feed and water consumption for the treated animals.

Table 1 and 2 bring an assessment of water and feed consumption, and the weights of organs in the animals, where it can be seen that there were decreases in water and feed consumption. However, there were no significant differences in the organ weights measured in the control group, and also there were no observed macroscopic changes in their organs, precluding the need for histopathological analyses of the organs. The animals showed no behavioral or physical changes, and as for the skin, fur, and eyes, all parameters remained normal, an indication that the extract did not affect the animals' growth. Thus, the analysis demonstrated that the Pg-EEtOH did not cause acute toxic effects, and has an LD_{50} of greater than 2000 mg / kg, for it did not cause the death of any of the animals after administration of the extract.

The results of the biochemical studies demonstrated no significant differences in biochemical parameters for the treated male rats as compared to the control group (Table 4). However, in the females we observed an increase in the levels of urea, that may be a result of increased protein metabolism. A decrease in creatinine levels also occurred, that may be related to decreased muscle mass, or increased renal function. The hematopoietic system is very sensitive to toxic compounds, and serves as an important index for physiological and pathological changes in both humans and animals[19]. After 14 days of treatment with Pg-EEtOH no changes in hematology parameters were noted (Table 3). Therefore, PG-EEtOH has low toxicity. This finding corroborates the safe use of this plant in traditional medicine by the population.

In assessing the anti-inflammatory activity of Pg-EEtOH we used the carrageenan induced paw edema model, which is widely used to assess this activity in many natural products. Species of the cactus family have been studied using this model, for example, *Opuntia dillenii* where the alcoholic extract of the flowers showed excellent anti-inflammatory activity, demonstrated with the use of this model[20].

Inflammation is a complex and essential process for the defense system of the organism. Excessive production of certain inflammatory mediators can lead to chronic diseases. Raw vegetable materials may have anti-inflammatory actions that affect various stages of the inflammation process, and inhibit the formation of eicosanoids, and cytokines, thus avoiding the onset of the inflammatory cascade[21].

In this study, since previous studies of this species do not exist, the initial dose to assess the anti-inflammatory activity was 25 mg / kg, based on the positive control (indomethacin 25 mg / kg), in order to compare the potency of the therapeutic extract with a commercial drug. From the initial dose, the dose was doubled to 50 mg / kg, and then again to 100 mg / kg in order to assess possible dose-dependent effects.

The results demonstrated that a dose of 25 mg / kg has anti-inflammatory activity comparable to indomethacin, for there were no significant differences between the two treatment groups, and the activity was not dose-dependent since in the other groups treated with Pg- EEtOH no significant effects were noted when compared to the control group. The dose of 100 mg / kg showed a significant effect only in the first hour. Many plant extracts and secondary metabolites inhibit the production of inflammatory mediators as well as the activity of second messengers, transcription factors, and expression of key pro-inflammatory molecules[21]. Some secondary metabolites provide relief from symptoms similar to allopathic medicines. Natural products and their derivatives are an attractive alternative for the treatment of various diseases such as acute inflammation, chronic asthma, and allergies[22].

CONCLUSIONS

The present study demonstrated that crude ethanol extract of the stems of *Pilosocereus gounellei* has low toxicity seeing that despite having observed some changes in the evaluated parameters in the treated animals, no deaths were caused in any of the treated animals. The extract displayed anti-inflammatory activity at a dose of 25mg / kg during the four hours assessed for the paw edema induced by the carrageenan model, thus confirming its popular use as anti-inflammatory. Despite the results, further studies are needed to assess the degree of toxicity with continued use of the extract in a chronic toxicity model, and also to confirm its anti-inflammatory activity, assessing the mechanism by which the *P. gounellei* extract acts.

ACKNOWLEDGMENTS

The authors would like to express their sincere thanks for the financial support provided by CAPES/CNPq of the Ministry of Education of Brazil.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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