Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 6 (4): 400-408, 2011
ISSN 1816-496X / DOI: 10.3923/jpt.2011.400.408

An Evaluation of Acute and Sub Chronic Toxicological Effects of *Hymenocardia acida* Leaf Extract in Adult Wistar Rats

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ABSTRACT

Hymenocardia acida leaf extract is used folklorically as remedy for fever, pain and respiratory diseases. It has also been shown to possess antisickling, antioxidant, antiulcer, anti-arthritic and mutagenic properties, among others. This study was undertaken to ascertain the acute and sub chronic toxicity profile of *H. acida* ethanol leaf extract administered orally in rats. Acute toxicity test was carried out using modified Lorke's method. Four groups of seven rats each were used for sub chronic toxicity evaluation. The first group served as control, while groups two, three and four received 25, 50 and 100 mg kg^{-1} extract respectively for twenty-nine days. In the acute toxicity test, the extract did not cause any signs of toxicity or produce mortality in rats. Results also showed that sub chronic administration of the extract did not significantly ($p>0.05$) affect food consumption, body weight and haematological parameters at the doses used. Water intake was observed to increase significantly ($p<0.01$) in the groups administered 25 and 50 mg kg^{-1} extract during the first and fourth weeks of administration. Relative spleen weights were significantly ($p<0.05$) lowered in groups given 50 and 100 mg kg^{-1} extract while relative brain weight reduced significantly ($p<0.05$) in the group administered 100 mg kg^{-1} extract. Serum triglyceride levels were also significantly ($p<0.01$) elevated in 50 and 100 mg kg^{-1} extract-treated groups, without significant alterations of other biochemical parameters. Microscopically, mild cortical-tubular cellular oedema in kidneys of extract-treated groups was observed. This study shows that although *H. acida* leaf extract is safe acutely, its long-term use of may be associated with mild renal toxicity.

Key words: Haematology, histopathology, renal toxicity, serum biochemistry, triglyceride, water intake

INTRODUCTION

The use of herbal medicines for disease management is on the increase globally, owing to their efficacy, availability and affordability. The Wold Health Organization estimates that about 75% of the world population rely on herbs to meet health care needs (WHO, 1991) and many drug classes used today include a prototype from a natural product (Gilani *et al*., 1992). Many African countries still rely on traditional medicines to meet different health needs (Ouedraogo *et al*., 2007). To ensure the safety of these products given their rapidly increasing use, there is a need to assess the risk associated with herbal medicines and products derived from them (WHO, 2005).

The herb, *Hymenocardia acida* (Euphorbiaceae) is widely employed in African traditional medicine. Growing as a shrub or tree in savannah regions of Africa, its leaves, stem bark and root

preparations are used as remedy for different ailments. Young leafy shoots and fruits of the plant have a characteristic acid taste and are sometimes eaten as supplementary food in Nigeria and Senegal (Burkhill, 1994). A decoction of the leaves or root is a popular medicine for fever and pain in Ghana and Nigeria, where the leaves are applied in form of a powdered snuff for the relief of pain. The root decoction is used for treating fever, pain, respiratory disorders, conjunctivitis and trachoma, biliousness, for sexual stimulation and as an antiseptic wash for wounds (Sofidiya *et al*., 2009). The plant is also used in the treatment of skin diseases and diabetes (John and Alexander, 2008). Scientific reports on the antiulcer, antisickling, antiarthritic, antimicrobial and antioxidant properties of the plant have been documented (Ukwe, 1997; Mpiana *et al*., 2009; Sackeyfio, 1988; Muanza *et al*., 1994; Ogbunugafor *et al*., 2010). The methanol extract of leaves of the plant was also shown to inhibit tracheal smooth muscle contractility *in vitro* (Sarr *et al*., 2010). Phytochemical components found present in the alcoholic leaf extracts include alkaloids, saponins, tannins, flavonoids (Ibrahim *et al*., 2007; Obidike *et al*., 2011).

A study carried out by Sowemimo *et al*. (2007) demonstrated the cytotoxic and mutagenic effects of the ethanol stem bark extract. It was moderately lethal to brine shrimp and its administration (200 mg kg^{-1} b.wt.) to rats caused 80% chromosomal damage in lymphocytes. This finding raises concern regarding the long-term use of the plant as medicine and no scientific report on the toxicological effects of the leaf extract was found in literature. Therefore, the present study investigates the acute and sub chronic toxicological effects of *Hymenocardia acida* ethanol leaf extract administered to rats, by assessing the physical, biochemical and histopathological effects it produces in the rats.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Hymenocardia acida* were collected from Suleja, Niger State in June 2009 by Ibrahim Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (NIPRD/H/6411) was prepared and deposited in NIPRD herbarium. The leaves were air-dried under shade for one week and milled to coarse powder in a mechanical grinder.

Extraction of plant material: Two hundred grams of the plant material was extracted by maceration in 2 L ethanol (70% v/v) with intermittent agitation (8 h day^{-1}) for 2 days using a mechanical shaker (GFL 37, Germany). Afterwards, the mixture was filtered using filter paper (Whatman) and the filtrate concentrated by rotary evaporation. The concentrate was dried to a constant weight on a hot water bath at 50°C to afford the Ethanol leaf Extract (ELE). The dark brown product was finely powdered in a porcelain mortar, transferred to an airtight bottle and refrigerated till use. The extract was poorly soluble in water and was reconstituted before use in an aqueous vehicle containing 0.1% w/v tragacanth powder.

Animals: Eight to ten week old Wistar rats of either sex weighing 90-110 g were used. The rats were obtained from and housed in the animal facility centre, NIPRD. Prior to the study, they were acclimatized to laboratory conditions for seven days. The rats were fed with standard rodent feed and allowed free access to clean drinking water. All animal experiments were conducted according to NIH guidelines (National Research Council, 1985) and NIPRD standard operating procedures.

Acute oral toxicity and determination of median lethal dose: Acute toxicity test and calculation of median lethal dose was done using a modification of Lorke's method (Lorke, 1983).

Sub chronic toxicity

Experimental design: Twenty-eight rats were divided into four groups of seven rats each (n = 7). The first group served as control group and received 1 mL vehicle kg^{-1} b.wt. orally. On day zero, groups two, three and four were administered 25, 50 and 100 mg kg^{-1} b.wt. ELE, respectively. Treatment was done daily for twenty-nine days during which food consumption and water intake of the groups was measured daily. Body weight of all rats in the groups was recorded on a weekly basis. The rats were also inspected daily for physical manifestations of toxicity. At the end of the 29th day, the rats were fasted overnight but allowed free access to water.

Haematology and serum biochemistry: On day 30, the rats were euthanized by chloroform inhalation and blood was withdrawn from the heart using a needle and syringe. One portion was transferred immediately into EDTA-containing tubes for measurement of haematological parameters such as Red Blood Cells (RBC), Hemoglobin (HB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelets (PLT), Lymphocytes (LYM), Monocytes (MXD) and Neutrophils (NEUT) using an automated haematology analyzer (Sysmex America Inc., USA). Another portion of blood was put in plain tubes for serum biochemical analysis. The blood was centrifuged and serum analyzed to determine cholesterol, triglycerides (TAG), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Total Protein (TP), albumin (ALB), total and direct bilirubin, aspartate transaminase (AST), Alanine Transaminase (ALT), alkaline phosphatase (ALP), urea and creatinine levels using Hitachi 902 analyser (Roche Diagnostic, GmbH, Germany). Sodium, potassium, chloride and bicarbonate ions were measured using Ilyte auto-analyser.

Relative organ weight and histopathology: The liver, heart, lungs, kidneys, stomach, spleen, gonads and brain were excised, carefully examined for gross pathological changes and weighed. Relative Organ Weight (ROW) was calculated by expressing absolute organ weight as a percentage of the total body weight. The organs were subsequently fixed in a cold solution of 10% formal saline for tissue processing and histopathological examination. Tissue slices were embedded in paraffin and 5 μm tissue sections stained with hematoxylin and eosin. Light microscopic examination of multiple tissue sections from each organ in all groups was performed in all groups.

Statistical analysis: Results were expressed as Mean±SEM. Statistical analyses was carried out by one-way ANOVA (Prism 3.0), data was further subjected to dunnett's post hoc test (Motulsky, 1999; Konate *et al.*, 2011) and differences between treated groups and control accepted as significant at $p<0.01$ and $p<0.05$.

RESULTS

No physical sign of intoxication or mortality was observed in groups that were administered 10-1000 mg kg^{-1} ELE within 24 h. In the second phase however, rats that received 2500 and 5000 mg kg^{-1} b.w portrayed initial signs of hyperactivity but appeared calm afterwards. No mortality was recorded in these groups during the 24 h observation period and one week

Table 1: Effect of *Hymenocardia acida* ethanol leaf extract on weekly food consumption in rats

		Food consumed (g)			
Treatment	Dose (mg kg^{-1})	Week 1	Week 2	Week 3	Week 4
Control	-	82.83±7.96	104.0±16.72	98.57±7.86	83.43±7.48
ELE	25	87.67±8.06	105.0±17.68	94.57±6.03	89.00±7.98
	50	91.67±8.86	97.57±12.88	107.1±9.61	97.14±5.68
	100	87.67±6.21	100.0±14.04	95.43±7.96	87.57±6.25

ELE = *H. acida* ethanol leaf extract

Table 2: Effect of *Hymenocardia acida* ethanol leaf extract on body weight of rats

		Body weight (g)				
Treatment	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 21	Day 28
Control	-	91.43±4.21	97.71±3.60 (6.42)	106.1±3.35 (13.83)	120.0±4.35 (23.80)	125.4±4.41 (27.09)
ELE	25	90.57±1.85	98.29±2.06 (7.85)	106.6±3.24 (15.04)	118.7±3.87 (23.70)	129.3±3.76 (29.95)
	50	93.86±3.69	102.9±3.36 (8.79)	109.1±2.19 (13.97)	122.9±1.72 (23.63)	135.2±2.70 (30.58)
	100	96.00±4.05	99.43±4.06 (3.45)	107.3±4.10 (10.53)	119.9±5.23 (19.93)	134.3±6.02 (28.52)

ELE = *H. acida* ethanol leaf extract. Values in parenthesis represent percent (%) increase in body weight calculated relative to day 0

Table 3: Effect of *Hymenocardia acida* ethanol leaf extract on weekly water intake in rats

		Water intake (mL)			
Treatment	Dose (mg kg^{-1})	Week 1	Week 2	Week 3	Week 4
Control	-	141.8±5.14	163.6±4.55	144.9±8.65	112.6±7.78
ELE	25	190.5±12.25**	188.7±12.67	180.3±13.77	147.1±9.47*
	50	207.2±10.99**	175.9±10.85	166.9±10.31	148.4±4.57*
	100	155.7±8.12	162.3±7.34	178.6±14.46	135.9±10.4

*Significantly different from control at $p<0.05$. **Significantly different from control at $p<0.01$, ELE = *H. acida* ethanol leaf extract

afterwards. No mortality was witnessed during sub chronic ELE administration. However, behavioral and physical changes observed were aggressive behaviour and prominent penile erection in male rats that received 50 and 100 mg kg^{-1} ELE.

Table 1 shows that the effects of ELE on food consumption in treated groups was insignificant ($p>0.05$) compared to the control group. Food consumption was observed to decrease in the third week of treatment in all the groups except 50 mg ELE/kg group that showed increased food consumption from 97.57±12.88 g to 107.1±9.61 g during the 29-day treatment period.

From the results displayed in Table 2, there were no significant ($p>0.05$) changes in food consumption and body weight in ELE-treated rats relative to the control group. The rats in all the groups gained weight in a similar manner during the study, although the % weight gains in the 100 mg kg^{-1} b.wt. group was lower in the 1st, 2nd and 3rd weeks of treatments compared to other treated groups.

Table 3 depicts a significant increase ($p<0.01$) in water intake in groups treated with 25 and 50 mg ELE kg^{-1} body weight in the first week of treatment. Thereafter, no significant difference was observed until the fourth week when water intake increased significantly ($p<0.05$) to 147.1±9.47 and 148.4±4.57 mL in rats administered 25 and 50 mg kg^{-1} ELE respectively, against 112.6±7.78 mL in the control group.

Table 4: Serum biochemical parameters of rats treated with *Hymenocardia acida*

Parameters	Control	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}
Cholesterol (mmol L^{-1})	80.14±7.70	86.86±4.01	95.17±4.34	102.50±6.29
TAG (mmol L^{-1})	114.00±15.38	154.10±17.55	203.20±23.47**	208.00±5.69**
HDL (mmol L^{-1})	42.00±4.37	38.00±4.14	34.33±3.57	50.00±5.69
LDL (mmol L^{-1})	15.43±3.64	18.14±4.18	20.17±4.78	28.75±9.72
TP (g L^{-1})	68.86±2.09	70.71±1.21	70.17±1.30	72.25±1.49
ALB (g L^{-1})	33.43±1.63	36.29±0.61	37.17±1.30	36.50±1.19
Direct Bilirubin (µmol L^{-1})	2.01±0.35	2.17±0.32	1.90±0.28	1.78±0.22
Total Bilirubin (µmol L^{-1})	6.06±1.03	6.54±0.98	5.73±0.85	5.30±0.67
AST (µmol L^{-1})	381.30±49.03	540.00±163.6	360.30±101.8	394.50±70.01
ALT (µmol L^{-1})	64.71±6.48	141.40±59.38	204.80±103.1	94.25±7.32
ALP (µmol L^{-1})	166.90±22.52	174.10±16.85	171.00±27.81	194.30±17.01
Sodium (mmol L^{-1})	133.30±1.70	131.10±0.51	128.00±4.87	130.50±2.90
Potassium (mmol L^{-1})	9.51±1.53	10.49±0.34	11.77±1.21	11.70±0.68
Chloride (mmol L^{-1})	95.86±0.94	95.14±0.26	93.33±3.02	95.75±1.38
Bicarbonate (mmol L^{-1})	25.29±0.36	24.29±0.78	23.83±0.48	25.00±0.91
Urea (mmol L^{-1})	11.13±0.80	10.51±0.83	10.33±0.83	11.68±1.22
Creatinine (µmol L^{-1})	61.71±2.09	60.57±4.02	61.17±2.60	67.50±2.22

**Significantly different from control at $p<0.01$

Table 5: Effect of *Hymenocardia acida* ethanol leaf extract on organ: body weight (%)

Organs	Control	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}
Brain	1.08±0.05	1.00±0.04	0.97±0.05	0.89±0.03*
Lungs	0.80±0.06	0.68±0.03	0.71±0.03	0.81±0.04
Heart	0.36±0.01	0.38±0.01	0.35±0.01	0.33±0.02
Kidneys	0.62±0.01	0.62±0.02	0.60±0.02	0.56±0.02
Gonads	1.71±0.07	1.57±0.04	1.76±0.11	1.68±0.08
Spleen	0.52±0.03	0.42±0.02*	0.41±0.02*	0.43±0.01*
Liver	3.40±0.11	3.45±0.08	3.30±0.09	3.06±0.10
Stomach	1.18±0.09	1.10±0.18	1.17±0.20	1.01±0.10

*Significantly different from control at $p<0.05$

The extract did not cause any significant change in the hematologic profile, the changes that were recorded was statistically insignificant ($p>0.05$) and within ranges comparable to that of the control group.

From the results shown in Table 4, the administration of ELE caused a significant elevation of serum triglycerides ($p<0.01$) In 50 and 100 mg kg^{-1} treated groups (203.2±23.47 and 208.0±15.69 mmol L^{-1}, respectively) while other serum lipids, enzymes and electrolytes did not differ significantly ($p>0.05$) from the control group.

No gross pathological changes were observed in the liver, heart, lungs, kidneys, stomach, spleen, gonads and brain of ELE-treated groups. Table 5 shows significant ($p<0.05$) reduction of ROW of spleen from a mean value of 0.52 g in the control group to 0.42, 0.41 and 0.43 g in 25, 50 and 100 mg ELE kg^{-1} treated groups respectively, while significant ($p<0.05$) reduction in ROW of brain was recorded only in the group that received 100 mg kg^{-1} ELE (0.89±0.03 g).

Microscopically, no significant pathological change was observed in brain, heart, spleen, gonads and liver tissues. Normal hepatic architecture was also preserved as no evidence of edema, necrosis,

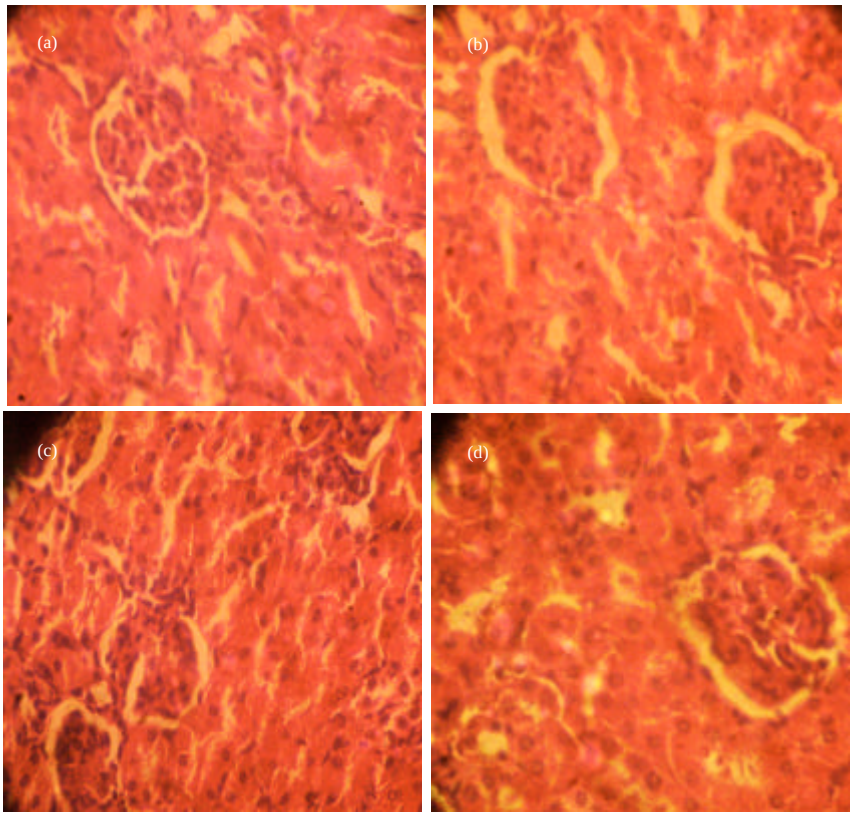

Fig. 1: Photomicrographs of kidney (×400) from rat treated with the aqueous vehicle, 25, 50 and 100 mg kg^{-1} ethanol leaf extract of *Hymenocardia acida*. Fig. 1a shows normal tubular architecture, Fig. 1b shows cortical oedema while Fig. 1c and 1d show oedema and considerable tubular distortion

fatty change or significant vascular response was observed. The most consistent finding was tubular oedema in kidneys of ELE-treated groups. Compared to the control group shown in Fig. 1a, the administration of 25 mg kg^{-1} ELE caused mild tubular oedema, with preservation of normal cellular architecture (Fig. 1b). Considerable distortion of tubular architecture was evident in 50 and 100 mg kg^{-1} treated groups (Fig. 1c,d).

DISCUSSION

From the results of the acute toxicity test, the median lethal dose of the extract was estimated to be greater than 5000 mg kg^{-1} b.wt. The physical and behavioral changes observed in groups that received ELE sub-chronically may be attributed to sexual stimulatory action of the plant, which is traditionally used as aphrodisiac medicine (Burkhill, 1994). The extract did not suppress food consumption and body weight relative to the control group, showing that it did not affect appetite. Body weight is an indicator of adverse drug effect and has been used to assess response to drug therapy (Winder *et al*., 1969), hence it may be deduced that treatment with the extract did not adversely affect treated groups.

The increased water intake in ELE-treated rats may have been caused by increased thirst and dehydration in these groups. This may be caused by increased water excretion by the kidneys or through the skin, resulting in the observed increased in water intake as a compensatory

mechanism. Endogenously, hypothalamic stimulation of thirst and polydypsia may occur centrally in the paraventricular nucleus of the hypothalamus when there is a lack of antidiuretic hormone, or in response to peripheral nephrogenic stimulus (Nosiri *et al.*, 2010). Therefore, the observed effect of the extract on water intake may have been brought about by brought about by disruption in ADH secretion in the hypothalamus, or by a direct effect on the kidneys.

The haemoglobin, erythrocyte and haematocrit profile of ELE-treated groups which was statistically comparable with the control group, eliminates the occurrence of anaemia or adverse effects on normal erythrocyte and hemoglobin functions. White blood cells, platelets, lymphocytes and neutrophils are mediators of immunity and contribute to immunoprotection against inflammation (Tripathy *et al.*, 2010). The levels of these cells were within normal levels in ELE-treated groups, showing that administration of the extract did not elicit inflammatory changes in these groups. These observations are also supported by an earlier report on the antiinflammatory and antiarthritic properties of *Hymenocardia acida* in rodents (Sackeyfio, 1988).

Serum levels of transaminases (ALT, AST) and phosphatase (AP) which reflect the state of hepatic function (Konan *et al.*, 2007) were not significantly altered in ELE-treated groups; this is an indication that the extract did not affect normal hepatic function. This is further strengthened by the observation that levels of protein, albumin, direct and total albumin in these groups were not adversely affected, implying normal synthetic and excretory functions of the liver in these groups. This can be associated with the antioxidant properties of the extract and is supported by a study conducted by Ogbunugafor *et al.* (2010) who reported antioxidative effects of the plant in Wistar rats.

The observed elevation in serum triglycerides may have occurred due to increased production and secretion of triglycerides by the liver, or due to nephrotic disease. However, apart from the significant increase in TAG, serum lipid profile was not significantly altered by treatment with ELE and this suggests that the extract does not impair lipid metabolism. The observed elevation of triglycerides in groups that received the extract (50 and 100 mg kg^{-1}) may also be a mild sign of renal toxicity produced by the extract but this may not present a complete reflection of renal damage as serum creatinine and urea levels which are indicators of renal function were preserved within normal limits preserved in the treated groups.

The decreased ROWs of spleen and brain in extract treated groups may be an indication that treatment with the extract may have adversely affected the spleen and brain. A decrease in organ weight has been linked to disintegration of cytoplasmic material and necrotic changes and is usually accompanied by a fall in protein (Malathi and Gomaz, 2008). However, no macroscopic or microscopic alterations were observed in these organs and protein levels in treated groups were not significantly decreased. Therefore, the observed decreases in ROWs of spleen and brain in ELE-treated groups may not be likened to a significant adverse effect.

The pathology noticed in the kidneys may not be unexpected. This finding is important as an abnormality in histoarchitecture is the most reliable outcome of toxic manifestations (Singh *et al.*, 2009). The kidney, being a major excretory organ is exposed to toxic principles that may be present in the extract that can incite injury (Abdulrahman *et al.*, 2007). Indeed, some herbal products have been noted for producing degenerative changes to renal architecture (Padmanabhan *et al.*, 2003; Khorshid, 2008). Phytochemical compounds such as tannins and saponins reportedly present in the extract (Ibrahim *et al.*, 2007; Obidike *et al.*, 2011) may account for its toxic effect on kidney tubules. These compounds produce renal tubular toxicity as chronic intake of diets rich in these compounds have been shown to exert necrotic changes in renal tubules

(Jovanoic *et al.*, 1991). This may not be unrelated to the increase in water intake and serum triglycerides in extract treated groups observed during from the study. Elevations in triglyceride levels may also contribute to oxidative stress in the kidneys and promote renal insufficiency (Bhalodia *et al.*, 2010). High water intake has been reported to attenuate tubular-interstitial injury in sub totally nephrectomised rats (Sugiura *et al.*, 1999) and the observed changes in water intake and serum triglycerides could be an indication of mild renal impairment caused by the extract.

CONCLUSION

The findings of this study indicate that the ethanol extract of *H. acida* leaves is acutely safe when administered orally. However, its effects in long term use, especially on renal function, needs to be further investigated.

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