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Acute Toxicity Studies of Aqueous and Ethanol Extracts of *Oldenlandia herbacea* Roxb. in Albino Rats

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The present work is to evaluate the acute toxicity of aqueous and ethanol extracts of *Oldenlantia herbacea* Roxb. in albino rats as per the OECD guidelines. Acute toxicity study of aqueous and ethanol extracts was carried out by administration of 1,3 and 5g/kg bw of *Oldenlantia herbacea* Roxb. to rats in the respective groups at a single dose. While rats in the control group received 0.5 ml of normal saline. At the end of the experiment, blood samples were collected for hematology and clinical evaluations. Body weight and food consumptions were also noted. The main organs of the body like liver, kidney and lungs were collected for measurement of their weight. In the acute toxicity study of both aqueous and ethanol extracts showed no toxicological signs observed on body weight, organ weight and food consumption of rats. There were no significant changes observed in the liver markers, kidney markers and haematological parameters. From the above findings concluded that aqueous and ethanolic extracts of *Oldenlantia herbacea* Roxb. at a dose of 1,3 and 5g/kg body weight does not produce any adverse effects and it may be considered as promising therapeutic applications in drug discovery.

Key words: Acute toxicity, Oldenlantia herbacea Roxb., OECD, Haematological parameters, Body weight

Toxicity is described as a component of pharmacology which offers with the harmful effects of bioactive substances on living organisms. Toxicological research and experiments are very crucial to establish the safety and efficiency of any new drug and to choose whether or not this drug ought to be followed for medical use or not (Anisuzzaman et al., 2001; Alam *et al.*, 2006). Toxicological studies can be classified into three categories based on the length of time that animals are exposed to the drug: acute, sub-acute, and chronic investigations (Baki et al., 2007). Acute and chronic toxic effects differ primarily in terms of the amount of chemical substance involved and the amount of time that passes before the effect manifests (Timbrell, 2002). Acute effects are usually noticed quickly after exposure and are caused by the ingestion of significant doses of the substance. Acute effects occur shortly after exposure and are caused by the ingestion of significant amounts of toxin in a single dose. Chronic effects, on the other hand, are frequently found over a long period of time, during which exposure may be continuous or intermittent, but at levels that are clearly too low to generate an acute effect (Loomis and Hayes, 1996; Pascoe, 1983). The main goal of a toxicity investigation is to look into and document any negative effects that a test chemical may have when used or consumed by humans. Extrinsic, intrinsic, or other extra causal factors could contribute to the progression of toxicity from herbal medicine (Muhammed Ashraf *et al.*, 2021). Herb-herb and herb-drug interactions are critical in the activation of adverse effects.

The major goal of toxicological testing is to look for negative effects of plant-derived pharmaceuticals and to define the dosage limits of exposure levels at which such effects can occur. The nature and significance of the antagonistic action are two significant aspects considered in the safety assessment of all plant-derived medications. Toxicity testing can show some of the dangers that may be connected with the use of medicinal herbs, particularly in sensitive populations. Similarly, detecting the harmful effect of plant extracts or compounds obtained from them in the pre-clinical and clinical phases of drug discovery and development from various plant sources is an equally important goal for toxicity studies. These phases can aid in the discovery of numerous toxicants that can be disposed of or adjusted during the process, as well as provide an opportunity for a thorough evaluation of safe, promising substitutes for the toxic substances (Gamaniel, 2000). Modifications such as structural or chemical group changes, as well as dosage decrease, may help to increase tolerability.

Herbs are considered alternative medications for the treatment of a variety of disorders because of their perceived acceptance, efficacy, affordability, safety, and low cost. There is also a growing trend in the public's use of herbal formulations, owing to the widespread perception that these products are natural and thus safe to employ in the treatment of diseases. However, due to the way herbal medicines are made, they may contain pollutants including heavy metals, aflatoxins, and dangerous bacteria. There's also the assumption that because herbal treatments are sourced from nature, they don't have the same negative or harmful side effects as manufactured pharmaceuticals utilized in traditional medicine. Toxicity should be investigated for proper and documented herbal medical items, just as it is for conventional orthodox drugs that have been thoroughly researched and created; the toxicity of traditional herbal treatments is rarely assessed. As a result, users frequently focus on the medical benefits of herbal medications while overlooking their harmful effects on other organs.

Oldenlantia herbacea Roxb. a Rubiaceae family annual plant, is an upright, glaborus annual herb that grows in temperate and tropical climates. This plant's aerial component is used to treat wounds (Kirtikar, Basu, 1989; Shobana *et al.*, 2019). Ethnomedically, this plant is known as one of the ingredients in the treatment of jaundice. The herb's decoct can be used to treat rheumatoid arthritis and swellings. The herb is cooked with oil, and the resulting oil is used to treat elephantiasis and other bodily aches and pains. Asthma sufferers have used the leaves as an expectorant. This plant has been shown to contain ursolic acid, kaempferol-3-o-arabino pyranoside, kaemferol-3-o-rutinoside, 23-ethyl-cholest-23-en-3-ol, and 9, 9-dimethyl

hexacosane.

The goal of this study is to see how the aqueous and ethanol extracts of *Oldenlandia herbacea* L. affect some kidney and liver functions.

MATERIALS AND METHODS

Collection and authentication of plant material

Oldenlantia herbacea Roxb. aerial parts were obtained in and around Trichy. The specimen (Voucher No – S002) placed at Rapinat Herbarium, St. Joseph's College, Trichy was used to identify and authenticate the material.

Preparation of Aqueous Extract

200g of dry powder from a *Oldenlandia herbacea* L. plant component was combined with 1200 ml of water and boiled. The heating was cut by a third, and the water was filtered. The filtrate was dried by evaporation. The extracted paste was kept at 4°C in an airtight container.

Preparation of Ethanol Extract

Weighing 250g of dry powder from specific plant *Oldenlandia herbacea* L. portion and transferring it to a stoppered flask. A sufficient amount of ethanol was poured to completely submerge the powder. The flask was shaken every hour for the first 6 hours, then set aside for 24 hours before being shaken again. After three days, the process was repeated, and the content was filtered. The filtrate was collected, and the solvent was removed using distillation. The extracted paste was kept at 4 degrees Celsius in an airtight container.

Acute toxicity study

The acute toxicity of aqueous and ethanol extracts of the aerial part of Oldenlandia herbacea L. was investigated by giving a single dose of various concentrations of the plant species' aqueous and ethanol extracts to overnight fasted Wistar albino rats. Individual animals were examined for a total of 14 days following dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention provided during the first 4 hours, and daily thereafter.

Experimental Animals

Biogen in Bangalore provided healthy adult wistar albino rats that were two to three months old and weighed 100g-120g. Prior to the trial, the animals were given 5 days to acclimate under laboratory conditions. Standard polypropylene cages were used to keep the animals in. The rats were fed rat chow pellets and water ad libitum from Sai Durga Foods and Feeds in Bangalore, India. After receiving appropriate approval from the committee **(Approval No: 790/03/ac/CPCSEA),** all investigations were carried out in accordance with CPCSEA's ethical principles.

Experimental Design

Group I	A single dosage of normal saline was given to the animals orally.
Group II	Animals were given a single dose of aqueous extract of *Oldenlandia herbacea* L. (1g/kg bw) orally
Group III	Animals were given a single dose of aqueous extract of *Oldenlandia herbacea* L. (3g/kg bw) orally
Group IV	Animals were given a single dose of aqueous extract of *Oldenlandia herbacea* L. (5g/kg bw) orally
Group V	Animals were given a single dose of ethanolic extract of *Oldenlandia herbacea* L. (1g/kg bw) orally
Group VI	Animals were given a single dose of ethanolic extract of *Oldenlandia herbacea* L. (3g/kg bw) orally
Group VII	Animals were given a single dose of ethanolic extract of *Oldenlandia herbacea* L. (5g/kg bw) orally

After 14 days, all the animals were subjected to gross necrops. Blood was collected for analyzing liver and kidney markers, hematological parameters.

Parameters studied for toxicity studies:

Physical Parameters	
i Body weight	
ii Organ weight	
iii Food Consumption	
Haematological Parameters	
iv. Determination of hemoglobin	(Armour *et al.*, 1965)
v. Determination of Red Blood Cells Count	(Armour *et al.*, 1965)
vi. Determination of White Blood Cells count	(Armour *et al.*, 1965)
Kidney Function Test	
vii. Estimation of creatinine	(Bonsnes and Taussky, 1945)
viii. Estimation of Uric Acid	(Caraway and Seligson 1963)
ix. Estimation of Urea	(Natelson *et al.*, 1951)

Liver Function Test		
	xi. Estimation of alanine transaminase	(King, 1965)

xii. Assay of aspartate transaminase	(King, 1965)

xi. Estimation of serum alkaline phosphatase	(King, 1965)

xii.	Estimation of protein	(Lowry *et al.*, 1951)

RESULT AND DISCUSSION

Effect of aqueous and ethanolic extracts on body weight

Table 1 shows the influence of aqueous and ethanolic extracts of Oldenlandia herbacea L on experimental animals' mean body weight. When compared to normal rats, experimental rats given aqueous and ethanolic extracts of the chosen plant had a slight rise in body weight. Evidence has demonstrated that several herbal bioactive compounds have detrimental effects that are exacerbated by secondary metabolites, necessitating toxicity testing of herbal treatments. Changes in animal body weight have been used as an indicator of medication and chemical adverse effects, according to Mukinda and Syce (2007), and it would be significant if the body weight reduction was more than 10% of the starting body weight. It is well understood that gaining or losing weight can result in major physiological changes, such as hormone shifts and decreased protein, amino acid, and other nutrient absorption (Belakredar *et al.*, 2020). The body weight of the normal and treated groups was gradually increased in the current investigation, and there was no significant difference in mean body weight between the different treated groups and the normal group over the 14-day experimental period. This demonstrated that both extracts of the selected plant had little toxicity on animal growth and did not interfere with the animals' natural metabolism.

Table 1: Effect of Aqueous and Ethanolic Plant Extracts on Body Weight

	Initial Body weight (g)	Final Body weight (g)	% of body weight	% of body weight
I (Control)	125±7.64	140±5.55	12.00±1.00	+
Aqueous Extract				
II	130±5.77	134±7.50	3.07±0.08	+
III	142±6.32	156±8.70	9.85±0.12	+
IV	146±5.17	155±7.25	6.61±0.28	+
Ethanol Extract				
V	129±3.29	124±6.30	4.03±0.05	-
VI	137±4.12	130±3.57	5.28±0.08	-
VII	135±5.02	129±5.47	4.65±0.10	-

Values are expressed as mean ± SEM n=6; P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

\+ - Represents the increased body weight - - Represents the decreased body weight

Table 2. Effect of Aqueous and Ethanol Plant Extracts on Food Consumption

Mean Food consumption (g)/6 rats per day/week	
Groups	**Aqueous Extract**
I	55.10±1.64
II	61.10±1.98
III	56.25±1.75
IV	55.05±1.52
Ethanol Extract	
V	58.00±1.88
VI	53.12±1.71
VII	49.10±1.48

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Table 3. Effect of Aqueous and Ethanol Plant Extract on Organ Weight

GROUPS	**Aqueous Extract**

	Liver (g)	Kidney (g)	Lungs (g)
I	4.68±0.04	1.42±0.03	1.28±0.03
II	4.62±0.02	1.50±0.01	1.22±0.02
III	4.55±0.04	1.39±0.05	1.25±0.02
IV	4.64±0.05	1.43±0.04	1.23±0.02
Ethanol Extract			
V	3.68±0.05	1.27±0.01	1.07±0.03
VI	3.84±0.02	1.28±0.01	1.07±0.02
VII	3.77±0.04	1.17±0.04	1.10±0.02

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII**) compared with normal group **(Group I)**

Table 4. Effect of aqueous and ethanol plant extracts on liver markers

GROUPS	Aqueous Extract		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I	40.61±0.98	40.83±0.46	92.56±0.43
II	30.13±0.81	31.83±0.56	82.43±0.47
III	32.56±0.46	33.15±0.52	84.30±0.60
IV	35.79±0.63	36.8±0.37	86.33±0.25
Ethanol Extract			
V	32.53±0.57	31.23±0.23	82.35±0.40
VI	35.56±0.70	32.15±0.46	84.45±0.45
VII	38.79±0.35	36.82±0.42	87.15±0.59

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Table 5. Effect of aqueous and ethanol plant extracts on kidney markers

GROUPS	Aqueous Extract				
	Urea(mg/dl)	Uric acid(mg/dl)	Creatinine(mg/dl)	Total Bilirubin (mg/dl)	Direct bilirubin (mg/dl)
I	19.12±0.12	5.752±0.25	0.89±0.003	0.50±0.019	0.36±0.007
II	17.85±0.13	5.15±0.52	0.73±0.001	0.39±0.013	0.29±0.008
III	18.5±0.77	6.18±0.50	0.76±0.004	0.43±0.009	0.32±0.008
IV	19.35±0.57	6.75±0.90	0.82±0.001	0.52±0.025	0.42±0.006
Ethanol Extract					
V	20.66±0.52	5.31±0.49	0.50±0.003	0.38±0.01	0.30±0.02
VI	20.95±0.14	6.68±0.51	0.56±0.002	0.32±0.02	0.24±0.01
VII	23.54±0.17	7.54±0.49	0.59±0.001	0.36±0.02	0.26±0.02

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Table 6. Effect of aqueous and ethanol plant extract on hematological parameters

GROUPS	Aqueous Extract

	HB (%)	WBC (10^3 Cells/mm^3)	RBC (10^6 Cells/mm^3)	PLATELETS (10^3 cells/mm^3)
I	13.47±0.16	6.25±0.08	7.25±0.01	255±3.1
II	13.13±0.05	6.65±0.06	6.98±0.02	235±5.10
III	14.47±0.09	7.20±0.08	8.1±0.01	223±2.6
IV	15.03±0.05	7.05±0.08	7.56±0.02	260±1.16
Ethanol Extract				
V	9.46±0.08	7.84±0.11	6.20±0.02	227±4.69
VI	8.92±0.02	7.94±0.06	5.71±0.01	197±4.25
VII	10.26±0.06	8.49±0.07	6.83±0.02	185±1.75

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Table 7. Effect of aqueous and ethanol plant extracts on Differential Leucocytes

GROUPS	Aqueous Extract				
	Neutrophils (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)	Lymphocytes (%)
I	14.56±0.09	3.65±0.08	10.45±0.08	0.30±0.10	69.5±0.76
II	16.37±0.13	3.28±0.10	12.35±0.08	0.33±0.09	67.55±0.80
III	19.15±0.19	3.93±0.08	10.25±0.11	0.35±0.13	70.45±0.80
IV	18.26±0.11	3.57±0.09	11.48±0.09	0.42±0.14	72.65±0.80
Ethanol Extract					
V	15.07±0.11	2.42±0.07	10.45±0.08	0.39±0.12	46.21±0.52
VI	14.19±0.12	2.12±0.08	11.18±0.07	0.45±0.08	45.38±0.62
VII	16.97±0.08	2.77±0.06	9.08±0.10	0.49±0.12	48.28±0.62

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Table 8. Effect of aqueous and ethanol plant extracts on total protein

Total Protein (g/dl)	
GROUPS	**Aqueous Extract**
I	5.68±0.61
II	5.63±0.48
III	5.75±0.46
IV	5.88±0.65
Ethanol Extract	
V	5.21±0.50
VI	4.51±0.47
VII	4.63±0.45

Values are expressed as mean ± SEM n=6

P< 0.05 statistically significant when plant treated groups **(Group II to Group VII)** compared with normal group **(Group I)**.

Effect of Aqueous and ethanolic plant Extracts on Food Consumption

Administration of aqueous and ethanol extracts of *Oldenlantia herbacea* L. (Leaves) on food consumption

showed slight alteration in the food consumption in both normal control and treated rats. The data were represented in **Table 2.**

Determination of food consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional conditions (Steven and Mylecrdfaine, 1994; Iversen and Nicolaysen. 2003**).** The slight variation in food consumption on each day was noted in both control and treated rats. However, these differences were not statistically significant. Thus, the present study reported that food consumption was not affected by administration of the both the extracts and it did not induce appetite suppression and had no deleterious effect. It indicates that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen *et al.*, 2001**).** It also reveals that the extracts did not interfere with the nutritional benefits of ad libitum food and water, such as weight increase and appetite stability, which are predicted in animals who are constantly fed and watered. Furthermore, in terms of folkloric practice in the administration of therapeutic plants, these extracts are safe when taken orally. As a result, up to a greater dose, the studied extracts of chosen plants can be regarded non-toxic.

Effect of aqueous and ethanolic plant extracts on organ weight

Table 3 shows the organ weights of the normal, aqueous, and ethanol extracts of selected plants. It reveals that the weight of important organs such as the kidney, liver, and lungs in plant-treated animals does not differ significantly from that of normal animals. Organ weight is a measure of an animal's physiological and pathological health. The relative organ weight is critical for determining whether or not the organ weight was affected by the injury. The lungs, liver, and kidney are the principal organs impacted by toxicant-induced metabolic reactions (Dybing *et al.*, 2002). The administration of various doses of aqueous and ethanol extracts of all selected plants did not result in substantial weight changes in the important organs (liver, kidney, and spleen) in the current investigation, which indicates the non-toxic nature of plant species selected under study.

Effect of aqueous and ethanolic plant extracts on liver markers

The effect of aqueous and ethanol extracts of the plants on the levels of liver marker enzymes was shown in Table 4. When the aqueous and ethanolic extract treated groups were compared to the normal group, there was no significant difference in liver enzymes (ALT, AST, and ALP). When assessing the hazardous effect of plant extracts, it's also crucial to look at biochemical indicators. Biochemical indicators, in fact, may provide significant information on specific organs such as the kidney and liver, which are essential for an organism's survival (Nfozon *et al.*, 2019). The enzymes ALT, AST, and ALP are well-known indicators of liver function and biomarkers for identifying potential toxicity. In the present study, there are no variations in the levels of ALT, AST and ALP in plant treated groups indicated that the aqueous and ethanol extracts of selected plants did not cause the inflammatory cellular leakage and damage of cell membrane to cells in the liver.

Effect of aqueous and ethanolic plant extracts on kidney markers

The effect of aqueous and ethanolic extracts of chosen plants on kidney indicators is shown in Table 5. When the levels of kidney indicators including urea, uric acid, and bilirubin in the aqueous and ethanolic extract of selected plant treated groups were compared to normal control, the results showed no significant differences. The kidney is known to be hugely susceptible to damage caused by various toxic elements as large volume of blood flows through it and the toxins filtered usually gets concentrated in the kidney tubules (Ghosh *et al.*, 2019**).**One of the major roles of kidneys is to filter out metabolites, such as creatinine, urea and electrolytes from the plasma through the glomeruli. Since creatinine and urea are normally filtered from the plasma and only re- absorbed or secreted by the proximal tubule to a minor extent, both have been used as indices of renal clearance (Alawode *et al.*, 2020**).** Urea, uric acid and bilirubin are known to be important markers of renal dysfunction. In the present study, there

was no significant difference was observed in the levels of kidney markers between the treated and control groups probably indicated that both the aqueous and ethanol extracts did not interfere with the renal capacity to excrete metabolites.

Effect of Aqueous and Ethanolic Plant Extracts on Hematological Parameters

The examination of blood parameters is particularly beneficial for determining the anomalies caused by a plant extract. It also aids in the provision of information on a medicinal agent's toxicity mechanism and safety (Yamthe *et al.*, 2012; Tousson et al., 2011). Hematopoiesis is a life-sustaining process that can be harmed by both conventional and natural medicines (Essiet *et al.*, 2019). Table 6 shows the results of haematological parameters for aqueous and ethanolic extracts of selected plant treated and control groups. When compared to the normal control, the plant treated groups showed modest to moderate alterations in haematological markers like total RBC, WBC, and platelet counts, as well as haemoglobin.

In animals and mammals, the hematopoietic system is one of the most sensitive targets for hazardous substances and an important indicator of physiological and pathological condition. The amount of the harmful effect of plant extract on an animal's blood can be determined using haematological measures. It can also be used to illustrate how a plant extract or its components affect blood flow (Yakubu and Musa, 2012). Furthermore, when data from animal research are translated into human toxicity, changes in the hematological system have a higher prognostic value for human toxicity. The non-significant effect of both extracts on total red blood cells, haemoglobin, total WBC count, and platelets indicates that the aqueous and ethanol extracts of ***Oldenlandia herbacea* L.** does not affect the erythropoiesis, morphology, or osmotic fragility of the red blood cells, leukopoiesis and also had no effects on circulating blood cells or on their production.

Effect of aqueous and ethanolic plant extracts on Differential Leucocytes

The percentage of the differential leucocytes of aqueous and ethanolic extracts of selected plants were represented in the **Table 7.** The results of the present study suggested that differential counts were not significantly different in the control and plant treated groups. Differential counts of leucocytes are used to determine the type of infection. Allergies, anaphylactic shock, and parasitism are all indicators of leukopenia, while a high number of white blood cells implies the presence of a recent infection (Konmy *et al.*, 2020). The lack of substantial alterations in the plant-treated groups shows that the aqueous and ethanolic extracts of the selected plant materials are not allergenic, and that they do not affect the animals' immunological condition.

Effect of aqueous and ethanol plant extracts on total protein

Table 8 shows the total protein levels of experimental animals treated with aqueous and ethanolic extracts of several plant species, as well as a control group. In this study, there were no significant differences in total protein levels between the normal and plant-treated groups. The purpose of determining serum protein levels is to look into the liver's secretory and synthetic functional capacity (Balaji and Ganesan, 2020). Albumin and total protein concentrations in the blood may be reduced as a result of impaired hepatic function. The lack of significant differences in serum total protein concentrations between the treated and control groups shows that the liver's synthetic functions are unaffected by any of the test doses of the aqueous and ethanol extracts of selected plant sources.

CONCLUSION

The results of the present study can be concluded that the aqueous and ethanolic extracts of *Oldenlandia herbacea* L. can be considered as safe and they did not produce any toxicant effects in all the doses (1,3 and 5mg/kg bw).And also those extracts did not create antagonistic effect on the liver and kidney functions as supported by the results of both haematological and clinical parameters. Further in-depth studies should be carried out to evaluate the sub acute and chronic toxicity by using this plant source to explore the safety of the plant.

ETHICAL STATEMENT

The study protocol was approved by the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA)

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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