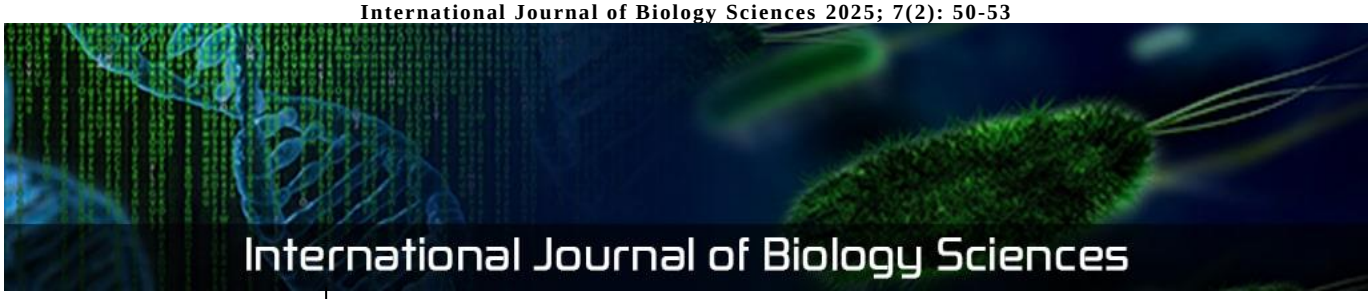

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Acute toxicity study of oral polyherbal formulation having immunomodulatory property in Wistar albino rats

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Abstract
This study investigates the acute oral toxicity of a polyherbal formulation with immunomodulatory properties in female Wistar Albino rats. The formulation, comprising *Asparagus racemosus*, *Withania somnifera*, *Andrographis paniculata*, *Ocimum sanctum*, *and Piper nigrum*, was prepared using a decoction method. The polyherbal formulation was prepared by the decoction method of extraction with the excipients to extend the shelf life. The acute toxicity study, conducted following OECD guidelines, involved administering single doses (0 mg/kg, 300 mg/kg, and 2000 mg/kg) and observing the rats for 14 days. The findings showed the polyherbal formulation, at a dose of 2000 mg/kg, exhibited no signs of toxicity or mortality. Body weight, behavioral patterns, and hematological parameters remained unaffected. Gross pathological examination revealed no internal organ lesions. The LD_{50} was determined to be over 2000 mg/kg, classifying it as GHS category 5. The polyherbal formulation, consisting of *Asparagus racemosus*, *Withania somnifera*, *Andrographis paniculata*, *Ocimum sanctum* and *Piper nigrum*, was found to be safe at the maximum tested dose of 2000 mg/kg in acute oral toxicity study, demonstrating its potential for further exploration as an immunomodulatory agent.

Keywords: Acute oral toxicity, polyherbal formulation, immunomodulatory properties, Wistar albino rats, OECD guidelines, LD_{50}

Introduction
Immunomodulators are agents which can stimulate, suppress or modulate any aspect of the immune system including both adaptive and innate immune systems (Kumar *et al*., 2012) [7]. There is much-growing interest in the use of medicinal plants as modulators of the complex immune system. Numerous therapeutic consequences of plant extracts have been recommended to be because of their extensive assortment of immunomodulatory effects and persuade on the immune system of the human body (Porwal *et al*., 2021) [12]. In traditional medicine whole plants or mixtures of plants are used rather than isolated compounds. Due to synergism, polyherbalism confers some benefits which are not accessible in single herbal formulations. Polyherbal formulations express high effectiveness in numerous diseases with safe high dose. The plants selected for the polyherbal formulation (PHF) includes *Asparagus racemosus* (Bopana and Saxena, 2007) [2], *Withania somnifera* (Paul *et al.*, 2021) [11], *Andrographis paniculata* (Nety *et al*., 2018) [9], *Ocimum sanctum* (Harichandan *et al*., 2019) [4], *Piper nigrum* (Shingate *et al.*, 2013) [15] showed the immunomodulator action by enhancing cell mediated and humoral responses. *P. nigrum* was used to enhance the bioavailability of PHF.
This study is done 2016to evaluate the safety of the polyherbal formulation by doing acute oral toxicity study as per OECD (2002) Test No. 423 guidelines[10]. From the acute toxicity study, LD_{50} of the PHF can be determined.

Materials and Methods
Collection and identification of Plants
The selected plants were sourced from the Ethnoveterinary Herbal Products Research and

Development Centre at the Veterinary College and Research Institute in Orathanadu. To ensure the botanical identity and quality of the plant samples, the Siddha Central Research Institute, under the Ministry of AYUSH, Government of India, conducted a thorough authentication process.

Development of polyherbal formulation

The oral polyherbal formulation was prepared by the decoction method of extraction (Shailaja *et al.,* 2017) [14]. The plant materials were taken in equal ratio and distilled water was added, boiled and condense to get decoction of herbs. Syrup base (66.7%) was added to the filtrate in the ratio of 1:5 (decoction: syrup base) to get the syrup consistency. Additionally, specific quantities of methyl paraben sodium, propyl paraben sodium, sodium benzoate, bronopol, citric acid, saccharin sodium, and a flavoring agent were added to the mixture (Rowe, 2006) [13].

Animals

For acute toxicity study, the female Wistar Albino rats, non-pregnant, nulliparous animals are preferred as per the OECD 423 guidelines. The Wistar rats were purchased from the Laboratory Animal House, Madhavaram milk colony, Chennai - 51. The animals were kept for acclimatization for a period of 7 days. The female Wistar rats aged 8 weeks with an average body weight of 180 – 200 gms.

Dose administration

The acute toxicity study is a single dose administration and observed for a period of 14 days. The rats were fasted overnight and the dose was administered with 0 mg/kg (Control), 300 mg/kg and 2000 mg/kg with 3 rats in each group. Additionally, 2000 mg/kg was given to another group of 3 rats. The dose were administered using an oral gavage.

Observation

The animals were observed individually for first 30 min upto 4 hours with attention, then for the first 24 h and daily thereafter, for a total of 14 days to observe toxicity signs like changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and for behavioural pattern.

Body weight

The body weight of rats was taken on the day of dosing (day 0) and weekly once (day 7 and 14).

Gross pathological examination

On day 15, the animals were sacrificed under anesthesia by using intraperitoneal administration of xylazine and ketamine at the dose rate of 10 mg/kg B.W and 80 mg/kg B.W respectively. The internal organs were examined grossly for pathological changes.

Hematological analysis

The blood was collected at the end of the study for hematological analysis.

Results and Discussion

The acute oral toxicity study didn't show any signs of toxicity or mortality during the period of study at the dose of 2000 mg/kg. This indicates that the polyherbal formulation was safe to administer. This study correlates to the many of the previous study. Aiyalu and Ramasamy, (2016) [1] reported that the aqueous extract of *C. heteroclita* was safe to administer at the dose of 2000 mg/kg with no signs of mortality or toxicity. Elamaran *et al.* (2023) [3] reported that phytogenic feed additives loaded herbosome comprising of different herbal ingredients were safe to administer at the dose of 2000 mg/kg.

The body weight of the animals showed no significant changes during the study period indicates that it does not affect the growth and development of animals. The changes in body weight and weekly body weight gain data were listed in table 1 and 2. It is similar to the previous studies, in which there is no change in body weight by administering phytogenic feed additives loaded herbosome (Elamaran *et al.,* 2023) [3].

The behavioural and general observations were done during the period of study including fur, eye, respiration, temperature, stool colour, urination, and other changes. There was no visible changes noticed during the 14 day trial period and the observations are listed in table 3. Aiyalu and Ramasamy, (2016) [1] reported that no significant changes noticed during acute toxicity study which includes breathing, sense of touch/sound, central nervous systems, behaviour pattern and locomotor activity and there was no signs of convulsion, tremor, excessive salivation, diarrhea, sedation and edema.

The gross examination showed no pathological lesions in the internal organs and on the body cavities indicating that the polyherbal formulation did not produce toxicity at the dose of 2000 mg/kg. Narayanmurthy *et al.* (2021) [8] reported that there was no gross pathological changes noticed by administering clevira syrup - polyherbal formulation at the dose of 2000 mg/kg.

The hematological analysis of the acute toxicity study showed that there is no significant changes in the blood parameters, indicates that the Polyherbal formulation is safe to administer. The hematological parameters are listed in table 4. Aiyalu and Ramasamy, (2016) [1] indicated that there was no abnormality observed in hematological parameters for aqueous extract of *C. heteroclita* treated groups compared with control groups indicating the extract is safe.

From the acute toxicity study, the LD_{50} of the polyherbal formulation was found to be more than 2000 mg/kg BW and the LD_{50} of the polyherbal formulation was classified as GHS (Globally Harmonized System) category 5 (LD_{50} >2000 mg/kg b.w) as per OECD Guideline No. 423, (2002).

Table 1: Acute oral toxicity study - Body weight of female Wistar rats

Group	Dose (mg/kg)	0 Day	7th day	14th day
I	Control	183±3.46	199.30±4.10	215.70±3.84
II	300	180±0.58	197.70±1.20	213.70±0.88
III	2000	174.30±0.33	191.30±0.88	207.30±0.88
IV	2000	177.70±3.18	195.70±3.18	212.70±4.33

Table 2: Acute oral toxicity study -Weekly body weight gain of female Wistar rats

Group	Dose (mg/kg)	0 - 7th Day	7th - 14th day	0 -14th day
I	Control	16.33±0.88	16.33±0.33	32.67±0.88
II	300	17.67±0.88	16±0.58	33.67±0.88
III	2000	17±0.58	16±0.58	33±0.58
IV	2000	18±0.00	17±1.16	35±1.16

Values are expressed as Mean ± Standard Error of the Mean (SEM); (n=3)
p>0.05; No statistical significance was observed among all the parameters mentioned.

Table 3: Behavioural and general observations of Acute toxicity study

Parameter	T_1	T_2	T_3
Feed consumption (24 h)			
Water consumption (24 h)	25 ml	24 ml	27 ml
Body temperature	Normal	Normal	Normal
Visible abnormalities	Nil	Nil	Nil
Rate of respiration	Normal	Normal	Normal
Drowsiness	Nil	Nil	Nil
Tremor	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil
Stool colour	Dark black	Dark black	Dark black
Urination	Normal	Normal	Normal
Diarrhoea	Nil	Nil	Nil
Mucoid stool	Nil	Nil	Nil
Eye colour / Pigmentation	Normal	Normal	Normal
Skin colour	Normal	Normal	Normal
Rashes	Nil	Nil	Nil
Paw jumping	Nil	Nil	Nil
Paw licking	Nil	Nil	Nil
Paw biting	Nil	Nil	Nil
Mortality	No	No	No

Table 4: Acute Oral Toxicity - Hematology of Female Wistar rats

Parameter	T_1	T_2	T_3
Hb (g/dL)	13.23±0.34	13.17±0.37	13.3±0.32
PCV (%)	40.77±0.43	40.97±0.27	41.07±0.71
RBC (10^6/µL)	6.70±0.26	6.74±0.24	6.80±0.23
WBC (10^3/µL)	2.73±0.22	2.63±0.22	2.56±0.28
PLT (10^3/µL)	638±46.49	641.3±43.35	663±24.21
MCHC	32.47±0.48	32.13±0.45	31.7±0.53
MCH	19.5±0.3	19.27±0.46	18.93±0.76
MCV	60.57±1.57	60.23±1.76	59.17±2.52
MPV	4.57±0.09	4.4±0.25	4.26±0.18
Granulocyte	0.77±0.09	0.9±0.06	0.93±0.19
Lymphocyte	1.87±0.19	1.97±0.09	2±0.06
Monocyte	0.03±0.03	0.13±0.03	0.2±0.06

Control; T_2 - PHF 300 mg/kg bw; T_3 - PHF 2000 mg/kg bw
Values are expressed as Mean ± Standard Error of the Mean (SEM); (n=3)
p>0.05; No statistical significance was observed among all the parameters mentioned.

Conclusion

Based on the results, it is conclude that the polyherbal formulation comprising of *Asparagus racemosus, Withania somnifera, Andrographis paniculata, Ocimum sanctum* and *Piper nigrum* having immunomodulatory action was safe at a single dose at the maximum level (2000 mg/kg, PO) in acute toxicity study**,** which does not show any changes in body weight, behavioural changes, gross pathology and hematology.

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