Oral Toxicity Evaluation of a Polyherbal Formulation in Rats: Acute and Subacute Effects

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ABSTRACT

Most of these investigations are performed to determine the toxicity of substances to humans, animals, or the environment; to learn more about the mechanism of hazardous chemicals; or to provide better methods for detecting certain chemically caused effects. In light of OECD recommendations, the current study set out to assess the acute and 28-day repeated oral toxicity of a polyherbal formulation. Animals in an acute oral toxicity study were given a dose of 2000 mg/kg of the herbal mixture and then monitored every 30 minutes for the next 1, 2, and 4 hours. Four groups of six rats each were used to conduct a repeated dose, 28-day oral toxicity investigation. The animals in Group 1 were used as a comparison. Animals in Group II were given a low dose of the herbal mixture (50 mg/kg), whereas those in Group III were given a moderate dose (100 mg/kg), and those in Group IV were given a high dose (200 mg/kg) (orally, once daily, for 28 days). Results from both the acute toxicity trial of the herbal mixture at a dose of 2000mg/kg and the recurrent oral toxicity study over 28 days showed no harm or mortality. There were no discernible alterations in haematological and biochemical markers, relative organ weight, gross necropsy, or histological examination throughout a repeated oral toxicity studies of the herbal mixture over the course of 28 days. The current study's findings reveal that the newly developed Polyherbal formulation is entirely safe and non-toxic for therapy, with an LD50 of >2000 mg/kg.

Key Words: Polyherbal formulation, Acute toxicity, Sub Acute toxicity, WBC, Organ Weight

Introduction

Recent years have seen a rise in the popularity of medicinal plants due to rising concerns about the potential side effects of conventional medicine as well as the enhanced potency of medications derived from plants. Combinations of herbs have shown more effective than individual herbs, with less negative side effects [1].

In both developing and developed countries, where modern medicines are popular practice, the World Health Organization estimates that over 80% of the population relies on these "alternative" plant-derived treatments as their primary medical need [2]. Herbal treatments have proven highly effective in recent years, and their history of use has aided in the development of novel pharmaceuticals. In underdeveloped countries, doctors often turn to herbal remedies and natural medications to treat a wide range of conditions [3,4].

The main goals of toxicology are the identification, prevention, and treatment of poisonings, hence its name comes from the study of the deadly effects of medications and other chemicals. Once data on a compound's potential hazards have been collected, the levels at which it can be used safely, or the degree to which it is safe, can be determined; this is known as the compound's Biosafety level [5]. Providing information on potential health hazards that may occur from a short-term exposure is the primary goal of acute toxicity testing in animals, which is often the first stage in the assessment and evaluation of health effect characteristics of a test material.

The prevention, diagnosis, and treatment of many diseases and conditions are all areas where traditional and alternative medicine sees widespread use. It has garnered public attention during the past 2-3 decades as these drugs are freely accessible in some regions [6]. Therapeutically, medicinal plants have become increasingly important due to the large range of minerals, vitamins, and other chemicals they provide.The discovery of new medication leads

for the prevention and treatment of disease is greatly aided by natural products [7]. Many people utilize herbal medications as self-medication, thus it is important to evaluate their efficacy and safety. Increased efforts to clarify the health advantages and hazards of herbal medications are warranted in light of the paucity of data available on the safety of regularly used herbal mixes. Toxicology studies on herbal medicines, both acute and chronic, are urgently required [8]. Plant based formulations accessible with a range of indications such as liver protective, appetite and growth promoters, gastrointestinal and hepatic regulator; regardless of extensive use, there is a lack of scientific data on their quality, safety and efficacy of many herbal remedies. Although many herbal remedies are safe, several herbs that are commonly utilized as medicines are really quite hazardous when used abruptly or chronically [9,10]. Since there aren't many experimental data on the safety of plant-based medicines despite their widespread use, it's crucial to perform direct toxicological evaluation on natural herbal products [11,12].

Herbal remedies are becoming widely used. acceptance as all-natural treatments for conditions like diabetes, arthritis, liver and kidney illness, obesity, and heart disease. Herbal combinations have been shown to be more therapeutically beneficial than the individual herbs used alone. These synergies are used to treat a wide range of persistent diseases. Finding safe, less toxic, and cost-effective polyherbal therapies that can be helpful against many chronic diseases, such as diabetes, obesity, and liver dysfunction, is an urgent global need right now. In this case, we created a polyherbal combination consisting of three commonly used Indian medicinal plants. We are optimistic that the newly produced herbal medicine will prove to be a successful treatment for a wide range of persistent illnesses. As a result, establishing the formulation as a medicine requires standardizing the preclinical safety and efficacy studies on animal model for further therapeutic exploration. The polyherbal formulation included herbs with documented effects against diabetes and its consequences, including antidiabetic,

antioxidant, antihyperlipidemic, and cardio tonic properties.

The selected plants for preparation of polyherbal formulation Includes *Cleome gynandra*. Its leaves are widely used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. Abundant pre-clinical studies have documented its anti-diabetic and hypoglycaemic effects through various postulated mechanisms

Plants are chosen for use in making a polyherbal remedy Including the *Cleome gynandra*. Native peoples in Asia, South America, India, and East Africa all use their leaves to treat diabetes and its complications. Anti-diabetic and hypoglycemic effects have been established in numerous pre-clinical trials via a number of different hypothesized pathways [12].

Gibson's barleria, or *barleria Gibsonii* (Acanthaceae), is a plant used for its antihyperglycemic, antihyperlipidaemic, anti-inflammatory, cardioprotective, and antioxidant properties in the treatment of cardiovascular and metabolic diseases. The presence of bioactive chemicals including phenols, flavonoids, and tannins in various plant tissues is responsible for these effects [13].

Due to its many therapeutic characteristics, *Pulcaria wightiana* (Asteraceae), also known as sontikli, has been extensively employed in traditional systems of medicine. Bael exhibits antibacterial, antifungal, radio protective, antipyretic, analgesic, antioxidant, hepatoprotective, wound healing, and many other actions. It has a significant potential to treat disorders like diabetes, hyperlipidemia, ulcer, inflammation, diarrhoea, cancer, constipation, and many morem [14].

Although the safety of each of these plants is well established, it is unknown how these herbs when combined will behave. Thus, prior to their usage in humans, it is required to assess the toxicity and safety of the herbal combination (PHF). To establish a safe dose for human

trials, preclinical toxicity studies are required. Therefore, the purpose of the current study is to evaluate the safety of a polyherbal formulation in albino Wistar rats using acute oral toxicity (single dosage, 14 days) and sub-acute oral toxicity (repeated doses, 28 days). The toxicity study was carried out in accordance with OECD Guidelines 423 and 407, respectively, for economic cooperation and development.

Materials and Methods

Plant Materials

In Kadpa, Andhra Pradesh, India, leaves of *Cleome gynandra*, *Barleria Gibsonii*, and *Pulcaria wightiana* were collected and authenticated by a botanist from Yogi Vemana University.

Preparation of Polyherbal Formulation

Cleome gynandra, *Barleria Gibsonii*, and *Pulcaria wightiana* leaves were gathered, dried in the shade, and then ground into a coarse powder. The dry powder was screened through filter No. 40 and kept for use in extraction. The 500 g of powdered plant material were placed in the soxhlet extraction assembly and extracted continuously for 48 hours at a high temperature using solvents of increasing polarity. The cold-maceration method was used to perform the aqueous extraction.

Experimental Animals

Male and female wistar rats weighing 150–175 g were obtained from the animal house of the PRRM College of Pharmacy, Kadapa, in good health. All of the animals were housed in spotless polypropylene cages with 12 hours of light/dark cycles, well-ventilated temperatures and humidity, and free access to food (a normal pellet diet) and tap water, with the exception of when fasting was necessary during the study. The experimental methods were completed in accordance with CPCSEA Regulation (1423/PO/Re/S/11/CPCSEA) and IAEC guidelines.

Evaluation of the Acute Toxicity Study

The acute toxicity research was carried out in accordance with OECD guideline 420, 2001, which changed the fixed dosage approach for acute toxicity testing. The PHF was administered to two groups of five healthy albino wistar rats of either sex (3-month-old, 150-200 g b.wt.) and the animals were observed for mortality and clinical signs for the first hour, then hourly for the following three hours, and finally periodically until 48 hours for changes in skin and fur, eyes, and mucous membranes, behavior pattern, tremors, salivation, diarrhea, sleep, coma, mortality, moribund, or any The number of rats that died throughout the study period was counted, and all experimental animals were kept under strict observation for a total of 14 days. If three or more rats survived, it was projected that the LD_{50} would be higher than 2000 or 5000 mg/kg [15].

Sub-Acute Toxicity Study

The subacute oral toxicity study was conducted in accordance with OECD Standard OECD-407. Rats were split into four groups of ten (five males and five females), and the body weights of each group were noted. Groups II through IV received PHF at doses of 50, 100, and 200 mg/kg, respectively, whereas control rats (group I) received distilled water. Every treatment was administered orally once day for 28 days. Throughout the course of the treatment, animals were watched for indications of abnormalities. Additionally, the animals' final treatment weights were recorded. Animals were kept in individual metabolic cages for 24 hours on the 28th day of therapy. For ion and biological analysis, excreted urine was collected and stored at a temperature of 20°C. Animals were given a free night of fasting after the procedure but were still given access to water. They were then given an ether anesthesia before blood samples were taken by retro-orbital puncture, with and without the use of an

anticoagulant (ethylene diamine tetra acetate), for hematological and biochemical examination, respectively.

Liver Function Test

The blood samples were further analysed for glucose, triglyceride, cholesterol, Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH), total bilirubin, creatinine,urea, protein and albumin by using different biochemical kits (Erba Diagnostics, New Delhi) in semi-automated biochemical analyser (Maxlyzer, Avecon model no: NB-201).

Histopathology

Rats were decapitated after blood samples were taken, and their internal organs, including their hearts, livers, kidneys, lungs, and spleens, were removed, blotted dry, and weighed in comparison to controls. Histopathology tests required the organs to be preserved in NaCl buffer containing 10% formaldehyde.

Statistical Analysis

All results were expressed as mean ± SEM. Data obtained from the toxicity studies were analysed by Student's t-test using Graph Pad prism 9.0 to determine significant difference between the means of control and test groups. P value < 0.05 was considered statistically significant

Results and Discussion

There has been a worldwide surge in the use of traditional medicine, and its popularity is only growing. Despite this, hepatotoxicity and nephrotoxicity findings, as well as other concerns, have recently called herbal medicine's safety into question. Patients still dispute the safety and efficacy of traditional herbal remedies despite the availability of numerous such products [16,17].

Skin, fur, eyes, and mucous membranes are all tracked in an acute toxicity study. Tremors, convulsions, sedation, stereotypic behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, pilo erection, muscular co-ordination, muscular grip, posture, gait, limb paralysis, lethargy, sleep, coma, and mortality are all examples of CNS, CVS, and ANS toxic symptoms that were carefully recorded (Table 1). According to the data collected, none of the treated animals died or showed toxicity symptoms (Table 2). Both male and female rats' weight changes were tracked and compared to the control group. The LD_{50} was determined to be larger than 2000 mg/kg b.wt., and there were no obvious pathological changes.

Table 1: Clinical observations of rat at 2,000 mg/kg dose of Polyherbal Formulation (PHF)

Observations	**Day 1**	**Day 7**	**Day 14**
Skin	Normal	Normal	Normal
Eyes	Normal	Normal	Normal
Salivation	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil
Sleep	Normal	Normal	Normal
Coma	Nil	Nil	Nil
Convulsion	Absent	Absent	Absent
Tremors	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent
Mortality	Nil	Nil	Nil

Table 2: Acute toxicity study of polyherbal formulation

Groups	**Treatment**	**Mortality**			**Toxicological Profile**
		After 24 hr	**After 7 days**	**After 14 days**	
Control	Distilled Water 10 ml/kg p.o.	None	None	None	Safe
PHF Treated	2000 mg/kg p.o.	None	None	None	Safe

The repeated administration of PHF for up to 28 days did not result in any clinical symptoms of toxicity or mortality, according to the sub-acute toxicity study. When compared to the control groups, the treated groups' changes in body weight, food intake, and water intake were determined to be minor. All of the rats' body weights continued to rise during the duration of the experiment. To the contrary, there was no statistically significant change in body weight between the treatment groups and the control, suggesting that it had no deleterious impact on the parameter used to evaluate the success of pharmacological therapy. No deaths occurred at any point during the experiment. Different dose groups were observed to consume a similar amount of food and drink as the control group throughout the dosing period and on the final day. There were no unusual occurrences to report

Table 3: Effect of PHF on relative body weight of rats.

Treatment	**Changes in Body weights (gm)**			
	1st week	**2nd week**	**3rd week**	**4th week**
Control	128.3±2.18	145.0±2.35	140.0±1.67	142.0±2.32
PHF (50 mg/kg p.o.)	151.8±2.93	162.0±1.95	168±2.98	169±1.16
PHF (100 mg/kg p.o.)	176.0±3.16	181.50±1.55	185.24±1.98	188.4±1.54
EEAL (200 mg/kg p.o.)	163.7±1.75	172.30±1.31	180±1.63	182±1.26

Table 4: Effect PHF on relative organ weight of rats.

Organ	**Relative organ weight (%)**			
	Control (Distilled water)	**PHF**		
		(50 mg/kg)	**(100 mg/kg)**	**(200 mg/kg)**
Liver	3.48 ± 0.09	3.14 ± 0.21	3.09 ± 0.04	3.40 ± 0.11
Heart	0.31 ± 0.02	0.36 ± 0.02	0.34 ± 0.01	0.28 ± 0.01

Spleen	0.41 ± 0.02	0.46 ± 0.02	0.42 ± 0.03	0.44 ± 0.02
Kidney	0.80 ± 0.06	0.89 ± 0.03	0.86 ± 0.02	0.87 ± 0.04
Lungs	0.69 ± 0.02	0.72 ± 0.07	0.66 ± 0.01	0.71 ± 0.01
Brain	1.54 ± 0.06	1.62 ± 0.01	1.58 ± 0.05	1.60 ± 0.02

Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Platelets, Haemoglobin (Hb), Mean Cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Neutrophils, Eosinophil's, Basophil's, Lymphocytes, and Monocytes were all found to be within the normal physiological limits for rodents, and no significant differences were found when compared with the control groups.

Table 5: Effect of PHF on haematological values of rats.

Parameters	**Haematological parameters**			
	Control (Distilled water)	**PHF**		
		(400 mg/kg)	**(400 mg/kg)**	**(400 mg/kg)**
HB g/L	12.47 ± 0.21	12.71 ± 0.13	12.26 ± 0.24	12.19 ± 0.15
RBC 10^6/µL	8.29 ± 0.07	8.34 ± 0.43	8.61 ± 0.17	7.90 ± 0.26
WBC 10^3/µL	4.27 ± 0.09	4.88 ± 0.26	4.60 ± 0.06	4.57 ± 0.15
PLT 10^3/µL	1010 ± 32.36	1012 ± 20.02	1043 ± 19.67	1052 ± 16.59
LYMPH 10^3/µL	4.63 ± 0.02	5.28 ± 0.34	4.78 ± 0.39	6.81 ± 1.4
MONO 10^3/µL	0.15 ± 0.01	0.16 ± 0.02	0.35 ± 0.17	0.25 ± 0.09
BASO 10^3/µL	0.022 ± 0.009	0.052 ± 0.021	0.025 ± 0.006	0.055 ± 0.016
NEUT 10^3/µL	1.55 ± 0.11	1.47 ± 0.17	1.87 ± 0.06	1.97 ± 0.31

After 28 days of treatment with PHF, biochemical indicators including liver biomarkers (ALP, ALT, total bilirubin and AST), renal function markers (creatinine, urea), cardiac function markers (creatine kinase-MB), and others were all within normal ranges (total protein, glucose, and total cholesterol).

The histopathological examinations show no significant weight changes and normal architectural changes in the vital organs such as the heart, brain, kidneys, liver, lungs, and

spleen suggesting that the PHF is free from risk of serious organ degenerative potential at all dose levels

Table 6: Effect of PHF on biochemical values of rats.

Parameters	**Biochemical parameters**			
	Control (Distilled water)	**PEAL (400 mg/kg)**	**CEAL (400 mg/kg)**	**EEAL (400 mg/kg)**
AST (IU/L)	184.65 ± 5.14	177.68 ± 6.19	190.34 ± 4.93	193 ± 2.56
ALP (IU/L)	156.43 ± 1.09	150.13 ± 2.14	146.78 ± 1.73	159.73 ± 1.86
ALT (IU/L)	47.32 ± 1.34	52.09 ± 2.31	45.63 ± 1.24	49.60 ± 2.63
Total protein (g/dL)	6.84 ± 0.15	6.43 ± 0.20	6.14 ± 0.19	6.25 ± 0.23
Total bilirubin (mg/dL)	0.72 ± 0.02	0.67 ± 0.01	0.68 ± 0.03	0.70 ± 0.01
Glucose (mg/dL)	86.6 ± 1.87	83.19 ± 1.50	89.17 ± 2.03	81.17 ± 1.34
Creatinine (mg/dL)	0.76 ± 0.02	0.69 ± 0.01	0.62 ± 0.02	0.71 ± 0.01
Urea (mg/dL)	7.30 ± 0.67	6.78 ± 0.59	7.85 ± 0.45	7.13 ± 0.54
Total cholesterol (mg/dL)	103.43 ± 2.94	110.84 ± 1.84	113.54 ± 2.06	107.24 ± 3.71
CK-MB (IU/L)	289.32 ± 4.85	297.48 ± 5.67	285.58 ± 5.24	287.16 ± 2.98

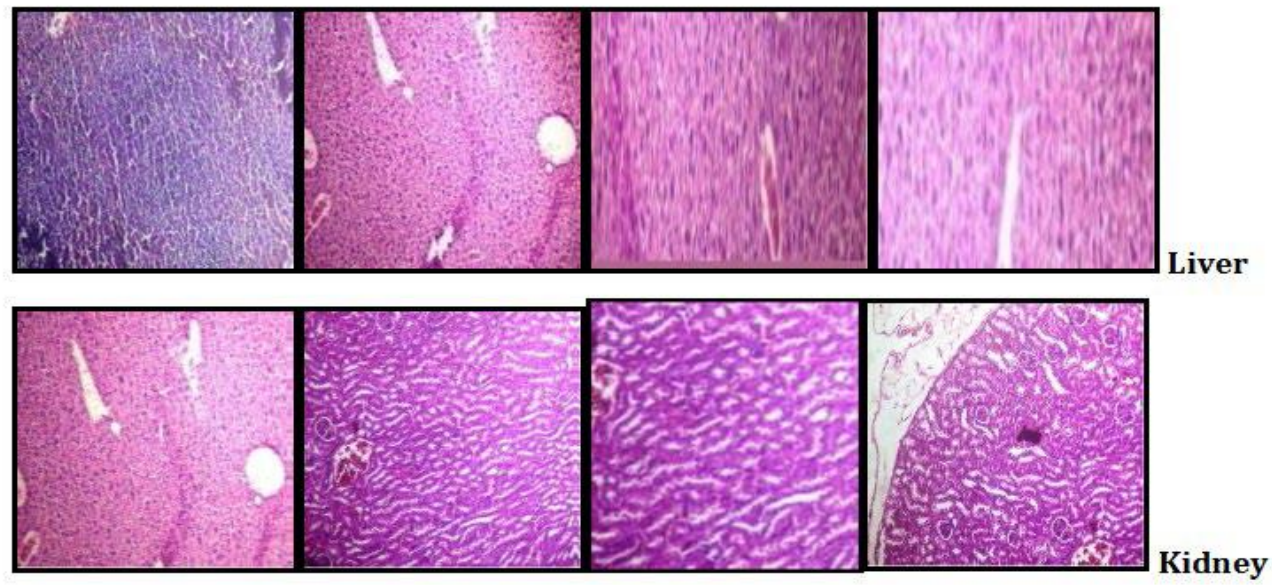

Figure 1: Histopathology (H&E stain10x) of treated groups rats after 28th days Control 50mg/kg 100mg/kg 200mg/kg.

World Health Organization estimated that 80% of the world's population still depends mainly on traditional medicines for their health care. The subcontinent of India is well-known for its major biodiversity centers with almost 45,000 plant species. In India, about 15,000 medicinal plants have been identified, which the communities used 7,000- 7,500 plants for the cure of different diseases and ailments. In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The 'Ayurvedic Literature Sarangdhar Samhita' highlighted the concept of polyherbal to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When multiple herbs are combined in a particular ratio, it will achieve better therapeutic efficacy and safety.

We have developed a herbal formulation which is prepared by mixing three well known Indian medicinal plants in a fixed ratio. The above study results clearly indicated that the newly developed poly herbal formulation was free from any risk of serious organ degenerative potential at all dose levels

Conclusion

The present Acute and sub-acute toxicity results suggest that LD_{50} of developed formulation was >2000mg/kg. Further studies on long-term toxicity and clinical trials may be rational to substantiate the study results.

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Conflict of Interest

Authors disclose no conflicts of interest for publication of the manuscript.

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