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ASSESSMENT OF THE ACUTE TOXICITY FOR ETHANOLIC EXTRACT OF POLYHERBAL FORMULATION IN SWISS ALBINO MICE

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ABSTRACT: The main objective of this study was to Assessment of the acute toxicity of ethanolic extract of Polyherbal formulation in Swiss albino mice. In this study, the acute oral toxicity study of ethanolic extract of PHF_1 was carried out according to Organization for Economic Co-operation and Development (OECD) guideline 423 and 407 on Swiss albino mice (20–30 g). In the study, the oral dose of 2000 mg/kg of PHF_1 extract was administered in the treated group. General behavior, morbidity, and mortality were determined up to 72 h and compared to control group. Further animals were investigated for a period of 3days assess any other toxic effect. Behavioral changes and other parameters such as body weight, hematological parameters, and biochemical parameters were evaluated and compared to the normal group. The result of the study was mentioned that In acute toxicity study, treatment groups revealed neither morbidity nor mortality or any significant changes in behavior only drowsiness, sedation, and lethargy were observe in the group having 2000 mg/kg of Polyherbal formulations extract. In the acute toxicity study, no change in organ weight, hematological, biochemical parameters were observed when compared to the control group. The result concluded that the oral administration of Polyherbal formulations extract didn't produce any significant toxic effect in Swiss albino mice. Hence, Polyherbal formulations extract can be utilized safely for the treatment of Parkinson's disease (PD).

INTRODUCTION: Toxicology is defined as a stem of science of poisons, as well as a toxin is defined as some material that cause a dangerous outcome when administer both by accident or propose, to a living being.

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Toxicity may be acute or chronic and can vary from one organ to another as well as vary with age, genetics, sex, diet, physiological situation, or the health status of the living being [1, 2]. Toxicity depends on the route of entry into the body, whether through the alimentary canal, the lungs, or the skin [3].

Acute Toxicity Studies: Acute toxicity is generally defined as the adverse change(s) happening immediately or a little time following only a short time of exposure to material or substances [4].

An adverse effect is "any outcome to results in useful injury and/or biochemical lesions" that may affect the show of the complete organism or that decrease the organ's capacity to respond to a supplementary challenge" [5, 6]. Studies of acute systemic toxicity effort to decide the dose-dependent adverse outcome that may occur, and different suitable data may be collected when determining the complete acute toxicity profile of a substance [7, 8]. Acute toxicity evaluation of lead/drug is followed by using the OECD Guideline no. 420, 423, and 425. Acute systemic toxicity is assessed following oral, dermal and/or inhalation exposure(s) - depending upon the expected routes of personal exposure to the material [9].

MATERIALS AND METHODS:

Plant Collection and Extraction of Plants: In this study, different parts of plants were used. The whole plant of BM, fruits of EO, a seed of MP, and root of WS were taken. The selected parts of plants were collected in and around Central Institute of Medicinal and Aromatic Plants (C-MAP -CSIR) Lucknow, Uttar Pradesh, India. These plants were authenticated by Botanical Survey of India (BSI), Dehradun vide authentication number Tech./Herb/ 2018-19/118502-05. The voucher specimens of plants are placed in department of pharmacognosy at our institution. Four plants (MP, BM, WS and EO) were extracted by different solvents using soxhlet apparatus. Different extracts were obtained by using different solvents like n-hexane, chloroform, ethyl acetate and ethanol. The extracts were evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary evaporator. The percentage yield of all extracts was calculated and extracts were stored in air-tight desiccators for further analysis [10].

Development and Optimization of PHF: PHFs were prepared by using the extracts in different ratios. Each 1g of PHF contains a different amount of extracts of BM, EO, WS and MP. The prepared PHFs were optimized by using DPPH assay [11].

Test Animals: Male albino mice of Swiss strain were used for the study. Animals were received from the animal house of Goel Institute of Pharmacy and Sciences, Lucknow, India. Experimental animals were handled according to regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (2014/PO/Re/S/18/CPCSEA). The animals were maintained under standard conditions of humidity, temperature (25±5°C) and light (12 h light/dark). The animals were acclimatized to animal house conditions and fed with standard pellet diet and water *ad libitum*. The mice were maintained their life cycle.

Acute Oral Toxicity: For the acute oral toxicity study, of ethanolic extract of PHF_1 was evaluated according to Organization for Economic Co-operation and Development (OECD) guideline 423 on Swiss albino mice. The animals were maintained at 22±5 ºC with humidity control and as well on an automatic dark and light cycle of 12 hours. The animals were fed by the standard feed and provided *ad libitum* drinking water. In every group, 6 animals were taken (approx. bodyweight 20–30 g in mice). The experimental animals were subjected for acclimatization for 3 days prior to experiments. Fixed-dose procedures were followed for the experimental animals, while control animals received only vehicles. The test substances were administered through gastric gavage. The animals were observed for any toxic effect for the first 4 h after the treatment period. Further animals were investigated for a period of 3 days for any toxic effect [12, 13]. Behavioral changes and other parameters were also considered for the study, such as body weight, urination, food intake, water intake, respiration, convulsion, tremor, temperature, *etc.* [14, 15]

Observational, Hematological and Biochemical Study: In the acute oral toxicity, the animals were checked for mortality and every sign of ill health at hourly distance on the day of administration of test substance, and thereafter a daily general case side clinical examination was carried out, including changes in the skin, mucous membrane, eyes, occurrence of secretion and excretion, *etc.* Also, change in gait, posture, and response to treatment were also to be recorded [16]. In addition to the observational study, body weights were to be recorded, and blood samples to be collected from all the animals on the 7th day of the experiment in acute oral toxicity. The samples were to be analyzed for total RBC, WBC, differential leucocytes count (DLC), hemoglobin percentage, and biochemical parameters similar to ALP, SGPT,

SGOT, total cholesterol, triglycerides, creatinine, bilirubin, serum protein, and tissue protein activity. Weights of vital organs like liver, heart, kidney, *etc*., were to be recorded [17].

Effect of PHF Extract on Hematological Parameters: Red blood cell count, hematocrit, mean cell volume, hemoglobin, white blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, monocyte, neutrophil, lymphocyte, and platelet count of the control and plant treated groups were determined and compared with control group using an automatic hematology analyzer (Sysmex K21, Tokyo, Japan) [18, 19].

Effect of PHF Extracts on Serum Biochemical Parameters: The biochemical analysis were done on serum after centrifugation of collected blood and the following parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, high-density lipoprotein, total bilirubin (T- BIL), total protein, albumin, urea, and creatinine level were determined for both control, and PHF extract treated groups. All analyses were determined on using a clinical chemistry analyzer (Vital Scientific, Netherlands) [20, 21].

Statistical Analysis: Effect of PHF_1 for acute toxicity study in control and treated group, the experimental animals on various parameters studied through Student T-test and were analyzed through instat software program (Mean ± SE; n=6).

RESULT: PHF_1 showed potent Anti-Parkinson activity *via* an *in-vivo* model. Hence its preclinical safety evaluation was carried out in Swiss albino mice. In this context, acute oral toxicity was conducted in accordance with OECD test guidelines no. 423 and 407.

Acute Oral Toxicity: In this toxicity study, the experimental animals were given a single dose of the extract at 2000 mg/kg body wt., the experimental animals were observed for 7 days & sacrificed on the 7th day. The animals were studied for different observational, behavioral, hematological & biochemical indices.

Observational Parameter: Animals treated with PHF_1in acute oral toxicity study were observed from 0 days to 7th day of experiment for mortality, morbidity, *etc*. No observational changes, morbidity, and mortality were recorded throughout the experimental period in all the groups of experimental animals.

TABLE 1: BEHAVIORAL AND GENERAL OBSERVATIONS OF ACUTE TOXICITY STUDY FOR CONTROL AND TREATED GROUPS

Observation	Control group	Treatment of PHF (2000mg/kg)
Temperature	Normal	Normal
Food intake	Normal	Normal
Urination	Normal	No effect
Rate of respiration	Normal	No Effect
Body weight	Normal	Slightly change
Eye color	No effect	No effect
Diarrhea	Not present	Not present
Sedation	No effect	Observed
Change in skin	No effect	No effect
General physique	Normal	Lethargy
Coma	Not present	Not present
Death	Alive	Alive

Body Weight: The body weights of animals were recorded at the 0 day of the experiment from all the groups of animals and non- significant changes was found in PHF_1 treated groups when compared with vehicle control **Fig. 1**.

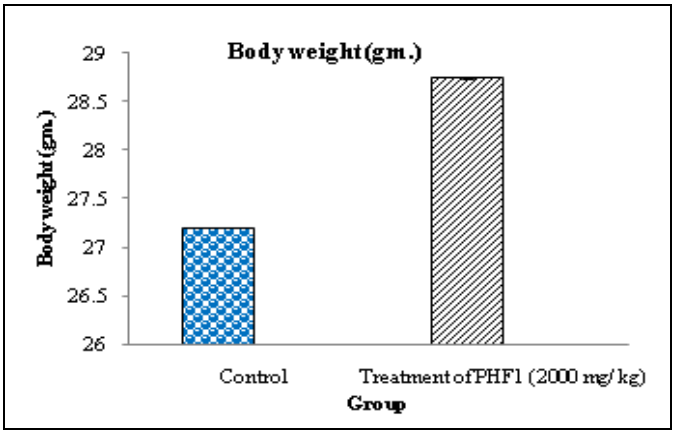

FIG. 1: CHANGES OF BODY WEIGHTS OF ANIMALS AFTER-TREATMENT OF PHF_1

Organ Weight: The animal was necropsied for gross pathological changes of vital organs &no gross changes were observed in the organs studied like liver, kidney, and brain.

The organ weight showed non- significant changes among all experimental groups both in absolute and relative terms and was presented in **Table 2** and **Fig. 2**.

TABLE 2: EFFECT OF PHF_1 AT 2000 mg/kg BODY WEIGHT ONCE ORALLY ON ABSOLUTE ORGAN WEIGHT (G)

Organ	Average organ weight	
	Control group	Treatment (2000 mg/kg)
Liver	1.24±0.029	1.387±0.021
Brain	0.33±0.0085	0.335±0.0053
Kidney	0.37±0.0146	0.38±0.008
Average body weight on sacrifice day	27.067±0.592	28.912±0.683

(Mean ± SEM, n=6)

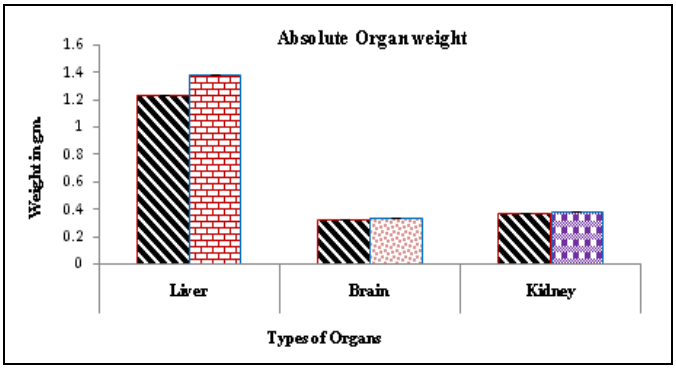

FIG. 2: EFFECT OF PHF_1 AT 2000mg/kg ON ABSOLUTE ORGAN WEIGHT (g). # Values are expressed as mean ± SEM. *P* > 0.05 when compared to normal control group.

Hematological Parameter: Blood samples were collected from all the groups of animals at the end of the experiment. All hematological parameters like hemoglobin level, total RBC counts, total WBC counts, DLC were studied & non-significant changes were found in treated groups when compared to control. RBC, WBC, and Hemoglobin, were presented in **Table 3** & **Fig. 3**.

Biochemical Parameter: Serum samples collected from all experimental groups were tested for SGOT, SGPT, ALKP, creatinine, cholesterol triglyceride, albumin, and serum protein levels. There were non-significant changes in all biochemical parameters studied in the experimental group (2000 mg/kg) when compared to the control. These parameters were presented in **Table 4**.

TABLE 3: EFFECT OF ORAL ADMINISTRATION OF ETHANOLIC EXTRACTS OF POLY HERBAL FORMULATIONS ON HEMATOLOGICAL PARAMETERS

Parameters	Control group Treatment (2000 mg/kg)	
Total RBC (million/mm^3)	10.83±0.075	9.97±0.047
Hemoglobin (g/L)	8.712±0.175	10.95±0.258
WBC (thousand/mm^3)	4.76±0.087	5.21±0.044
Neutrophil (%)	33±2.48	36.833±1.02
Lymphocyte (%)	55.125±2.32	50.014±1.39
Monocyte (%)	7±1.05	6.4±0.67
Eosinophil (%)	0.322±0.212	0.162±0.16

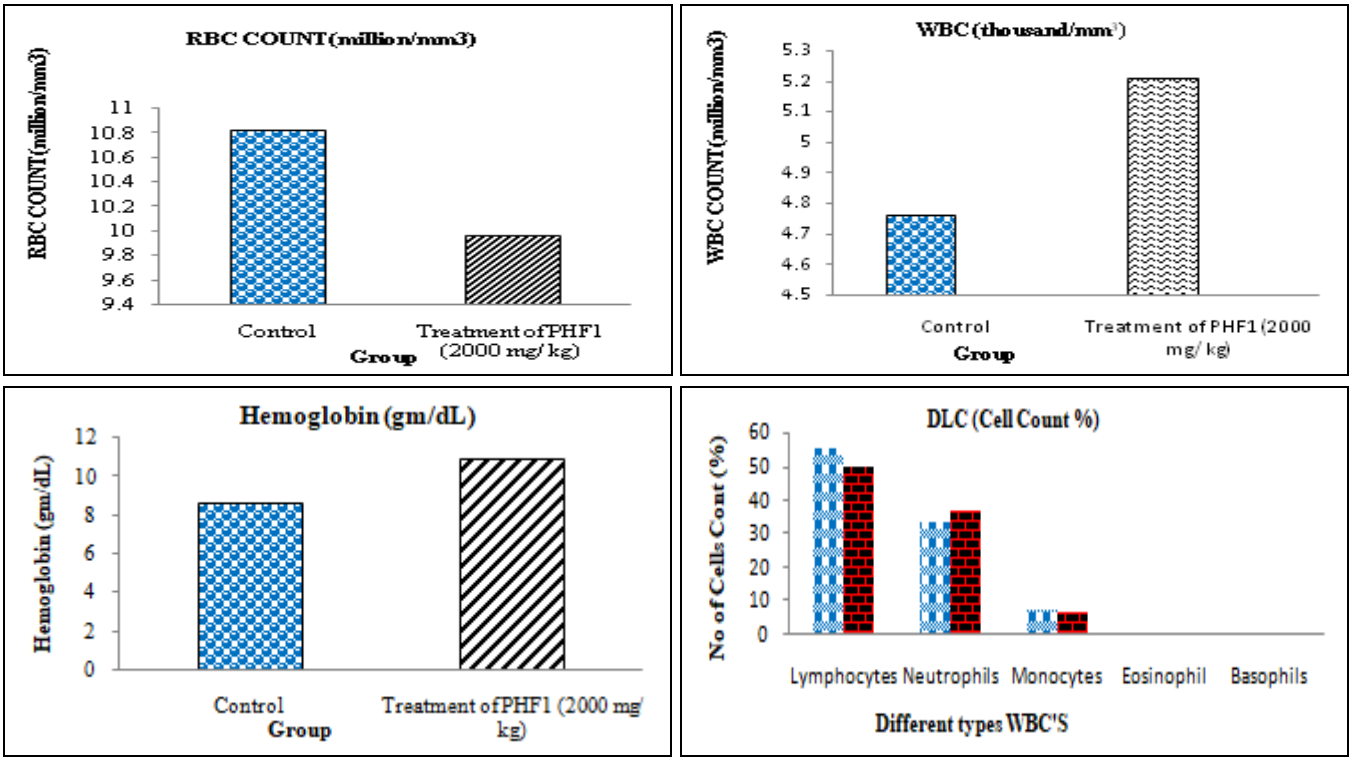

FIG. 3: HEMATOLOGICAL PARAMETERS FOR EXTRACT OF POLY HERBAL FORMULATIONS. [Effect of PHF_1 at 2000mg/kg body weight once orally on RBC Count, WBC Count, Hemoglobin and DLC], {# All values expressed as mean ± S.E.M (n=6) and P<0.05 is considered statistically significant. Statistical analysis determined by Student t-test. The treated group was compared against the vehicle control.}

TABLE 4: EFFECT OF ORAL ADMINISTRATION OF ETHANOLIC EXTRACTS OF POLY HERBAL FORMULATIONS ON BIOCHEMICAL PARAMETERS

Parameters	Control group	Treatment (2000 mg/kg)
Triglycerides (mg/dL)	62.23±0.437	75.15±0.478
Creatinine (mg/dL)	0.47±0.010	0.91±0.006
Serum protein(mg/dL)	7.29±0.093	6.98±0.054
Total cholesterol (mg/dL)	18.26±0.295	17.32±0.128
Bilirubin (g/dL)	0.38±0.005	0.52±0.011
Albumin (g/dL)	1.36±0.013	2.72±0.038
ALP (U/L)	78.58±0.288	55.01±0.453
SGOT (U/L)	28.79±0.276	45.48±0.201
SGPT (U/L)	34.75±0.274	26.17±0.181

(Mean ± SEM, n=6), # Values are expressed as mean ± SEM. $P > 0.05$ when compared to the normal control group

DISCUSSION AND CONCLUSION: The present research was aimed to evaluate the ethanolic extract of $PHF_1$for acute toxicity study and to identify the range of doses that could be used for further studies. The oral acute toxicity study of PHF_1 extract was carried out on Swiss albino mice at a single dose of 2000 mg/kg body weight and was continuously monitored for the first 4 h, followed for a period of 72 h for any toxic effect after the treatment period. No major changes in behavior and mortality were observed in the animal group. However, sedation and drowsiness were confirmed in 2000 mg/kg body weight in the treated group. The PHF_1 extract seems to be safe at a dose level of 2000 mg/kg, and the LD_{50} is considered to be >2000 mg/kg. Any pharmaceutical drug or compound with an oral LD_{50} higher than 2000 mg/kg could be considered safe and low toxic. This suggests that the ethanolic extract of $PHF_1$is practically non-toxic in a single dose of level 2000 mg/kg body weight [22, 23].

However, with respect to multiple doses uses in the treatment of the CNS disorder like Parkinson disease, Alzheimer disease, Epilepsy, whether it will be safe and have no effect on relative organ weight, hematological and biochemical parameters that can be confirmed from its toxicity study[24]. An acute toxicity study was carried out as per OECD guideline [25]. Decreases or increases in body weights are associated with the toxic effects of chemicals and drugs.

Scientific evidence confirmed that increases or decreases in the body weights are accompanied by accumulation of fats and physiological adaptation responses to the plant extracts rather than to the toxic effects of chemicals or drugs that lead to decrease appetite hence lower caloric intake by the animal [26]. The relative weight of the vital organs was found normal, indicating no toxic effect in both control and treated groups, and there were statistically non-significant differences ($P>0.05$). The bone marrow is responsible for the production of the blood cell, and some phytochemicals isolated from the plant have affected red blood cell levels. Hence, the tested PHF_1 extract may not have harmful effects on bone marrow function and justify the fact that all doses of PHF_1 does not induce anemia, making it safe. Similarly, estimation of serum biochemical parameters in treated animals showed non-significance ($P>0.05$) compared to control group [27].

The liver is the main target organ for drug or bioactive active compounds were exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the mice. However, the transaminases enzyme SGOT (AST) and SGPT (ALT) were observed positive and showed a remarkable significant elevation ($P<0.005$) in PHF_1 treated animals for 2000 mg/kg extract as compared to a respective control group. Increase (SGPT and SGOT) levels are responsible for causing inflammation, cellular leakage, and damage of cell membrane to cells in the liver [28]. These results showed that the use of plant extracts of $PHF_1$is safe and explained the extensive use of the plant as traditional medicine.

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