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Acute And Sub-Acute Toxicity Studies of a Poly Herbal Formulation Used for Management of Diabetes

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ABSTRACT

The polyherbal solution comprising of Karela (*Momordica charantia*), Daruharidra (*Berberis aristata*), and Gudmar (*Gymnema sylvestre*), Amlatas (*Cassis Fistula*), Jamun (*Syzyguium Cumin*), Guduchi (*Tinospora Cordifolia*), Vijatsara (*Pterocarpus Marsupium*), Dalchini (*Cinamomum Zeylenicum*), Methi (*Trigonella foenum graecum*), Pipal (*Piper Longum*), Gum Acacia (*Acacia Arabica*) has been used for the management of Diabetes mellitus empirically. However, there are no available toxicity data for this combination of herbal formulation. Therefore, our study focused on evaluating the acute and subacute toxicity of this polyherbal formulation on Wistar rat model. Briefly the experiment was performed in two phases. The acute toxicity analysis was performed for 7days in 3 Wistar rats and sub-acute toxicity was performed on 9 Wistar rats for 28 days. For acute toxicity a single dose of 800 mg/kg and 1200mg/kg of polyherbal solution was administered to rats. While for sub-acute toxicity 2 doses variations of 100 mg and 200 mg administered orally for 28 days. Biochemical and histo-pathological analysis suggested negligible toxicity and no mortality was reported in both acute and sub-acute doses signifying non-toxicity of the poly-herbal formulation.

Keywords: *Acute toxicity, Chronic toxicity, Poly-herbal formulation, Wistar rats*

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder primarily characterized by high concentration of blood glucose levels caused by insulin deficiency and can be combined with insulin resistance. Diabetes Mellitus can be of several categories including Type 1, Type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, Chronic usage of steroid medication, etc. The main subtypes of DM are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion (T1DM) and/or action (T2DM). T2DM is the most common type of Diabetes Mellitus, accounting for about 91% of cases[1]. Approximately 360 million of people worldwide predicted to be affected by Type 2 Diabetes Mellitus [2]. The microvascular and macrovascular problems linked to type 2 diabetes mellitus, which significantly increase the morbidity and mortality associated with the condition. Based on epidemiological statistics, individuals with type 2 diabetes mellitus apparently have increased chance of acquiring musculoskeletal, cardiovascular, and psychological diseases, as well as different types of cancer. [3].

The field of herbal medicine is currently undergoing significant expansion, and due to its natural origin and low side effect profiles, these formulations are becoming more and more popular in both developed and developing nations. All the plants included in the polyherbal formulation in our study have shown traditional evidence-based anti-diabetic effects[4]. Because of the widespread misconception that natural herbal remedies are safe, there are few research done to evaluate the toxicity and safety profiles of all traditional herbal remedies. However, the combined effect and safety of the herbal formulation is not well-known. Therefore, it is important to study the safety and toxic effects of polyherbal formulation before their usage. With this in view the present study focused on examining the acute and subacute toxicity of our polyherbal formulation. For experimental analysis Wistar rats were used. Acute doses of 800 mg/kg and 1200 mg/kg of poly-herbal formulation were administered for 7 days (single dose for 7 days consecutively) and sub-acute dose was 100 mg/kg and 200 mg/kg for 28 days. Biochemical analysis of collected blood samples and histo-pathological analysis were which signified negligible toxicity. Further, no mortality was observed throughout the experimental duration. The study was carried out as per animal ethic committee.

2. MATERIAL AND METHOD

2.1 Extraction and development of formulation:

For preparing the poly-herbal extract extract, the listed plant components, *Momordica charantia* (Fruit), *Berberis aristate* (Root), *Gymnema sylvestre* (Leaves), *Cassis Fistula* (Flower), *Syzyguium Cumin* (seed), *Tinospora Cordifolia* (stem), *Pterocarpus Marsupium* (bark), *Cinamomum Zeylenicum* (bark), *Trigonella foenum graecum* (seed), *Piper Longum* (Leaves), *Acacia Arabica* (Leaves) were purchased from a reliable source. Following a period of shade drying, the chosen plant materials were ground with a mixer grinder, sieved through 100 sieves, and then sealed in an airtight container. Each substance was coarsely powdered to 500 grams, and then extracted using 50% v/v hydro-alcohol through triple maceration (1 × 3 L) process. The generated hydro-alcohol extracts were vacuum-concentrated at 40° C using a rotating evaporator (removing alcohol). The concentrated extracts were freeze dried for 12 hours at -20ºC and thereafter lyophilized. The powdered lyophilized extract(s) were stored in desiccator under airtight condition. The hydro-alcoholic extracts of various herbs, lyophilized into powder form, were used for formulation development keeping the ratio of same for each plant extract.

2.2 Animals:

The experiment was carried out on young and healthy Wistar rats, weighing 150-250 gm. The animals were obtained from the animal house, Galgotias University, Greater Noida, Uttar Pradesh, India and all experiments were carried out there. The protocol was approved by the Animal Ethic committee of Galgotias University (2087/PO/ReReBi/S/19/CPCSEA). The 1964 Declaration of Helsinki and its subsequent amendments, as well as the ethical guidelines established by the institutional and/or national research council, were followed in this study involving animal subjects.

The animals were acclimatized to the laboratory setting for one week before starting the experiment. They were kept in normal atmosphere with a temperature 25^{O} Celsius and 12-hour light and 12-hour dark cycle.

2.3 Acute Toxicity Studies:

To evaluate the acute toxicity studies single ascending dose study design was applied. For this study two groups of Albino Wistar rats were used and each group consist of three rats kept fasted for 24 hours. Two doses in ascending order were tested in this study. Three Albino Wistar rats were given 800 mg/kg and 1200mg/kg of polyherbal formulation. The rats were kept under constant observation for the first four, twenty-four, and 48 hours respectively and then daily for next 14 days. During this period the rats were observed for any behavioural sign of toxicity (convulsion, breathing difficulty, abdominal rigidity, hypoactivity), changes in body weight, consumption of food and water, skin (fur), paralysis, nasal bleeding, central nervous system effects including tremors, sleepiness, and autonomic effects like lacrimation, salivation, piloerection.

2.4 Sub-acute toxicity studies:

Three groups of rats consisting three in each group were used to assess the sub-acute toxicity. Two groups received experimental doses of 100 gm/kg and 200 gm/kg, respectively, while the control group was given normal food and water and considered as the control group. The rats were weighed daily and observed for behavioural changes. On the 28th day of therapy, the animals were anesthetized by administering Ketamine 75mg/kg and Xylazine 5mg/kg (1:1) intraperitoneally. To get a blood sample, a heart puncture was carried out. A blood sample was drawn and placed in heparinized and EDTA tubes before being sent for biochemical and haematological analysis.

Groups	Acute Toxicity (No. of Rats/ Dose)	Sub-acute Toxicity (No. of Rats/Dose
Control	3	3
Group -1	3 (800mg/kg)	3 (100 mg/kg)
Group -2	3 (1200 mg/kg)	3 (200 mg/kg)

2.5 Statistical Analysis:

Statistical analysis of the collected data analysed using one way ANOVA and was representation as mean ± standard deviation. The p value is less than 0.05 was considered significant.

2.6 Biochemical and Histopathological Analysis:

Blood samples for haematological and clinical chemistry tests were taken from the infraorbital vein and placed in an EDTA-containing vacutainer tube. The automatic haematology and Biochemistry analyser (Vitreo 7600 integrated system) was used to evaluate various biochemical parameter. parameters such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean

corpuscular haemoglobin concentration (MCHC), and blood platelet count. Clinical chemistry parameters such as urea and creatinine were evaluated using an automated biochemistry analyse (Atellica IM 1600 analyser).

3. RESULTS

3.1 Acute toxicity:

3.1.1: Toxic syndrome and mortality: The animals used in the study were observed for 14 consecutive days after administering the dose and none of them showed any sign of toxicity. No mortality noted in the 14 days observation. The findings of acute toxicity study demonstrated that the poly herbal formulation was safe at 800 mg/kg and 1200 mg/kg. At the tested levels, there were no mortality or alteration in behaviour in the treated rats were noted. All observable parameters, including general appearance, grooming, posture, stride, and behaviour, was found to be normal during the study.

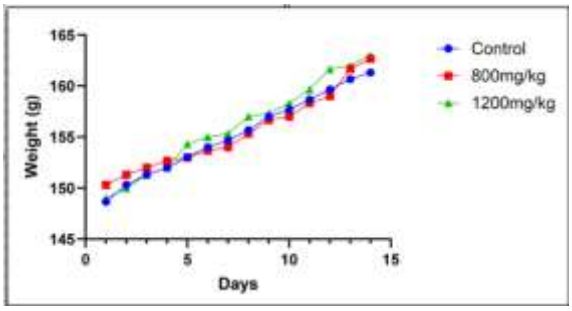

Fig 1: Effect of PHF on bodyweight of rats. There was no statistical difference between control group and different doses of PHF

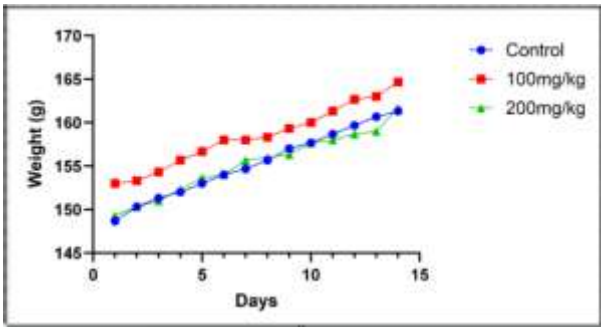

Fig 2: Effect of PHF on bodyweight of rats. There was no statistical difference between control group and different doses of PHF during subacute toxicity

3.1.2 Body Weight: Average body weight changes in the animals were monitored during the study. It is one of the important criteria that is used to assess the overall health of animal used in experiments. It was calculated by subtracting the body weight of the test animals on first day by the body weight on the fourteenth day, and then dividing the result by 14 days. Figures 1 and 2 display the average of the body weight changes. There was no discernible variation in body weight observed between the treatment and control groups.

3.1.3 Histopathological Evaluation: Histopathological examination showed no morphological variation in 14days

3.2 Sub-acute toxicity:

3.2.1 Mortality and Clinical symptoms of toxicity: No symptoms of toxicity or morality were noted during the treatment period.

3.2.2 Body weight: No abnormal change in the weight of the rats during acute toxicity tests with Polyherbal formulation were noted.

3.2.3: Haematology: Haematological variation is indicated in Table 3. There were no discernible alterations as noted compared to the control group. No treatment-related changes in these haematological markers were found by the end of the observation period.

3.2.5: Biochemical analysis: ALT, AST, GLU, TC, urea, and CRE levels did not differ between the dosage and control groups.

3.2.6: Histopathology. Histopathology analysis of organs such as brain, kidney, liver and spleen appeared normal without any distortion.

Table 1: Haematological Parameters of rats during subacute toxicity assessment

Parameters	Control	100 mg	200 mg
WBC (10*3/ul)	6.1 ± 0.21	5.9 ± 0.06	6.2 ± 0.071
RBC (10^6/ul)	6.4 ± 0.23	6.9 ± 0.05	6.3 ± 0.03
PLT (10*3/ul)	682 ± 0.34	695 ± 0.042	686 ± 0.002
Hb (mg/dl)	12.01± 0.21	13.34± 0.32	12.35± 0.32
Neutrophil	21.08 ± 0.01	22.54 ± 0.44	22.01 ± 0.22
Basophil	0.14 ± 0.01	0.13 ± 0.71	0.13 ± 0.34
Lymphocyte	76.03 ± 0.23	74.03 ± 0.03	73.03 ± 0.05
Monocyte	0.72 ± 0.31	0.71 ± 0.01	0.76 ± 0.02

There was no significant statistical difference between control group and different doses of PHF

Table 2: Biochemical Parameters of rats during subacute toxicity assessment

Parameters	Control	100 mg	200 mg
Blood Urea	29.3 ± 3.75	34 ± 2.64	31.66 ± 2.6
Creatinine	0.72 ± 0.23	1.66 ± 0.05	0.86 ±0.61
Uric acid	2.48 ± 0.96	3.33 ± 0.94	2. 66 ± 1.51
Triglyceride	69.2 ± 8.2	87.5 ± 7.72	73.8 ± 8.2
HDL	39.37 ± 10.05	32.5 ± 7.7	35.66 ± 5.6
LDL	24.02 ± 7.4	35.93 ± 8.1	28.26 ± 4.9
SGOT	38 ± 8.76	47 ± 8.5	48.33 ± 9.50
SGPT	67.8 ± 7.3	75 ± 9.6	85 ± 6.45

There was no significant statistical difference between control group and different doses of PHF

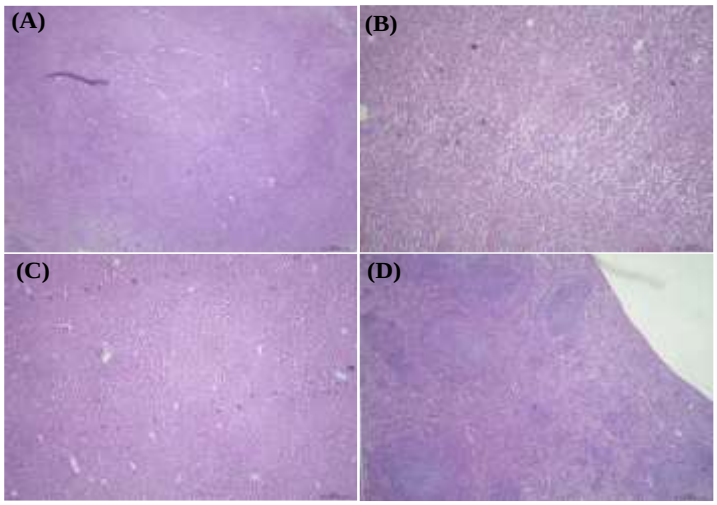

Figure. Histopathology analysis at 10X magnification (A) Brain (B) Kidney (C) Liver (D) Spleen

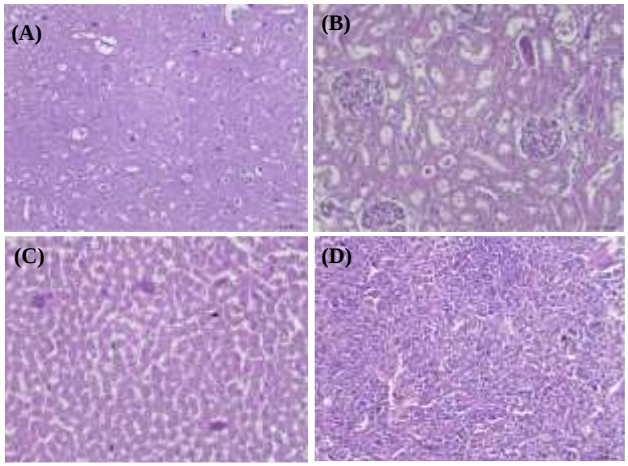

Figure. Histopathology analysis at 20X magnification (A) Brain (B) Kidney (C) Liver (D) Spleen

4. DISCUSSION

Natural products, especially medicinal plants, have long been the mainstay of treatment for a variety of illnesses and conditions. This study tried to determine the effect of combination of several herbal ingredients. In the traditional medicine systems, numerous plant species have been documented for their therapeutic efficacy in management of Diabetes Mellitus. The concept of polyherbal formulations is well-established in ancient medical texts, with evidence supporting their enhanced therapeutic potential. Polyherbal formulations, when compared to single-plant treatments, offer a broader and more sustained range of therapeutic effects due to the synergistic interactions[4]. *Gymnema sylvestre* (Asclepiadaceae), commonly known as "Gudmar," is an herb with significant pharmacological properties, particularly recognized in Ayurvedic medicine for its sugar-destroying effects. The key bioactive compounds found in laves of Gudmar is responsible for its sweet-suppressant activity are triterpene saponins, including gymnemic acids and gymnemasaponins, along with a polypeptide called gurmarin. G. sylvestre demonstrates a broad spectrum of therapeutic benefits, including the management of diabetes, arthritis[5]. *Withania coagulans*, belonging to the Solanaceae family, is an important plant in traditional Ayurvedic medicine, commonly known as "Paneer ke phool". It is a small herbaceous plant native to parts of India and has been traditionally used for various medicinal purposes, particularly in the treatment of conditions like diabetes, inflammation, and reproductive health issues [6]. *Berberis aristate* (Indian Burberry) commonly known as Daru haldi is a medicinal plant that has been traditionally used in Ayurvedic and other traditional medicine systems. It is well-regarded for its wide array of therapeutic effects, particularly its role in managing metabolic disorders like diabetes. The primary bioactive compounds in Berberis aristata include berberine, berberrubine, berberastine, and berberine alkaloids, which are largely responsible for its medicinal properties[7]. *Cassia fistula L.*, commonly known as the Golden Shower (Amaltas in hindi), is a deciduous, medium-sized tree renowned for its bright yellow flowers and elongated, rod-shaped fruits that contain pulp. This plant is widely used in various traditional medicinal systems, including Ayurveda and Traditional Chinese Medicine, for its diverse therapeutic properties. Its medicinal potential is significant, especially for managing conditions like diabetes [8]. *Syzygium cumini Linn*., commonly known as "Jamun" or black plum, is a tropical fruit-bearing tree native to the Indian subcontinent [9]. It has long been utilized in traditional medicine, particularly in the Unani system of medicine (USM), where it is recognized for its therapeutic benefits, especially in the management of diabetes mellitus and other metabolic disorders. including alkaloid like Jambosine and Jamboline, flavonoid, tannin, anthocynins, saponins and glocosides [10]. Assessing and evaluating the toxic characteristics of an herbal product extract is typically the first step in screening herbal formulation for pharmacological activity. The current study assessed the acute and subacute toxicity of a polyherbal formulation. In the acute toxicity 3 dosages of polyherbal formulation (800 and 1200 mg/kg b.w.) were given to test animals. There were no notable changes observed in the central nervous system, the skin and hair, mucous membranes, eyes, somatic motor system, autonomic nervous system, digestive system, and organs throughout the study. Mortality is a crucial parameter for toxicity evaluation. Animals in this investigation did not die or develop any toxic symptoms after receiving a single dose of polyherbal formulation up to 5000 mg/kg bodyweight. The findings of the acute toxicity test showed that in SD rats, the estimated tolerated dose of polyherbal formulation was higher than 5 g/kg. According to the OECD, the LD50 of polyherbal formulation found to be unclassified. In order to ascertain the impact of the polyherbal formulation on animal growth, mean body weights were measured. Nutrition from food and water intake is directly correlated with body weight. Stressors may also have an impact on body weight. []. One-way ANOVA statistical analysis revealed no significant differences between the groups ($P > 0.05$), suggesting that polyherbal formulation administration had no effect on the average body weights of all the animals. There was no discernible change on the amount of food and drink consumed. All groups saw variations in their meal and water intake. Individual energy requirements and environmental variables like temperature or light cycle may have an impact on food consumption [11]. The food intake only increases to keep its body temperature steady if the outside

temperature is lower than its typical temperature. High temperatures, stress, poor housing conditions, and humidity all contribute to a drop in food consumption. The state of the housing is an external element influencing water consumption. High humidity and temperatures will reduce the amount of water used. The most important indicators for assessing a substance's toxicity in both human and animals are haematological analyses. Haematological parameters were not affected by the product, as seen by the lack of significant differences between the dose and control groups. In clinical practice, biochemical measurements are also the noted primary diagnostic standards. It might reveal the negative impact if any the substance has in animal as well as human. Biochemical analyses play a very significant role to identify, and describe the harmful effects that toxic substances contain. The parameters used to assess kidney injury are UREA and CREA levels. Marker values like as ALT, AST, and TP can be used to assess liver function. The kidney and liver, which help in the removal of xenobiotics, are delicate organs that can be impacted by chemicals, including medications and plant extracts[12]. When compared to the control group, the polyherbal formulation had no effect on the levels of UREA, CRE, AST, ALT, TP, TC, and GLU. These findings showed that the polyherbal formulation had no effect on the liver's or kidney's functions. The histological investigation validated the findings of the biochemical analyses. The goal of a histological investigation is to find any anomalies in the organ and gross pathology [13]. There were no microscopic alterations in the lung, heart, kidney, liver, stomach, intestine, or lymph, according to the histological analysis of the essential organs.

5. CONCLUSION

This study examined polyherbal formulations toxicological profile. The result from the study showed that the poly herbal formulation was non-toxic to Wistar rats at administered doses. The clinical appearance, mean body weights, food and water intake, haematological parameters (WBC count, RBC count, platelet count, MCH, MCHC, MCV, Hb, and HCT), clinical biochemistry (AST, ALT, TP, TC, and GLU), urinalysis, relative organ weights, gross necropsy findings, and organ histopathology were all unaffected by the daily administration of polyherbal formulation for 14 days.

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