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# ACUTE AND SUB-ACUTE TOXICITY STUDIES OF POLYHERBAL POWDER TO AMELIORATE THE EFFECTS OF DIABETES MELLITUS AND ITS COMPLICATION

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## ABSTRACT

Due to the development of numerous complications such as vascular damage, neuronal damage, nephronal damage, and retinal damage, both IDDM and NIDDM are linked to increased mortality and morbidity. The aim of the present study is to assess the acute and sub-acute toxicity of ethanolic PP extract. In the acute toxicity, at a dose of 500 , 1000 mg/kg was administered to wistar rat which was then observed for physical symptoms and behavioral changes. In sub -acute toxicity repeated doses of the polyherbal powder were administered to wistar rat. The animals received three doses of polyherbal powder (500 mg/kg, 750 mg/kg 1000 mg/kg , 2000 mg/kg ) for the period of 28 days. On 29th day of experiment, blood sampling of animals was done for hematological and biochemical analysis. There was no morbidity and mortality with single dose administration in acute toxicity study in rat. In sub -acute toxicity there was change in the body weight of the treated rat when compared to control rat, there was morphological changes in liver, kidney, stomach of the treated rat was recorded at a dose of 2000 mg/kg. From the data obtained in this study, it can be concluded that though LD50 is greater than 1500mg/ kg b.w. but moderate toxicity signs appeared in liver, kidney, stomach at limit dose.

**Keywords:** -Toxicity study, Polyherbal Powder, Diabetes Mellitus

## Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, as well as changes in lipid, carbohydrate, and protein metabolism and a higher risk of vascular complications [1]. It is one of the most serious public health issues that has now turned into a global epidemic [2]. Polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision are all symptoms of marked hyperglycemia [3]. As reported by International Diabetes Federation (IDF), approximately 75–80% of people with diabetes die due to cardiovascular complications [4]. Its global prevalence was around 8% in 2011 and is expected to increase to 10% by 2030 [5]. According to estimates, India will have the highest number of diabetics by 2025 in the entire world [6]. The global population of diabetic patients is rapidly increasing, with India leading with 50.8 million diabetics; a WHO prediction stated that developing countries would be affected by the epidemic in the twenty-first century; the population predicted to be affected by this syndrome by 2010 was 285 million people (6.4 percent of the world's adult population) and expected to increase to 438 million by 2030

(7.8% of the world's adult population) [7]. According to a survey conducted by the International Diabetes Federation (IDF) in 2016, diabetes is a disease that affects 415 million people worldwide, with that number expected to rise to 642 million by 2040. According to Aroma World, 61.3 million people in INDIA have diabetes, with the 20-79 age group accounting for the majority of the population. By 2030, it may have more than doubled. INDIA is known as the world's diabetes capital, and it primarily affects rural and urban populations [8]. According to the Atlas guideline report published by the International Diabetes Federation (IDF), there are currently 352 million adults with impaired glucose tolerance who are at high risk of developing diabetes in the future. In 2017, 425 million people (20–79 years old) were estimated to have diabetes, with that number expected to rise to 629 million by 2045 [9].

Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are the two types of diabetes, with T2DM accounting for nearly 95% of cases [10]. Type 2 diabetes affects 2.4 percent of India's rural population and 11.6 percent of its urban population. Type 1 diabetes is an autoimmune disease in which the pancreas β-cells fail to produce enough insulin, a hormone that aids in the utilization of blood sugar (glucose) for energy [11]. According to the World Health Organization (WHO), more than 180 million people worldwide have diabetes. T2DM is expected to become pandemic, with the number of people with the disease increasing from 171 million in 2000 to 366 million by 2030. T2DM is the most common type of diabetes, accounting for 90% of all diabetes cases worldwide [12]. In South-East Asia, India accounts for the majority of T1DM children. According to the 6th edition of the International Diabetes Federation's diabetes atlas, India has three new cases of T1DM per 100,000 children aged 0 to 14 [13].

Diabetes can affect a variety of organ systems in the body, leading to serious complications over time [14]. Diabetes complications can result in damage to the eyes (retinopathy) leading to blindness. Nephropathy leads to renal failure and neuropathy leads to impotence and diabetic foot disorders (which include severe infections leading to amputation) [15]. It has been reported that the prevalence of diabetic complications is neuropathy 42.6%, cardiovascular diseases 23.6%, nephropathy 21.1%, retinopathy 16.6%, and foot ulcers 5.5% [16]. The incidence of diabetic complications is uncommon. CHD, peripheral vascular disease, CVA, retinopathy, cataract, and neuropathy were found to be prevalent in 32.3 percent, 11.5 percent, 6.9 percent, 15.4 percent, 20 percent, and 60 percent of diabetics, respectively. DM was found to be significantly associated with a variety of complications. Diabetics had a higher prevalence of coronary heart disease (32.3 percent) than non-diabetics (3.3 percent) [17].

Synthetic drug treatment of diabetes is frequently associated with negative side effects such as gastrointestinal reactions, weight gain, blood glucose fluctuations, increased risk of cardiovascular disease, and frequently fails to correct serious biochemical disorders and diabetic complications [18]. Despite the intense interest in developing new drugs to alleviate the disease's burden, the scientific community has focused on evaluating raw or isolated natural products in experimental studies; only a few have been tested clinically in humans [19]. Substitute to these synthetic agents' various herbal plants with hypoglycemic assets are identified since crosswise the planet. 21,000 plants have been listed by the World Health Organization (WHO), which are utilized for therapeutic rationale around the world [20]. Even though herbal drugs with antidiabetic activity have been praised for their therapeutic properties in traditional medical systems, they have yet to be commercially formulated as modern medicines [21]. Polyherbal formulations are those that contain multiple ingredients

from various herbal sources. Polyherbal formulations are primarily used to boost the activity of compounds derived from other plants or to counteract their toxic effects. Due to the presence of multiple ingredients, these formulations may have a synergetic effect. Different active constituents with different mechanisms of action are found in polyherbal formulations, which can produce a combined action against various diabetes complications [22].

## Materials and Methods

### Experimental section

### Collection and authentication of plant materials

Fresh barks of *Cinnamon zeylanicum*, leaves of *Withania somnifera*, the stem of *Tinospora cordifolia*, and seeds of *Trigonella foenum graecum* were procured and collected from the local market. The collected herbs were washed and air-dried. All of them were ground in a mixer separately. Then the grinded powder was mixed in equal quantities. It was then passed through a sieve (Sieve No.120) to obtain a fine powder

### Extraction of polyherbal powder

The extraction of PP was done through the Soxhlet apparatus. The powder was placed in a thimble (made of filter paper) and place into the extractor, the successive solvent used for extraction was ethanol 500 ml and temperatures were set to 45 -50ºC. The whole extraction run for 5 cycles the obtained extract was used for further analysis.

### Experimental Animals

Wistar rats (both sex), 9–12 weeks old weighing about 150–200 g was used in this study. The animals were maintained under standard environmental conditions (23–25 °C, 12h/12h light/dark cycle) and had free access to a standard pelleted diet, water ad libitum. Animals were acclimatized to the laboratory environment for a week before starting the study. The protocol used in this study was approved by the Institutional Animal Ethical Committee, CPCSEA New Delhi.

### Acute Toxicity Study

The acute toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) revised fixed-dose procedure for acute toxicity testing (OECD guideline 423). For acute toxicity studies, the rat was used for the study. The rat was divided into 2 groups containing 3 animals. The rat fasted overnight and the drug was administered orally. The rat was administered a limit dose of 500 and 1000 mg/kg of the PP ethanolic extract and the rat were observed for mortality and clinical signs for the 30 min, 4 hrs, 24 hrs than 1 week for changes in body weight, skin, and fur, eyes, and mucus membranes, behaviour pattern, tremors, salivation, diarrhea, sleep, coma, mortality, moribund, ill-health or any visible reaction to treatment. The rat was maintained under close observation for up to 14 days, and the number of rats dead within the study period was recorded.

$LD_{50}$ DETERMINATION: -

| Exp | Dose | Dose differences | No of animals | No of dead animals | Mean mortality | DD×MM |
|---|---|---|---|---|---|---|
| **1.** | 500 mg/kg | 0 | 3 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **2.** | 750 mg/kg | 250 | 3 | 0 | 0 | 0 |
| **3.** | 1000mg /kg | 200 | 3 | 0 | 0 | 0 |
| **4.** | 2000 mg/kg | 1000 | 3 | 3 | 1.5 | 1500 |

Mean mortality =Differences of two adjacent no. of dead animals /2

$\sum (DD \times MM)$=150/3

= 500

Finally, this value is subtracted from the minimum dose which produce the 100% mortality, i.e.,2000 mg/kg

$LD_{50}$ =2000-500 = 1500 mg/kg.

**Sub- Acute Toxicity Study**

The sub-acute oral toxicity study was carried out as per OECD guidelines OECD-407 For sub-acute toxicity studies, rats were divided into 2 groups of 3animals each. The rat fasted overnight and the drug was administered orally. The PP ethanolic extract at the dose of 250, 500,750mg /kg b.w., was administered once daily for 28 days. The parameters focused on were body weight, and, hematological parameters (WBCs, RBCs, PLT, Hb, HCT, MCH, MCV, and MCHC), liver function parameters (Bilirubin, ALT, AST), renal function (urea, uric acid, and creatinine).

**Hematological**

At the end of the experiment, the rat was euthanized, blood samples were collected through the retro-orbital sinus and placed in $K_2$EDTA containing tubes, various parameters were evaluated at Vet diagnostic lab lucknown. The hematological parameters like, red blood cell (RBC) count, hemoglobin (Hb) levels, the proportion of lymphocytes (LY), monocytes (MO), granulocytes (GR), and platelet count (PLT) were all measured [23].

**Serum Biochemical Parameters**

The serum separated was analyzed at Vet diagnostic Lab, Lucknow. The following enzymes were measured: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum albumin, bilirubin, total protein (liver), and urea, uric acid, and creatinine, phosphorus, calcium, potassium (kidney) [24].

**Histopathology study**

The rat was sacrificed on day 29$^{th}$ under ether anesthesia. Necropsy of a rat was carried out and the weights of the organs including liver, kidneys, stomach, heart, were recorded. Tissue samples of organs rat were preserved in 10% formalin for histopathological study. The histopathological study was performed at Vet Diagnostic Lab, Lucknow [25].

**Results**

The experimental animals were examined for any clinical signs, and other behavioral parameters. In the present study ethanolic extract of PP in rat at a dose of 500, 1000 mg/kg b.w. had no effects on mortality, examined clinical signs, overall observation.

TABLE 2 PARAMETERS

| **Parameters** | **30 min** | | **4 hrs** | | **24hrs** | | **1 week** | | **2weeks** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control (Saline) | Experimental | Control | Experimental | Control | Experimental | control | Experimental | control | Experimental |
| Skin &fur | N | N | N | N | N | N | N | N | N | N |
| Mucous membrane | N | N | N | N | N | N | N | N | N | N |
| Respiratory rate | N | N | N | N | N | N | N | N | N | N |
| Heart rate | N | N | N | N | N | N | N | N | N | N |
| Salivation & lacrimation | N | N | N | N | N | N | N | N | N | N |
| Lethargy | N | N | N | N | N | N | N | N | N | N |
| Piloerection | N | N | N | N | N | N | N | N | N | N |
| Urinary incontinence | None | None | None | None | None | None | None | None | None | None |
| Defecation | N | N | N | N | N | N | N | N | N | N |
| Tremors &convulsion | None | None | None | None | None | None | None | None | None | None |
| Mortality | None | None | None | None | None | None | None | None | None | None |

**N= Normal , Control- Saline**

**Effect on Body weight:**

The weight of the rat was determined and recorded on days 0, 7, and 14 of the study. Weight changes were calculated. The rat treated with PP ethanolic extract have shown decrease in the body weight when compared to control rat.

BODY WEIGHT

| S.no | Group | Bodyweight | | |
|---|---|---|---|---|
| | | Day 0 | Day 7 | Day 14 |
| 1. | Group 1 | 165 ±2.35 | 160±2.35 | 160±6.23 |
| 2. | Group 2 | 150±1.88 | 165±4.71 | 150±7.01 |
| 3. | Group 3 | 155±2.35 | 165±2.35 | 150±4.08 |
| 4. | Group 4 | 175±4.71 | 150±2.35 | 165±2.35 |

**Sub-Acute Toxicity**

**Effect on Bodyweight**

Sub-acute toxicity in which rats given ASME at a dose of 500,750 mg/kg 100 mg/kg, 2000 mg /kg b.w. demonstrated significant reduction in body weight at higher dose [26]. Body weight change serves as a sensitive indication of general health status of animals [27]. In the present study the body weight changes of rats are recorded on 0 days, 7 days, 14 days, 28 days represented in table 4 and figure 2. The rat treated with PP ethanolic extract have shown s decrease in the body weight when compared to control rat.

BODY WEIGHT

| S.no | Group | Bodyweight | | | |
|---|---|---|---|---|---|
| | | Day 0 | Day 7 | Day 14 | Day 28 |
| 1. | Group 1 | 200±4.35 | 200±181.6 | 195±176.6 | 195±173.3 |
| 2. | Group 2 | 200±2.35 | 195±2.35 | 185±2.35 | 175±2.35 |
| 3. | Group 3 | 200±9.4 | 195±0.94 | 185±2.35 | 180±2.35 |
| 4. | Group 4 | 225±11.78 | 200±2.35 | 195±2.35 | 185±2.35 |

**Effect on Hematological Parameters**

Evaluation of hematological parameters is used to determine the extent of deleterious effect of the tested PP ethanolic extract on the blood-related functions in rat [28]. In the present study the evaluation of hematological parameters showed no changes in treated rat when compared with control group. The effects of subacute administration of PP ethanolic extract on hematological parameters presented in Table 5. Most hematological parameters, like hemoglobin, Total RBCs, neutrophiles, monocytes, lymphocytes in treated rat shows no differences different from the control group.

HEMATOLOGICAL PARAMETERS

| Ethanolic extract | | | | | |
|---|---|---|---|---|---|
| | Saline | 500 mg /kg | 750 mg /kg | 1000 mg /kg | 2000 mg /kg |
| Hb (g/L) | 12.6± 12.4 | 12.7±0.26 | 13.7±0.25 | 13.9±0.12 | 14.9±0.355 |
| RBC (milli/mm3) | 5.6±0.12 | 4.6±0.29 | 7.21± 0.43 | 7.09±0.43 | 6.7±0.27 |

| | | | | | |
|---|---|---|---|---|---|
| PCV (L/L) | 37.5±0.16 | 38.9±2.91 | 47.3±5.7 | 46.3±3.41 | 47±0.87 |
| MCV (fl) | 19.5±0.24 | 62.8±1.15 | 61.8±0.77 | 60.7±4.12 | 69.5±2.7 |
| MCHC(g/l) | 32.0± 0.5 | 32.5±0.96 | 35.8±0.81 | 34.3 ±1.58 | 31.7±0.12 |
| MCH (Induction pg) | 19.5±0.24 | 23.7±0.77 | 20.6±1.31 | 21.2 ±1.19 | 22±0.94 |
| RDW CV (%) | 0.115±0.0015 | 0.129±0.002 | 0.121±0.004 | 0.116±0.006 | 0.121±0.007 |
| Neutrophiles | 3.3±0.065 | 4.11±0.46 | 4.59±0.63 | 7.56±0.31 | 7.84±0.008 |
| Lymphocytes | 1.01±0.01 | 1.4 ±0.24 | 2.5±0.24 | 1.34±0.13 | 1.57±0.133 |
| Eosinophiles | 0.1±0.04 | 0.11±0.021 | 0.19±0.016 | 0.19±0.02 | 0.20±0.020 |
| Monocytes | 0.1±0.20 | 0.34±0.18 | 0.11±0.10 | 0.17±0.016 | 0.20±0.02 |

**Effect on biochemical Parameters**

Biochemical parameters are evaluated to see if PP has any effects on hepatic and renal functions. The evaluation of liver and kidney function is critical because these vital organs in the body have a variety of mechanisms for removing toxic substances from the body through the liver and kidney to reduce their toxic effects [28].

**Effect on liver Parameters**

In the present study the effect of subacute administration of PP ethanolic extract on liver parameters There were differences in serum Alkaline phosphate, Alanine aminotransferase, Aspartate aminotransferase level of treated group when compared to control group. No relevant changes were found in albumin, globulin, and total protein.

LIVER PARAMETERS

| **Ethanolic extract** | | | | | |
|---|---|---|---|---|---|
| | **Saline** | **500 mg /kg** | **750 mg /kg** | **1000 mg /kg** | **2000 mg /kg** |
| Albumin (g/dl) | 2.1±0.28 | 2.5 ± 0.12 | 2.1 ± 0.21 | 3.0 ±0.08 | 3.3±0.12 |
| Globulin(g/dl) | 2.1±0.21 | 2.5±0.08 | 2.8±0.08 | 3.1 ±0.12 | 3.3±0.12 |
| Alkaline phosphaste (U/L) | 199±5.88 | 200±27.7 | 388±20.8 | 426±3.09 | 430 ±3.68 |
| Bilirubin total (mg/dl) | 0.4±0.08 | 0.06±0.012 | 0.9±0.12 | 0.2±0.08 | 0.1±0.12 |
| Bilirubin direct (mg/dl) | 0.04± 0.016 | 0.01±0.012 | 0.03±0.08 | 0.04±0.008 | 0.05±0.008 |
| Bilirubin indirect (mg/dl) | 0.04±0.40 | 0.01±0.01 | 0.04 ±0.012 | 0.03±0.008 | 0.05±0.016 |
| Aspartate aminotransferase (U/L) | 180±8.16 | 199±5.79 | 210± 13.81 | 300 ±8.21 | 323 ±1.2 |

| | | | | | |
|---|---|---|---|---|---|
| Alanine aminotransferase (U/L) | 75±1.6 | 85±4.08 | 89±1.24 | 90 ±0.94 | 91±1.247 |
| Total protein (g/dl) | 3.4±0.16 | 4.3±0.21 | 0.19±0.016 | 0.19±0.02 | 0.20±0.020 |

**Effect on Kidney Parameters**

In the present study the effect of subacute administration of PP ethanolic extract on Kidney Parameters are presented in Table 7. The PP ethanolic extract had no effect on serum electrolytes ($Na^{+,}$ $K^{+,}$ $Cl^{-).}$ The kidney function parameters like urea, creatinine, uric acid, did not reveal any changes in treated group as compared to control group as depicted in figure 6,7 and Blood urea nitrogen and Sodium , chloride also do not reveals any changes

KIDNEY PARAMETERS

| **Ethanolic extract** | | | | | |
|---|---|---|---|---|---|
| | **Saline** | **500 mg /kg** | **750 mg /kg** | **1000 mg /kg** | **2000 mg /kg** |
| Blood Urea (mg/dl) | 18 ±0.047 | 27.4± 0.49 | 40.2± 0.50 | 55.6 ±0.75 | 57.4±0.8 |
| Blood urea nitrogen (mg/dl) | 23.8± 0.47 | 24.3 ±0.47 | 25.5±0.63 | 25.8 ±1 | 26.8±0.3 |
| Uric acid (mg/dl) | 1.7 ± 0.21 | 2.3 ±0.14 | 2.8 ±0.12 | 3.0 ±0.16 | 3.3±0.26 |
| Calcium(mg/dl) | 7.6 ± 0.77 | 8.7± 0.49 | 9.1 ±0.16 | 10.5±0.12 | 10.8±0.12 |
| Phosphorus (mg/dl) | 8.5 ±0.81 | 8.7 ±0.26 | 8.9 ±0.12 | 9.1 ± 0.12 | 9.8±0.12 |
| Sodium (mE/ql) | 140.2± 5.91 | 143.1±4.97 | 150.1±5.33 | 150 ±8.4 | 156.0±0.24 |
| Potassium (mE/ql) | 4.5±0.54 | 4.5±0.20 | 4.9± 0.12 | 4.9±0.12 | 5.2±0.081 |
| Chloride (mE/ql) | 110.2±3.81 | 110.1±4.21 | 113.3±2.223 | 110.3 ±1.75 | 11.4±0.32 |

**Histopathological**

The microscopic examination of liver tissue of normal control rats showed normal histological features. While the rat treated 2000 mg/kg PP ethanolic extract showed marked pathological changes in liver showing severe congestion of portal and degeneration of hepatocytes and mild fatty acid changes. The microscopic examination of kidney tissue of normal rat shows normal histological features. while the rat treated with 2000 mg/kg PP ethanolic extract shows the presence of mild browman's atropy, moderate to severe acute proximal tubular necrosis as depicted in figure 11.The microscopic examination of stomach tissue of normal rats showed normal histological features, while the rat treated rats with 2000 mg/kg of PP ethanolic extract shows Desquamation of gastric mucose leading to erosion of gastric wall Histopathological examination of rat heart tissue shows normal appearance of cardiac fibres and no evidence of necrosis or myocardial damage .

**Discussion**

In recent years, diabetes and its complications have become devastating health problems that affect millions of people around the world. Although synthetic drugs are capable of treating diabetes to some extent, unfavorable side effects have overshadowed their popularity [28]. Due to their low risk of side effects, herbal medicines and their formulations have been thought to be safe and effective for centuries. This assumption may have had a significant impact on the rural population's indiscriminate use of these formulations. These formulations are commonly given over a long period of time without expert dosage monitoring or

knowledge of the potential toxic effects of such prolonged use. As a result, scientific understanding of oral toxicity is critical, as it will not only aid in the identification of future doses, but also reveal the potential clinical signs elicited by agents under investigation [27]. Polyherbal formulations are widely used in developed countries for the treatment of a variety of ailments when compared to allopathic medicine [24]. Despite the fact that medicinal plants are widely used for health care in developing countries, scientific data on their toxicity and side effects is scarce. Several studies have discovered that some medicinal plants can have a variety of toxic effects that are potentially harmful to human health. To ensure the safety of plant products for human use, systematic studies are required to predict toxicity risks and provide scientific information for selecting safe doses in humans [29]. The FDA and WHO emphasize the importance of conducting scientific studies to validate the efficacy and safety of herbal therapies. To ensure the safety of herbal medications, a preliminary toxicological evaluation is required [30]. Natural products, such as medicinal plants, have been used to treat a variety of diseases for hundreds of years. Despite being safe drugs, herbal preparations must be validated through various toxicity studies before being consumed [31]. Acute toxicity is a short-term assessment and evaluation of a test substance's potential hazard or the effects of a single dose of a test substance. The assessment and evaluation of the toxic characteristics of a natural product extract or compound is usually the first step in screening natural products for pharmacological activities. Regardless of PP pharmacological benefits, there is a scarcity of information about the poisonous effects of this well-known PP. As a result, the current study was carried out in rats to assess and focus on the acute and subacute toxicity of PP [32]. Acute toxicity testing can be used to assess the risk of chemicals to humans and non-target organisms in the environment. Acute toxicity studies are better known as LD50 studies, which are defined as the dose that kills 50% of animals [33]. Acute toxicity showed that PP did not cause any change in animals throughout the experimental period of 14 days. The toxic effect compromised changes in liver function leading to alterations in the normal physiological function and weakening of the immune system of animals. There was no change in hematological parameters, kidney functions. Wistar rats are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed [34]. All observations are systematically recorded with individual records being maintained for each rat. Observations include changes in body weight, skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma [35]. In the present study the ethanolic extract of PP shows slight decrease in the body weight of the rat as shown in table 3 .The ethanolic extract of PP also show no change in skin , eyes , fur and mucous membrane as shown in table 2 As per the literature MTE (Ethanolic extract of *Marsdenia tenacissima* leaves )at a dose of 5000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation [27] .In the present study the PP extract at a dose of 500 , 1000 mg/kg has no adverse effects on the treated rats in up to 14 days observation.

Subacute toxicity is a repeat-dose study used to reveal any harmful changes in organ, hematological, and biochemical indices that may occur after repeated administration of a test substance. The study usually lasts weeks to months [36]. As per the literature review the sub-acute toxicity study showed that repeated administration of PHF up to 28 days didn’t produce any clinical signs of toxicity or death [37]. In the present study the PP ethanolic extract shows

no clinical signs of toxicity or death and there was slight decrease in the body weight of the rat.

Clinical biochemistry and hematological parameters are important in detecting drug-induced toxicity [38]. The liver is an important organ that is involved in drug biotransformation. Serum liver biomarker enzyme levels are biochemical parameters that are commonly used to assess any toxic effects on the liver. Increases in serum Aspartate aminotransferase and Alanine aminotransferase levels are linked to liver toxicity caused by drugs or any other hepatotoxin [39]. The standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10–55 U/L), Aspartate aminotransferase (10–40 U/L), and Alanine aminotransferase (45–115 U/L) [40]. In the present study the the ethanolic extract of PP shows slight changes from the normal value. The value of Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase are 91 U/L, 323 U/L, 420 U/L at a dose of 2000 mg/kg. In kidney damage, there is build-up of nitrogenous metabolic substances such as blood urea nitrogen and serum creatinine. Creatinine and urea concentrations were normal and this is an indication of how well the integrity of the kidney cells well preserved [41]. In the present study the Ethanolic extract of PP shows no changes in level of creatinine and urea level and all other parameters show no changes.

The analysis of hematological parameters reveals that the extract has toxic effects on the blood of rats due to physiological or pathological changes. Blood is the only way for nutrients and foreign bodies to get around the body. If an extract causes toxicity in the body, it affects red blood cells, white blood cells, platelets, and hemoglobin directly. These components' ranges either decrease or increase significantly when compared to normal. It was discovered that the extract's toxicity can affect the body's immune system as well as organ function [42]. When toxic doses of chemical compounds are administered, blood parameters often change, indicating hematological disorders such as anaemia (low hemoglobin count) [43] .In the present the ethanolic extract of PP at a dose of 2000 mg/kg shows no changes in the blood parameters.

The liver and kidneys are two of the body's major internal organs that serve a variety of functions. Only serious diseases show symptoms of disorder in those organs. Clinical blood chemistry tests were performed on female and male rats to see if the substance destroys and impairs liver and kidney functions [44].

The presence of a pro-inflammatory substance in the tissues and cells of the liver, lungs, and kidneys was confirmed by histological analysis of the liver, lungs, and kidneys, confirming the hypothesis that a different toxin is present in these extracts [45]. As in the present study the ethanolic extract of PP art a dose of 2000 mg /kg the histopathological analyses shows the presence of congestion of portal and degeneration of hepatocytes and mild fatty acid changes.

The effect of the test substances on the cellular architecture of the kidney revealed that the female control has good histoarchitecture, distinct proximal and distal tubules, and distinct vascular and urinary poles, with few additional mitotic cells [46]. As in the present study the ethanolic extract of PP at a dose of 2000 mg/kg the histopathological analysis shows the presence of mild bowman’s atrophy, moderate to severe acute proximal tubular necrosis.

Histopathological studies serve as supporting evidence for hematological and biochemical analyzes. In the present study, the histological evaluation performed in the subacute test

showed that animals treated with EE (ethanolic extract) or FA (alkaloid fraction) from *A. nitidum*. did not show changes in color, shape, size, and texture of the heart, when compared to matched control groups [47]. As per the literature in both the control and DC (*Dracaena cinnabari*) treated female and male rats, the heart shows normal cardiac muscle fibers [48]. In the present study the ethanolic extract of PP at a dose of 2000 mg /kg. The present study of the ethanolic extract of PP shows at a dose of 2000 mg /kg shows s normal appearance of cardiac fibres and no evidence of necrosis or myocardial damage seen.

As per the literature Sections of the stomach from both sexes of rats showed normal findings in the control treated groups. In the extract treated group, female rats showed normal cellular architecture with normal mucosa, submucosa, muscularis externa and serosa, whereas in male rats mild polyp formation or hyperplastic changes were observed [49]. In the present study the ethanolic extract of PP at a dose 2000 mg /kg shows Desquamation of gastric mucose leading to erosion of gastric wall.

**Results**

In the light of these findings, we may conclude that PP ethanolic extract is not toxic in all doses studied herein and did not produce any evident symptoms in the acute and subacute oral toxicity studies. Furthermore, the data of acute and subacute toxicity studies of PP were obtained in order to increase the confidence in its safety to humans for the use in the development of pharmaceuticals. No death or signs of toxicity were observed in rat treated with extract at doses 500 and 1000 mg/kg body weight, thus its safety in use. The present Acute and sub-acute toxicity results suggest that LD50 of developed formulation was >1500 mg/kg. Further studies on long term toxicity and clinical trials may be rational to substantiate the study results.

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