Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG

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**Abstract**

**Background:** *S. munja* roots have been used in ethno medicines for the treatment of different ailments. Despite its beneficial uses no studies on its toxicity potential have been reported.

**Objective:** The study was designed to evaluate acute toxic potential of aqueous ethanolic extract of *S. munja* roots according to OECD TG No. 425.

**Material and methods:** Female mice were divided into two groups (n = 5). One group served as control while the other as treated group that received 2000 mg/kg b.w. of *S. munja* roots ethanolic extract orally. Then both groups were observed for 14 days. Then the blood samples were collected by cardiac puncture, under chloroform general anesthesia and were subjected to hematological and biochemical analyses. The vital organs of anesthetized animals were preserved for histopathological examination.

**Results:** The data revealed that LD₅₀ of the extract was greater than 2000 mg/kg b.w. There was no significant alteration found in body weight and organ to body mass index. In comparison with control group, there was significant increase in levels of ALT, AST, total proteins, globulin levels, serum urea, cholesterol, triglycerides, LDL, platelet count, MCV, MCH, WBC count and lymphocytes whereas ALP and MCHC levels were reduced significantly.

**Conclusions:** From the data obtained in this study, it can be concluded that though LD₅₀ is greater than 2000 mg/kg b.w. but moderate toxicity signs appeared in liver, kidney, lipid profile and CBC also showed blood dyscresias at limit dose.

1. Introduction

Before the development of synthetic or semi-synthetic medicines, folklore use of herbals was very common in rural areas, the use of herbal preparations for the treatment of various ailments is still very common [1]. According to a survey reports in United States (US), herbal medicines were used by almost 12% of the population in 1993 as an alternative and complementary source which is significantly higher than 1990’s report [2,3]. Since natural herbal remedies are being used on large scale, it is now the major focus of the researchers to conduct studies on efficacy and safety of medicinal plants [4]. The plants having medicinal activity should have low toxicity because of their long-term use in humans. However, various medicinal plants used in folklore medicines have been reported to exhibit toxic effects [5,6]. Paracelsus, known as father of toxicology, has given a statement which is often quoted: “All substances are poisons; there is none which is not a poison. It is the right dose which differentiates remedy from poison” [7]. A large number of modern medicines are produced from the natural sources. Out of them many preparations rely on the use of agents in traditional medicines [8].

*Saccharum munja* (*S. munja*) is the common name of a wild grass found in Pakistan, Afghanistan and India along the river banks and in arid areas [9]. The plant is commonly known as sarkanda, Kana or moonja. The flowering and fruiting season is on annual basis which starts from October and ends in January. The buffalos and cattles use the young leaves of the plant as a fodder because of the large tufted grass [9–11]. The roots of the plant are used to treat dysuria, giddiness and vertigo. Fever and inflammation is also treated with its roots. *S. munja* grass is used in the form of gauze-pad to stop blood flow [12].

2. Materials and methods

2.1. Collection of plant material

*S. munja* was collected from suburbs of Southern Punjab- Pakistan. After identification and authentication by a taxonomist Prof. Dr.

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Zaheer-ud-Din Khan, Botany Department, Government College University, Lahore- Pakistan, a voucher specimen (Ref. No. 2937) containing leaves, flowers and roots was deposited to herbarium. The roots were separated, cleaned and washed with tap water and dried under shade for seven days. The dried roots were ground to fine powder.

2.2. Preparation of crude extract

Roots powder was soaked in 70% ethanol and 30% distilled water for seven days. Extract was filtered through muslin cloth and then passed through whatman’s filter paper for getting clear filtrate. The excess solvent was removed with rotary evaporator at 40 ℃.

2.3. Approval from animals ethics committee

The study was performed after getting approval from Animals Ethics Committee of Riphah International University. Ref. No. REC/RIPS/2017/002.

2.4. Acute toxicity assay

In accordance to OECD Test Guidelines 425 (Up and Down Procedure), nulliparous and non-pregnant female albino mice, weighing 28 ± 4 g having age 8–10 weeks were randomly selected. Animals were kept under standard conditions for five days. Limit test was performed at 2000 mg/kg p.o. as single dose and mice were kept without food for 3–4 h prior to dosing but had access to water ad libitum. The dose was administered to a single female mice according to body weight. The animals were closely observed for first 30 min, then for 4 h. Food was provided after 1–2 h of dosing. After survival of treated mouse, 4 additional mice were administered with the same dose under same conditions. The same procedure was followed for vehicle treated group of 5 mice to whom 1% Carboxymethyl cellulose (CMC) gel was administered in same volume as that of treated group. Both the groups were observed closely for any toxic effect within first 6 h and then at regular intervals for a total period of 14 days. Surviving mice were observed to determine the toxic reactions onset. Weights of animals were monitored and documented as well. At the end of study, animals were weighed and blood samples were collected by cardiac puncture under anesthesia with isoflurane and serum was separated for biochemical and hematological evaluations. Vital organs were excised after killing mice by cervical dislocation; weight of organs was noted and preserved in 10% formalin for histopathological evaluation.

2.5. Biochemical analysis

Urea, creatinine, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate, total protein, albumin, globulins were measured by using Randox kits.

2.6. Hematological analysis

The blood samples from animals (both treated and vehicle control groups) were collected in EDTA containing tubes for hematological study. CBC parameters, hemoglobin (Hb), total RBC, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cells (WBC) count, neutrophils (N), lymphocytes (L), monocytes (M), and eosinophils (E) were determined with humalyzer.

2.7. Histopathological study

The vital organs isolated from sacrificed mice were fixed in 10% formalin, then after processing embedded in paraffin wax. Paraffin sections were made at 5 mm and stained with hematoxylin and eosin. The slides were studied under a light microscope and captured the magnified images of tissues structure for further study.

2.8. Statistical analysis

Experimental results were presented as mean ± SEM and the statistical significance between the groups was analyzed by means of one way ANOVA followed by Tukey’s multiple comparison test. P ≤ 0.05 was considered as statistically significant.

3. Results

When limit test was conducted with dose of 2000 mg/kg b.w. of S. munja roots extract by using 1% CMC gel as a vehicle, no mortality was observed. Test animal was observed with special attention for first 30 min and then for 4 h. Observations were recorded at regular time intervals throughout the study period i.e. 14 days. All results are as follows:

3.1. Behavioral pattern and body weight

The body weights of test animals of both control and S. munja roots extract treated groups were increased progressively throughout the study period as showed in Table 1. Behavioral observation of the test animals after dosing showed elevated respiration rate for first 30 min in the extract treated group and also an increase in somatomotor activity was observed for first 2 h in this group. Drowsing and sleepy effects were noted in both groups at intervals for first 4 h. Convulsions and tremors were observed in the extract treated group frequently in first 4 h and then at intervals for 48 h, itching and shivering were also noticed. Sometimes itching in both groups was observed in first week of this study. Behavioral observations are summarized in Table 2.

3.2. Organ to body weight index

No lesion was found on examination of isolated vital organs such as heart, kidney and liver from testing animals. Organ to body weight index was calculated and summarized in Table 3 which shows that there was no significant variation present among the groups.

3.3. Biochemical analysis

No change in serum creatinine level was observed whereas serum urea level in acute toxicity group was higher when compared to vehicle control group (Table 4). There were significant (p < 0.05) changes in biochemical markers of liver function test as summarized in Table 5. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and globulin levels were raised while alkaline phosphatase decreased in acute toxicity group as compared to vehicle control group. No significant changes were observed in total bilirubin, albumin and

| Table 1 | Effects of the extract on body weight of mice in acute toxicity study. |
|---------|-----------------|-----------------|-----------------|-----------------|
| Groups  | 1st Day Body Weight (gm) | 7th Day Body Weight (gm) | 14th Day Body Weight (gm) |
| Vehicle control | 26.11 ± 0.495 | 27.68 ± 0.590 | 28.76 ± 0.691 |
| 2000 mg/kg SMRE | 25.94 ± 0.624 | 27.57 ± 0.575 | 29.32 ± 0.690 |

SMRE: S. munja roots extract; Values are presented as mean ± SEM; N = 5.
albumin to globulin ratio among the groups. It was observed that when the extract treated group at limit dose was compared with vehicle control group, H.D.L, V.L.D.L and cholesterol to H.D.L ratio among the groups have no variations whereas there was significant (p < 0.05) increase in cholesterol, triglycerides and L.D.L (Table 6).

3.4. Hematological analysis

The data in Table 7 presents the hematological potential of the extract.

| Parameters | Unit | Vehicle Control group (SMRE 2000 mg/kg) | Acute Toxicity Group (SMRE 2000 mg/kg) |
|------------|------|----------------------------------------|----------------------------------------|
| Hb         | g/dl | 11.8 ± 0.075                           | 12.8 ± 0.121                           |
| Total RBC  | x10^12/l | 7.45 ± 0.173                         | 7.91 ± 0.058                           |
| HCT        | %    | 33.7 ± 0.693                           | 42.91 ± 0.577                          |
| MCV        | Fl   | 45.2 ± 0.035                           | 54.1 ± 1.155                           |
| MCHC       | g/dl | 35 ± 0.254                             | 29.9 ± 1.193                           |
| Platelet Count | x10^12/l | 245 ± 0.269           | 245 ± 0.269                           |
| WBC Count (TLC) | x10^9/l | 3.2 ± 0.115                     | 5.16 ± 0.208                           |
| Neutrophils | %    | 10 ± 0.058                             | 10.23 ± 0.052                          |
| Lymphocytes | %    | 86 ± 2.309                             | 88 ± 0.745                             |
| Monocytes  | %    | 3 ± 0.144                              | 4 ± 0.098                              |
| Eosinophils | %    | 1 ± 0.115                              | 2 ± 0.040                              |
| MCH        | Pg   | 16 ± 0.577                             | 16.2 ± 0.017                           |

SMRE = S. munja roots extract; CMC 1%gel = 1% Carboxymethyl cellulose gel; Values are presented as mean ± SEM. * p < 0.05 when compared with the vehicle control group.
hematological profile. It can be seen that no remarkable alterations in levels of Hb, total RBC, MCH, neutrophils, monocytes and eosinophils when compared to vehicle control group. Nevertheless there were significant (P < 0.05) elevations in levels of HCT, MCV, MCHC, platelet count, WBC count and lymphocytes in comparison with normal control (Fig. 1).

4. Discussion

Medicinal plants are being used since centuries to treat different diseases [13]. Phytotherapy is gaining popularity as WHO encourages the appropriate ethnomedicinal use and signifies safety evaluation of herbal medicines [14–17]. FDA and WHO emphasize the validation of efficacious and safe use of herbal therapies through conduction of scientific based studies [14,18]. Preliminary toxicological evaluation is necessary for authentication of safety of herbal medications. Although Saccharum munja Roxb. roots have valuable pharmacological effects, the comprehensive awareness about its toxicity potential has been lacking. Therefore, the current study was conducted to assess the acute toxicity of aqueous ethanolic extract of S. munja roots in animal model by following OECD guidelines 425 [19] as the acute oral toxicity study is necessary to determine the safer dose range to manage the clinical signs and symptoms of the drugs [20]. In this study, mice rather than rats were used because it is scientifically documented that lethal dose data collected from mice might be more appropriately to anticipate the toxic effects in human beings [21].

The toxic outcomes of drugs on vital body organs are exposed by clinical signs and symptoms which are principal observations among
Conclusion

are supported by the study of mean corpuscular hemoglobin (MCH) levels and decline in mean corpuscular volume (MCV) and count (Table 7) indicating hemostatic activity of tested extract sample coagulation. This study showed remarkable elevated levels of platelet aqueous ethanolic extract of animals [40]. Blood platelets have a vital role in the process of blood changes in response to any environmental pollutant or toxic stress in evaluation [39].

Hematological parameters are sensitive markers of the physiological status of the body was evaluated by other biological parameters including serum biomarkers measurement. Liver injury caused by hepatotoxic drugs can result in elevated ALT, AST and total proteins levels [28–30]. Statistically significant elevation in ALT, AST, total proteins and globulin levels were observed in this study (Table 5). The present study data are in agreement with findings of Adedapo et al. and Adeoye et al. [31,32]. Hepato cellular damage may results in increased cell membrane permeability and cause release of amino transferases into blood stream [28,33,34]. ALP is considered as the standard marker of biliary tract obstruction [35]. In this study, there was significant decrease found in ALP levels (Table 5) which is indicative of plant hepato protective effect [20]. Multiple hyperlipidemias are often secondary to many factors e.g. diet, alcohol intake, therapies or to diseases such as nephrosis, diabetes, hyperthyroidism or tumors [36]. Increased levels of cholesterol, triglycerides and LDL (Table 6) were found in the treated group suggesting multiple hyperlipidemic effects of plant under study [37]. Renal function impairment is indicated by elevated levels of serum creatinine and urea [38]. In the present study, serum urea levels were found elevated (Table 4) showing that there is mild renal injury which is supported by patchy necrosis seen during histopathological evaluation [39].

Hematological parameters are sensitive markers of the physiological changes in response to any environmental pollutant or toxic stress in animals [40]. Blood platelets have a vital role in the process of blood coagulation. This study showed remarkable elevated levels of platelet count (Table 7) indicating hemostatic activity of tested extract sample [41]. In this study, increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels and decline in mean corpuscular hemoglobin concentration (MCHC) was observed (Table 7). Statistically, significant elevated WBC count and lymphocytes (Table 7) suggest its defending potential against the microorganisms and also its contribution to enhance cellular inflammatory process. These results are supported by the study of different researchers [31,42,43].

5. Conclusion

In the light of findings of acute toxicity testing it was concluded that aqueous ethanolic extract of Saccharum munja Roxb. roots is not devoid of toxic effects as it elevates LFT, RFT, lipid profile parameters and also showed blood dyscrasia. However, the preliminary results suggested that it should be further evaluated for long term use and repeated dose effects to ensure safety of this herb.

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[19] OECD, Acute oral toxicity: Up and down procedure, Guideline for the Testing of Toxicity to animals [40]. Blood platelets have a vital role in the process of blood changes in response to any environmental pollutant or toxic stress in evaluation [39].

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