EVALUATION of ACUTE AND SUB-ACUTE TOXICITY of *Lippia javanica in* RATS

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Abstract— The study was conducted to evaluate the safety dose range of methanolic extract from the leaves of *Lippia javanica* by acute and sub-acute oral toxicity study on rats. In acute toxicity study, a single oral dose of 1000mg/kg of the experimented plant extract was administered to three groups and adverse effect, mortality and general behavior were monitored for 15 days. In sub-acute study, the tested plant extract was administered orally at doses of 250 and 500 mg/kg for 30 days to the two animals groups and their body weight, organ body weight hematological, serum hepatic biochemical parameters were assessed and compared to normal group by sacrificing all group animals. In acute toxicity, all treated groups did not reveal any mortality nor any significant change in behavior. In sub-acute toxicity study, changes in hematological parameters (white blood cell, neutrophils, and monocyte) biochemical parameter (total bilirubin) and organ body weight (lungs, liver and kidney) were observed compared to the control group. The result specifies that the oral administration *Lippia javanica* extract produce some significant toxic effect in rats. Hence, the extract cannot be utilized safely for therapeutic use in pharmaceutical formulations without performing more in-depth investigation.

Index Terms— *Lippia javanica*, toxicology, hematological, biochemical.

1 INTRODUCTION

People are using medicinal plants for treating certain diseases since from more than 1000 years. According to World Health Organization (WHO), it is found that more than 80% of populations are using medicinal plants [1] and 40-90% of people of developing countries are using traditional medicine frequently [2]. In Bangladesh also, it is estimated that more than 500 species of medicinal plants exist and they are used Unani, Ayurveda, and Homeopathic preparation [3]. It is because herbal medicines are considered as effective and safe [4]. Due to this reason, many scientific researchers are directed to identify and isolate different bioactive principles from natural sources and researchers emphasize on optimizing and advancing the method of extraction so that maximum biological active material can be extracted [5].

However, many traditional plants exert toxic effect on both human and animals [6]. Some unwanted reaction can be observed due to overdose, over duration, tolerance, allergic and idiosyncratic reaction, mid-term and long term toxic effects and these reactions that necessitate toxicity evaluation [0]. It is therefore, for evaluate the safety or toxicity of the medicinal plants is needed to increase the confidence in its safety to human [6].

Lippia javanica is the indigenous Lippia shrubs and from family called Verbenaceae. The species is widely distributed throughout South Africa and also found in Botswana, Malawi, Swaziland, Mozambique, Tanzania, Zambia and Kenya. It has been found that The plant possess analgesic, anti-inflammatory and antipyretic activities [8]. We designed this study to determine or evaluate the toxicological effect of *Lippia javanica* on various organs of the animal model in order to provide information about its safety [9].

2 MATERIALS AND METHODOLOGY

2.1 PLANT MATERIALS COLLECTION AND IDENTIFICATION

From Chittagong hill tracts, the fresh leaves of sample plant materials were collected between March to April. Bangladesh national herbarium (Dhaka, Bangladesh) helped to identify materials of this plant. It is one the scientific organizations and the main function of this organization are to collect plant specimens from the different region of the country which are then preserved and documented as a reference material. After collecting the plant, it was sent to Bangladesh herbarium for verification. The organization identified the plant as *Lippia javanica* and provided the plant accession number DACB 45301.

2.2 EXTRACTION

The leaves were dried for seven days by using shed dried method. Then sieving was done to form coarse powder of the materials. After that, the powder was soaked into methanol for another seven days and occasional stirring was done simultaneously. Next, Whatman filter paper No.1 was used to filter the extract. Finally, the filtrate was evaporated under elevated pressure by using rotary evaporator at 40°C for drying.

2.3 EXPERIMENTAL ANIMALS

From Animal House of Jahangirnagar University, Savar, Bangladesh, all the experimental animals were collected. On average, 90-95 grams was the weight of each animal. Total animals were divided into different three groups and were caged at standard laboratory conditions temperature was 25±2° C, relative humidity was 60±5% and light and dark circle ration was 12:12. After starting the experiment, acclimatization of the animals were observed for five days. Due to handle the animals, a standard and well organized protocol was designed in accordance with the current established guideline. Moreover, an ethical guideline was established for studies of experiments in conscious animals [10].

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2.3 Acute toxicity testing

A single dose of 1000 mg/kg of the methanolic extract of *Sterculia coccinea* in normal saline which was administered to rats orally with the help of intra-gastric tube was taken to conduct acute toxicity test. All the rats were remained unfed for all night, before administration of extract. The control group received equal volume of water orally. In both control and experimental groups, there were 5 members. Both the control and experimental rats were observed for 1, 2, 4 and 24 hours in a periodical manner. Due to obtain mortality and delayed sign of toxicity, that process was continued fir 15 days. Different changes on different regions like in hair, skin, eyes, mucus membrane, food and water consumption, body weight, neurological and autonomic profile, behavioral and respiratory rate were observed. At the final stage of experiment, all the animals were sacrificed under anesthesia

2.4 Sub-acute toxicity testing

The animals were divided into three groups in such a way so that each group is consisting of five members. Group 1 was the control group in which water was administered orally with a dosing of respectively. The others two groups (group 2 and 3) were administered methanolic leaf extract of *Sterculia coccinea* with dosing of 250 mg/kg and 500 mg/kg respectively. The same process was continued for the next 30 days and in the meantime, commercial rodent fed and water were provided ad libitum to the experimental animals.

2.5 Sample collection

On the 31st day, all the animals were anaesthetized in an air tight dissection jar. Then, they were scarified by cardiac puncture using sterile needle syringes. One volume of blood was placed in bottles and the bottles were containing ethylediaminetetra acetic acid (EDTA) that helped to avert coagulation. Hematological test was performed by using this blood samples. Due to biochemical tests, collected blood was kept in bottles where no EDTA was present and it was then kept at 4 ℃ for 240 minutes to let it clot. Next, centrifugation was done at 1500 rpm for 15 minutes to achieve serum. This serum was chilled at 22 ℃ and it is used for biochemical assays later on. When all the blood samples were collected, the sacrificed animals were kept on dissecting board. At first vertical mid-line was cut from neck to pelvis to uncover peritoneum with the help of pair scissors. The organs of the rats like liver, spleen, lungs, kidneys, heart and pancreas were collected, washed and weighed by using the digital weighing balance machine.

2.6 Hematological test

Different parameters like hemoglobin (Hb), erythrocyte sedimentation rat (ESR), white blood cell (WBC), red blood cell (RBC), total platelet count, differential leukocyte count (neutrophils, lymphocyte, eosinophils and basophiles), hematocrit (Hct), mean corpuscular volume (MVC), red distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT) were analyzed by using hematology analyzer.

2.7 Biochemical test

For assay the liver and kidney indices, commercial kits were used. With the help of these kits, different parameter such as random blood sugar (RBS), serum creatinine, liver function test included serum bilirubin, plasma alanine aminotransferase (ALT), aspartate alanine aminotransferase (AST), alkaline phosphate (ALT), lipid profile that included serum total cholesterol, serum triglyceride, serum high density lipoprotein (HDL) and serum low density lipoprotein (LDL) were analyzed. Moreover, some others parameters like electrolytes including sodium, potassium, chloride, serum calcium, serum uric acid, serum protein, serum albumin, serum globulin were also analyzed.

2.8 Statistical analysis

All the collected data were stated as mean ± standard deviation (SD). Values with different superscripts were significantly different ($P < 0.05$, $P< 0.01$, $P< 0.0O1$, $P< 0.0001$). Graph pad Prism version7 was used for graphical representation and statistical analysis.

3 Results & Discussion

3.1 Acute oral toxicity study

There was an observational period of 15 days in order to find out clinical symptoms of the rats but the rats did not show any sign or symptoms of toxicity.

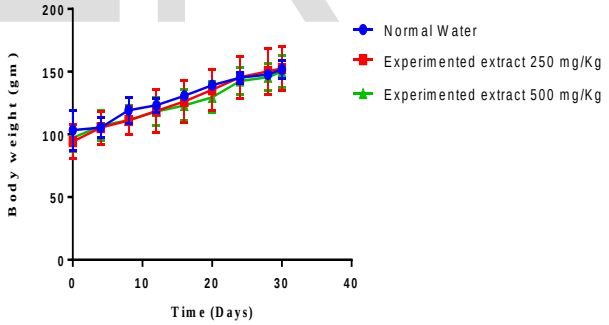

Figure 1: Effects of oral administration of methanolic leaf extract of *Sterculia coccinea* on body weight in rats (n=5)

3.2 Sub-acute toxicity study

Effects of extract on body weights and organ weights:

Rats were given *Sterculia coccinea* extract (250, 500 mg/kg) for 30 days but did not cause any mortality in rats. Changes were detected in body weight of extract treated groups while comparing with control group which was described through figure 1. Gains or losses of body weight indicate toxic properties of drugs and chemicals. However, it is scientifically confirmed that gains or losses of body weight is associated with fat accumulation [11]. The experimented group of this study did not gained body weight

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while comparing with control group that indicate less fat accumulation property of the experimented plant. Significant dose-dependent changes were found in organ body weight of lungs, liver and kidney (table1). Lungs, Liver and kidney weight decreased significantly while given 250 mg/kg of extract and 500 mg/kg extract (Compared to control group). To detect physiological status of animals, organ weight plays a vital role. Organ weight establishes weather organ is exposed to injury or not [12].

TABLE. 1: EFFECTS OF ORAL ADMINISTRATION OF METHANOLIC LEAF EXTRACT OF *LIPPIA JAVANICA* ON ORGAN WEIGHT IN RATS

Organs	Weight of the organs (gm)	Organs	Weight of the organs (gm)
	Group 1		Group 1
Lungs	1.4712 ± 0.157	Lungs	1.4712 ± 0.157
Heart	0.517 ± 0.044	Heart	0.517 ± 0.044
Liver	6.5076 ± 0.283	Liver	6.5076 ± 0.283
Pancreas	0.782 ± 0.134	Pancreas	0.782 ± 0.134
Spleen	0.773 ± 0.097	Spleen	0.773 ± 0.097

TABLE. 2: EFFECTS OF METHANOLIC LEAF EXTRACT OF *LIPPIA JAVANICA* ADMINISTRATION ON HAEMATOLOGICAL PARAMETERS ON RATS (*N* = 5)

Parameters	Name of the group		
	Group 1		Group 1
Hb (gm/dl)	12.86±0.472229	Hb (gm/dl)	12.86±0.472229
ESR (mm in 1st hour)	3.6 ± 1.140	ESR (mm in 1st hour)	3.6 ± 1.140
WBC (per cumm)	5300 ± 1106.797	WBC (per cumm)	5300 ± 1106.797
RBC (million per cumm)	6.468 ± 0.140	RBC (million per cumm)	6.468 ± 0.140
Platelet Count (per cumm)	632000 ± 108557.2	Platelet Count (per cumm)	632000 ± 108557.2
Neutrophils (%)	16 ± 0.008	Neutrophils (%)	16 ± 0.008
Lymphocyte (%)	83± 0.020	Lymphocyte (%)	83± 0.020
Monocyte (%)	3±0.009	Monocyte (%)	3±0.009
Eosinophil (%)	2± 0.006	Eosinophil (%)	2± 0.006
Basophil (%)	0.36	Basophil (%)	0.36
HCT (%)	37± 0.005	HCT (%)	37± 0.005
MCV (Fl)	58.22± 0.864	MCV (Fl)	58.22± 0.864
MCH (pg)	18.4±0.515	MCH (pg)	18.4±0.515
MCHC (g/dL)	32.34± 0.378	MCHC (g/dL)	32.34± 0.378
RDW-SD (Fl)	34.58± 1.96	RDW-SD (Fl)	34.58± 1.96
RDW (%)	18.62± 0.936	RDW (%)	18.62± 0.936
PDW (Fl)	14.74± 0.639	PDW (Fl)	14.74± 0.639
MPV (Fl)	7.3± 0.418	MPV (Fl)	7.3± 0.418

Effects of extract on hematological parameters in rats

Most of the hematological parameters did not show any significant dose-dependent changes except WBC, neutrophils, monocyte. These three parameters are changed significantly which indicate toxic property of plant. WBC parameter decreased significantly (P<.05) while given extract of 250mg/kg and 500mg/kg (compared to control). WBC is an impenetrable significative on inflammation for both acute and chronic time frame [13]. So, the observed significative decrease in WBC level may indicate an effect of extract leaf on immune system of treated groups. Both neutrophils level and monocyte level were decreased significantly (P<.0001 & P<.05) (in both treatment groups) compared to control group after administration of 250mg/kg and 500 mg/kg dose. Observed significant alleviation in neutrophils and monocyte level may be implied a wide range of inherited and acquired disorders as both of the parameters have phagocytic activity and decreased level could affect their activity of destroying cell wall materials, foreign particles as well as bacteria [14].

Table 3: Effects of extract of *Lippia javanica* on biochemical parameters in rats (*n* = 5)

Parameters	Name of the group		
	Group 1		Group 1
RBS (mmol/l)	3.4 ± 0.474	RBS (mmol/l)	3.4 ± 0.474
Serum urea (mg/dl)	21.4 ± 2.074	Serum urea (mg/dl)	21.4 ± 2.074
Serum creatinine (mg /dl)	1.067 ± 0.193	Serum creatinine (mg /dl)	1.067 ± 0.193
Serum bilirubin (total) (mg/dl)	0.194 ± 0.014	Serum bilirubin (total) (mg/dl)	0.194 ± 0.014
ALT(U/l)	57.2 ± 6.686	ALT(U/l)	57.2 ± 6.686
AST (U/l)	96± 7.906	AST (U/l)	96± 7.906
ALP (U/l)	298 ± 30.570	ALP (U/l)	298 ± 30.570

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Serum total cholesterol (mg/dl)	79± 2.550	Serum total cholesterol (mg/dl)	79± 2.550
Triglyceride (mg/dl)	45.6± 3.975	Triglyceride (mg/dl)	45.6± 3.975
HDL (mg/dl)	50 ± 4.637	HDL (mg/dl)	50 ± 4.637
LDL (mg/dl)	19.4 ± 4.561	LDL (mg/dl)	19.4 ± 4.561
Sodium (Na^+) (mmol/l)	148.8 ± 2.775	Sodium (Na^+) (mmol/l)	148.8 ± 2.775
Potassium (K^+) (mmol/l)	4.92 ± 0.466	Potassium (K^+) (mmol/l)	4.92 ± 0.466
Chloride (Cl^-) (mmol/l)	103.8 ± 3.962	Chloride (Cl^-) (mmol/l)	103.8 ± 3.962
Serum calcium (mg/ dl)	8.38± 0.526	Serum calcium (mg/ dl)	8.38± 0.526
Serum uric acid (mg/dl)	1.84 ± 0.404	Serum uric acid (mg/dl)	1.84 ± 0.404
Serum protein (total) (g/dl)	6.64 ± 0.219	Serum protein (total) (g/dl)	6.64 ± 0.219
Serum albumin (g/dl)	3.08 ± 0.602	Serum albumin (g/dl)	3.08 ± 0.602
Serum globulin (g/dl)	3.4± 0.255	Serum globulin (g/dl)	3.4± 0.255

Effect of extract on biochemical parameters in rats

Most of the serum biochemical parameters (table 3) of the treated groups did not show any significant dose-related changes compared to control group. Howsoever; extract doses of 250 mg/kg and 500 mg/kg expressed significant dose-related changes in serum bilirubin (total) level. The level of bilirubin increased significantly ($P<0.01$ & $P<0.001$) while given doses of 250 mg/kg and 500 mg/kg. Bilirubin is a breakdown production of aged red blood cell (RBC). Total bilirubin comprised both the conjugated and unconjugated (free) forms and elevation of total bilirubin is generally indicative of hemolysis or liver damage [15].

4 Conclusion & Future Scope

Current study exposes important information regarding acute and sub-acute toxicity of methanolic leaf extract of *Lippia javanica* which is very advantageous for any further in-vivo and clinical study of this plant. In our study, changes in hematological parameters (white blood cell, neutrophils, and monocyte) biochemical parameter (total bilirubin) and organ body weight (lungs, liver and kidney) were observed compared to the control group. The result specifies that the oral administration *Lippia javanica* extract produce some significant toxic effect in rats. Hence, the extract cannot be utilized safely for therapeutic use in pharmaceutical formulations without performing more in-depth investigation.

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