

EVALUATION of ACUTE AND SUB-ACUTE TOXICITY of *Sterculia coccinea* in RATS

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Abstract— Global population is increasing day by day. Therefore, the demand of food will be doubled in next 30 years. With limited resources to meet this demand will be a great challenge for human. To increase the production of food, traditional agricultural system needs to be updated. This paper is proposing developed an automated agricultural system with internet of things (IoT) to solve traditional uneven ploughing problem, water management on the field, intruder's attack and temperature effect on crops. This system has two major part. One is automated agricultural system with an automated vehicle for ploughing and another part is storing real-time field data to the ThingSpeak cloud with stream live video of field situation to the YouTube via internet of things (IoT) technology. Several sensors like soil-moisture, temperature, passive infrared ray (PIR), Rain-drop sensor, IR sensor was used in this system. Based on this sensor's real time data from developed prototype system three programmable microcontroller (Arduino Uno, Arduino mega, Raspberry pi 3B) controls the whole system. To stream the live video to YouTube a 5MP raspberry pi camera module was integrated. The automated system has another two key features to solve the problem of improper water management and intruder's inclusion. Those are automated irrigation system and bird repeller. Stored data in the webserver can potentially be worthy for next year operation. By using this system, farmers can also connect multiple farm to the web at the same time and access all of the farms in one webpage and will be able to observe the farm in real time. All the key features and technologies can help to build a sustainable and productive farming system and enter the fourth industrial revolution.

Index Terms— *Sterculia coccinea*, toxicology, hematological, biochemical.

1 INTRODUCTION

Long before the discovery of microbes and its disease-causing potential, the understanding that certain plants contained healing properties was very well established [1]. Specific plants are used as the most common form of medicine for various aboriginal people [2]. Different sections of plants such as roots, leaves, barks, twigs and stem are frequently used for conventional medicines [3]. Approximately 60% of the pharmaceuticals are manufactured from plant origin [4]. In Bangladesh, medicinal plant holds an important part in the health care system [5]. The use of plant extracts as medicines is a better alternative to allopathic because of the less to no side effect [6]. They are also more economical [7]. Despite the extensive use of medicinal plants, their safety and effectiveness have not yet been completely examined and further comprehensive analysis is therefore is required for standardization and evaluation of the plant formulations [8].

To the extent of our knowledge, since there is no documented study of the specific plant extract of *Sterculia coccinea*, this study is designed to find out the acute and sub-acute toxicity, toxicological study of the plant extract, hematological, biochemical test in rats.

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 *Sterculia coccinea* and provided the plant accession number DACB 47695

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].

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 for 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 feed 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 ethylenediaminetetra 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 °C 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 °C 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 rate (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 phosphatase (ALP), 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 \pm standard deviation (SD). Values with different superscripts were significantly different ($P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$). 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.

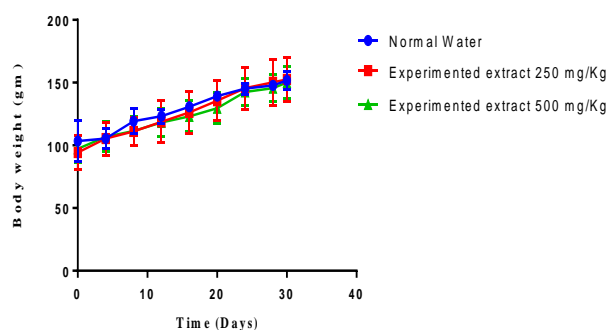


Figure 1: Effects of oral administration of methanolic leaf extract of *Sterculia coccinea* on body weight in rats (n=5)

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 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 whether organ is exposed to injury or not [12].

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

	Group 1	Group 2	Group 3
Lungs	1.4812 ± 0.167	0.858 ± 0.065***	1.107 ± 0.087*
Heart	0.501 ± 0.034	0.538 ± 0.070	0.546 ± 0.170
Liver	5.507 ± 0.273	5.493 ± 0.487***	5.232 ± 0.336***
Pancreas	0.772 ± 0.144	0.567 ± 0.180**	0.765 ± 0.079
Spleen	0.774 ± 0.007	0.823 ± 0.0779	0.843 ± 0.179
Kidney	1.153 ± 0.75	0.838 ± 0.042****	1.005 ± 0.184**

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

Parameter	Name of the group		
	Group 1	Group 2	Group 3
Hb (gm/dl)	11.86±0.479	13.2±0.4223	12.66±0.5495
ESR (mm in 1st hour)	3.6 ± 1.1240	4.4± 1.5217	5.6±1.517 *
WBC (per cumm)	5300 ± 11206.797	3920±649.625*	3180± 121.475*
RBC (million per cumm)	6.468 ± 0.150	7.17± 0.498**	5.162± 0.910*
Platelet Count (per cumm)	632000 ± 108557.2	541780± 147297.6	72960±47731.54
Neutrophils (%)	16 ± 0.018	8 ± 0.84****	8± 0.025****
Lymphocyte (%)	83± 0.0211	88± 0.05111	90±0.25021 ***
Monocyte (%)	3±0.119	1± 0.45*	1± 0.5*

Eosinophil (%)	2± 0.206	1±0.1055	1±0.005
Basophil (%)	0.37	1.56±0.0044**	.70± 0.013
HCT (%)	38± 0.005	45.46± 0.025***	36.28±0.026
MCV (fL)	58.24± 0.864	61.68± 2.107*	57± 3.45
MCH (pg)	18.4±0.525	18.78± 1.24	12.93 ± 7.429
MCHC (g/dL)	32.35± 0.378	34.18± 2.579	25.63 ±14.826
RDW-SD (fL)	34.68± 1.96	33.52± 2.682	27.981± 14.918
RDW (%)	18.63± 0.936	17.59± 0.005	15.67± 0.009***
PDW (fL)	14.75± 0.639	16.91± 2.528	16.47± 1.203*
MPV (fL)	7.4± 0.418	8.75± 0.619**	7.47± 0.398
PCT (%)	0.33± 0.0006	0.46±0.0003*	0.54± 0.0005**

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 ($P<0.05$) while given extract of 250mg/kg and 500mg/kg (compared to control). WBC is an impenetrable significant on inflammation for both acute and chronic time frame [13]. So, the observed significant 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 ($P<0.0001$ & $P<0.05$) (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 *Sterculia coccinea* on biochemical parameters in rats (n = 5)

Parameters	Name of the group		
	Group 1	Group 2	Group 3
RBS (mmol/l)	3.2 ± 0.474	5.24 ± 0.321****	2.70 ± 0.669
Serum urea (mg/dl)	21.2 ± 2.074	19.5 ± 2.191	24 ± 1.871**
Serum creatinine (mg)	1.07 ± 0.193	0.75 ± 0.080*	1.2 ± 0.426

/dl)			
Serum bili- rubin (total) (mg/dl)	0.174 ± 0.014	0.248 ± 0.008**	0.36 ± 0.0192***
ALT(U/l)	57.4 ± 6.686	64.3 ± 2.589	78 ± 5.916***
AST (U/l)	95± 7.906	103 ± 7.583	122.8 ± 12.677**
ALP (U/l)	297 ± 30.570	232 ± 18.881**	275 ± 19.837
Serum total cholesterol (mg/dl)	77± 2.550	75.6 ± 2.510	71 ± 11.091
Triglyceride (mg/dl)	44.6± 3.975	45.2 ± 1.643	42.8 ± 2.280
HDL (mg/dl)	53 ± 4.637	55.6 ± 7.021	57.6 ± 3.209
LDL (mg/dl)	19.5 ± 4.561	15.9 ± 4.439	19.4 ± 3.286
Sodium (Na ⁺) (mmol/l)	138.8 ± 2.775	147 ± 2.596	156±3.240*
Potassium (K ⁺) (mmol/l)	4.72 ± 0.466	5.76 ± 0.926	5.24± 0.654
Chloride (Cl ⁻) (mmol/l)	105.8 ± 3.962	103.2 ± 3.899	116.2 ± 5.975*
Serum cal- cium (mg/ dl)	8.18± 0.526	9.16± 0.434	8.5 ± 0.575
Serum uric acid (mg/dl)	1.74 ± 0.404	2.24 ± 0.320	2.8± 0.274**
Serum pro- tein (total) (g/dl)	6.54 ± 0.219	6.9 ± 0.235	6.25 ± 1.083
Serum al- bumin (g/dl)	3.18 ± 0.602	2.96 ± 0.503	3.77 ± 0.483
Serum globulin (g/dl)	3.7± 0.255	2.52 ± 0.602	4.8 ± 0.525**

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. However; 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 product 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 *Sterculia coccinea* 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 *Sterculia coccinea* 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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