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Exploring Acute and Sub-Acute Toxicity of Gramine Bioactive Molecule in Wistar Rats

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The study evaluated the toxicity of Gramine in Wistar rats using a specified dose for acute and subacute toxicity evaluation. Female rats were given the Single dose as specified in OECD Guideline 425. Subacute toxicity was assessed using doses of 13, 27.5, and 55 mg/kg p.o. compared to a control group. After treatment, the satellite group continued operating for 14 days to assess the recovery effects. No mortality was observed at doses of 175mg/kg, but mortality was observed at doses of 550 mg/kg. In a subacute toxicity investigation, rats given Gramine orally for 28 days showed significant weight loss, reduced food intake, and normal water consumption. No harmful effects were observed for haematological or biochemical parameters, and no evidence of deterioration was found in brain, kidney, or liver histopathological examinations. The results indicate that 13, 27.5, and 55 mg/kg were considered safe doses for both short- and long-term oral therapy.

Keywords: Acute; Gramine; Main Test; Subacute.

Since the introduction of additional sophisticated medical therapies in the decade following genomics, natural resources and their derivatives have become the most common sources of bioactive molecules in healthcare products. Traditional herbal medicines, as defined by the World Health Organisation (WHO), are those that have been used to treat an illness in regional or local healing practises and are derived from plant sources but have undergone minimal or no industrial processing.[1] Because of their natural origin and relatively few adverse effects, traditional systems of medicine and their formulations have seen extensive application in both developed and developing countries for a long period of time[2]. Very few varieties of plants have been examined in depth for their adverse effects, despite the fact that several pharmacological investigations have been carried out to identify the arbitrary conventional application of various therapeutic herbs. There are much more reports of effectiveness than of toxicity[3]. In order to incorporate findings of short- and long-term toxicity manifestations and to ensure efficient and open communication of such data, additional research into herbal remedies and phytochemicals is required. A new drug discovery

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relies heavily on toxicological testing, which makes a lot of sense. The Food and Drug Administration (FDA), the regulatory body, has made it clear that it is crucial to test potential new drugs for their toxicity and pharmacological effects in animals[4]. The recently recorded information tells us that traditional medicines also have adverse effects[5]. So it's necessary to perform toxicological studies and get the necessary safety data for specific drugs. The toxicity study evaluates the safety of test medications using a non-clinical approach and provides data on their toxicological parameters. To confirm the safety of the medications, toxicity screening of all chemicals is conducted prior to their use as test drugs in clinical trials. The best technique to determine a medicinal plant's toxicological effect is to examine its acute and sub-acute toxicity. It will increase drugs medicinal and pharmacological applications[6].

Gramine (Ga) is a naturally obtained Indol alkaloid; structurally, it is 3-(N, N-dimethylaminomethyl) indole and was isolated from different biological sources or plant sources[7,8] like mainly from A. sativa L., Hordeum vulgare L., Triticum aestivum L., etc. Gramine, also known as donaxine, is a popular ingredient in dietary supplements due to its wide range of pharmacological actions, many of which are similar to those of ephedrine. Gramine possesses many pharmacological properties, such as bronchial muscle relaxant, vasodilator, treatment of inflammation in the lungs, inflammation in the kidney, and bronchial asthma[9]. It is also a weak acetylcholinesesterase inhibitor, according to the literature[10]. Recently, it has been observed that when gramine is added to rats' diets, their feed intake decreases, resulting in weight loss[11]. Gramine possesses radioprotective qualities. Gramine is capable of blocking the activity of serotonin in the body[12]. Gramine has a low risk to human health and has no effects that may be classified as irritant, cumulative, allergic, or skin-resorbing[8]. Consideration was given to the current study due to its high potential and low toxicological profile. This investigation determines the acute and subacute toxicity of Gramine in rodents within 28 days by observing biochemical, histopathological, and haematological changes.

MATERIALS AND METHODS

Ethics approvals and Experimental Animals

Wister rats, both sexes, weighing 160–180 g, were obtained from LACSMI Bio-Farms, Pune, and Maharashtra, India. They were housed in rice husks at MET's BKC Institute of Pharmacy. 1277/PO/RcBt/S/09/CPCSEA is the CPCSEA registration number with all CPCSEA conditions and guidelines, such as a 12-hour light and 12-hour dark cycle, room temperature of 25–30 °C, and humidity of 50–70%. They were given unlimited access to filtered water and pelleted feed while being acclimatised to standard laboratory conditions for 7 days. Animal activity was performed after getting Animal ethical approval by the (IAEC) Institutional Animal Ethics Committee (IAEC/December 2021/04) of MVP's College of Pharmacy, Nashik, and dated December 18, 2021. After receiving institutional animal ethics committee (IAEC) approval (IAEC/December 2021/04) from MVP's College of Pharmacy, Nashik, established under CPCSEA, animal activity was carried out. Ethical standards were strictly complied with in accordance with the established broad standards in the Guide for Care and Use of Laboratory Animals.

Active constituents

Yucca Enterprises, Wadala (E), Mumbai 400037, India, from where Gramine was Purchased, the chemical name of Gramine is 1-(1H-Indol-3-yl)-N, N-dimethylmethanamine, Gramine, or donaxine, is a chemical Indole alkaloid with a molecular weight of 174.24. White to Off-white-coloured crystalline powder. Soluble in Methanol, Ethanol, DMSO, and DMF; soluble in water.

Experimental Design

Acute oral toxicity test

The OECD guideline 425 was followed to perform an acute oral toxicity study of Gramine.

Main test for Drug Gramine

Wistar female rats were utilised, as well as one control rat. As defined in the OECD 425 main test criteria, using the default progression factor, doses would be selected from the sequence of 17.5, 55, 175 and 550 mg/kg used for testing (up-and-down dose procedure). Prior to receiving Gramine orally by oral gavage, the rats had fasted overnight. The first female rat is orally administered 17.5 mg/

kg. If the animal survived after 48 hours, the dose was increased by a factor of 3.2, resulting in a dose of 55 mg/kg for the next animal. When the second animal lived after 48 hours, the next animal was orally administered 175 mg/kg. After the survival of the third animal, the animals received a 550 mg/kg dose. The testing was ended after the last three animals withstood the maximum dose, and all animals were observed for 14 days. Every Wellness pattern seen was identical to the limit test[24].

Oral Sub-Acute Toxicity Study (28 Days)

The investigation was undertaken for 28 days according to OECD Guideline No. 407. All Wister rats Males and females were separated into five groups, with n = 10 animals in each group: males five and females five. Nulliparous female rats (weighing 160–180 g b.w.) and male rats (weighing 180–200 g b.w.) aged 9–11 weeks were housed in separate cages for each group. Group I serves as the control, while Groups II, III, and IV were administered Ga at doses of 13, 27.5, and 55 mg/kg b.w. for 28 days. High doses of Ga from Groups V, which serve as a satellite group for follow-up observations, should be left untreated for at least 14 days in order to identify delayed occurrence, persistence, or recovery from toxic effects. All animals were dosed using oral gavage for 28 days. Animal weight was rhythmically measured while feed and water intake were recorded daily. As per day counting, the satellite group was totally observed for 42 days. After completing treatment for 28 days, groups I, II, III, and IV were considered first for weighting and then scarified by the cervical dislocation method. Biochemical and haematological parameters were performed on a blood sample, which is collected by the retro-orbital plexus method. Different organs like the liver, kidney, brain, lung, heart, pancreas, etc. were weighted, exterminated, and then fixed in 10% formalin.[25, 26].

Measurement of animas body and organ weights

Change in animal weight were measured every week and % weight was calculated by following formula,

$$\% \text{ weight} = Wf\text{-}Wi/Wi \times 100$$

Where, Wf = final weight, Wi = initial weight.

The heart, lungs, kidney, brain, pancreas, and liver were removed after blood was drawn. These organs were then weighed. The following equation was used to calculate the relative organ weight of each organ collection:

$$\text{Relative organ weight} = (\text{organ weight})/(\text{body weight}) \times 100$$

Estimation of haematological parameters

Haematology samples were stored in Eppendorf tubes, which already contain K2 ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. Autohematology analyzer was used to analyse the above parameters: RBC (Erythrocyte) (mill/cu.mm), HCT (haematocrite) (%), MCV mean corpuscular volume (fL), MCHC Low mean corpuscular haemoglobin concentration (%), WBC

Table 1. Behavioural observation on animal treated with drug Gramine in acute toxicity study

Parameter	30 Min	1Hr	4Hr	1 day	2 days	7 days	14 days
Fur and Skin	NL	NL	NL	NL	NL	NL	NL
Salivation	NL	NL	NL	NL	NL	NL	NL
Respiration	NL	NL	NL	NL	NL	NL	NL
Faces consistency	NL	NL	NL	NL	NL	NL	NL
Somatomotor activity	NL	NL	NL	NL	NL	NL	NL
Sleep	NL	Pr	Pr	NL	NL	NL	NL
Convulsion and tremors	NL	NL	NL	NL	N/A	N/A	N/A
Itching	N/A	N/A	N/A	N/A	NL	NL	NL
Mucous Membrane	NL	NL	NL	NL	NL	NL	NL
Coma	NL	NL	NL	NL	N/A	N/A	N/A
Mortality	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Note: NL= normal, Pr= Present, N/A = not found

leukocyte (103/mm3), NEUTRO (Neutrophil) (%), LYMPH (Lymphocyte) (%), MONOS (monocytes) (%), EOSINO (eosinophils) (%), PLT (platelet count) (103/mm3), Hb haemoglobin (g/dl), etc.

Estimation of serum biochemical parameters

A blood sample collected for biochemical parameters was placed sideways to avoid cell lysis. Serum was separated by centrifugation, which was used for biochemical estimations like glucose, Urea, Uric acid, Creatinine, Albumin, protein, SGPT, SGOT, Bilirubin, and cholesterol with the help of a semi-auto analyzer (Bench-top).

Histopathological investigation

On the 28th day, brain, liver, and kidney samples were taken and fixed in a 10% neutral formalin solution. Tissue samples were then fixed as normal by putting them in paraffin. Blocks were cut into 5 mm thick pieces and marked with hematoxylin and eosin for a histopathological study under a 40x microscope to look for damaged cells

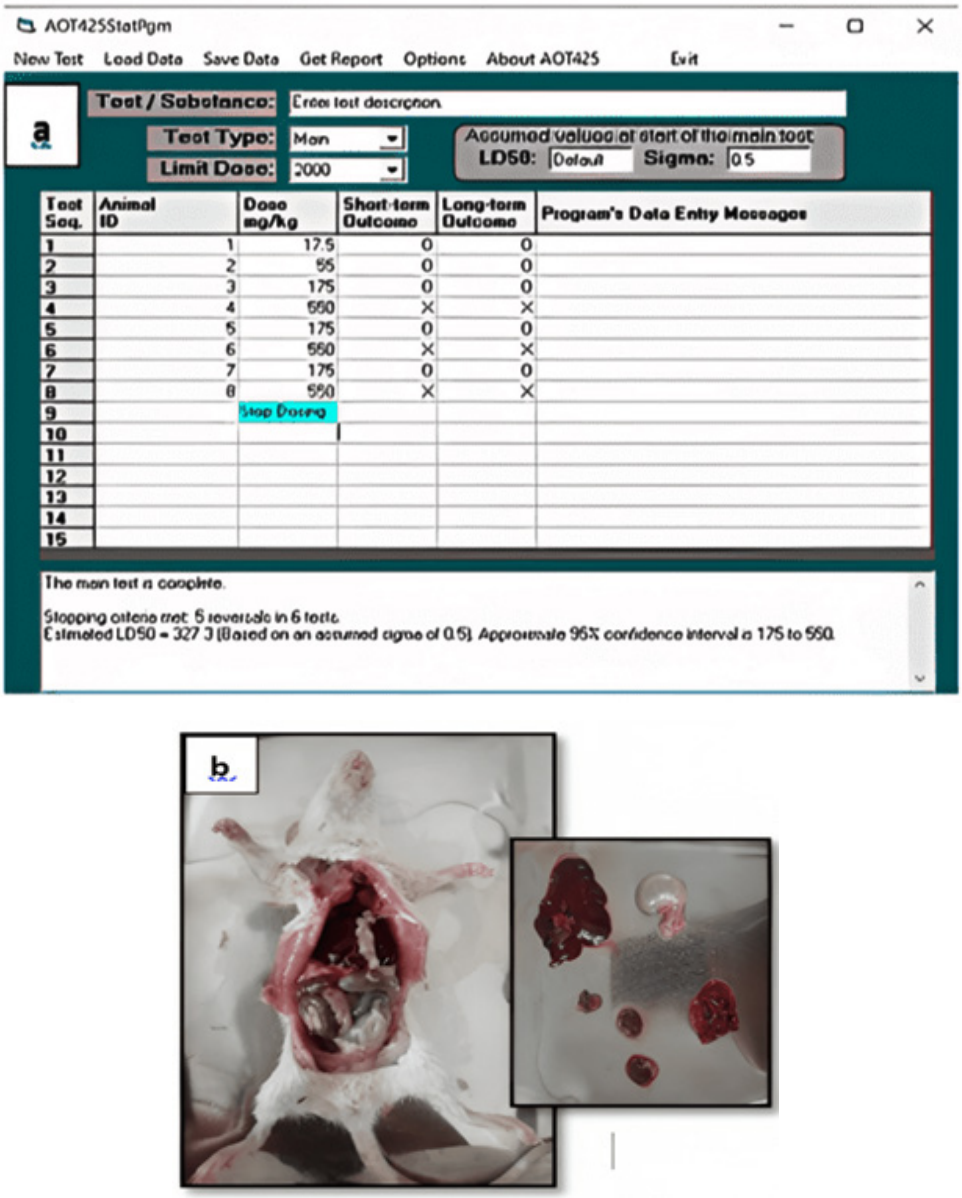

Fig. 1. a) Toxicity study on Rat treated with Gramine (Main study) (AOT425StatPgm); b) Gross Necropsy Study.

or changes in the shape of the tissue. All of the groups did the same thing[14].

Statistical analysis

The results obtained from this study were represented as a standard error mean value (± SEM) with the help of the software Graph Pad Prizam. Before performing a post-hoc analysis, an ANOVA was used to examine the variances among the various groups. Dunnett's Test for parametric multiple comparisons; significance was set at $p<0.05$; least significant difference between the control and treatment groups.

RESULTS

Acute Oral Toxicity Effects of Gramine

Drug Gramine acute toxicity was investigated using OECD Main Test 425 guidelines with (AOT425StatPgm). It was discovered that Gramine was safe and non-toxic at 17.5 mg/kg, 55 mg/kg, and 175 mg/kg BW in test animals. Following Gramine treatment, there were no changes in typical behavioural patterns and no signs or symptoms of toxicity (Table 1). There was

Table 2. Gramine effects of on food and water intakes during study

Groups	Avg. Food intake(Male)	Avg. Food intake(Female)	Avg. Water intake (Male)	Avg. Water intake (Female)
Control(mg/kg)	14.93± 1.24	12.89± 0.90	26.87± 1.41	19.50± 0.67
13mg/kg	14.17± 0.90	12.14± 0.60	23.77± 0.74	18.50± 0.33
27.5mg/kg	13.53± 0.63	11.77± 0.99	23.14± 1.66	16.63± 0.40
55mg/kg	11.70± 0.35	10.72± 1.01	21.04± 1.24	17.38± 1.15
Satellite	14.73± 0.90	12.81± 1.68	24.66± 1.279	18.28± 1.60

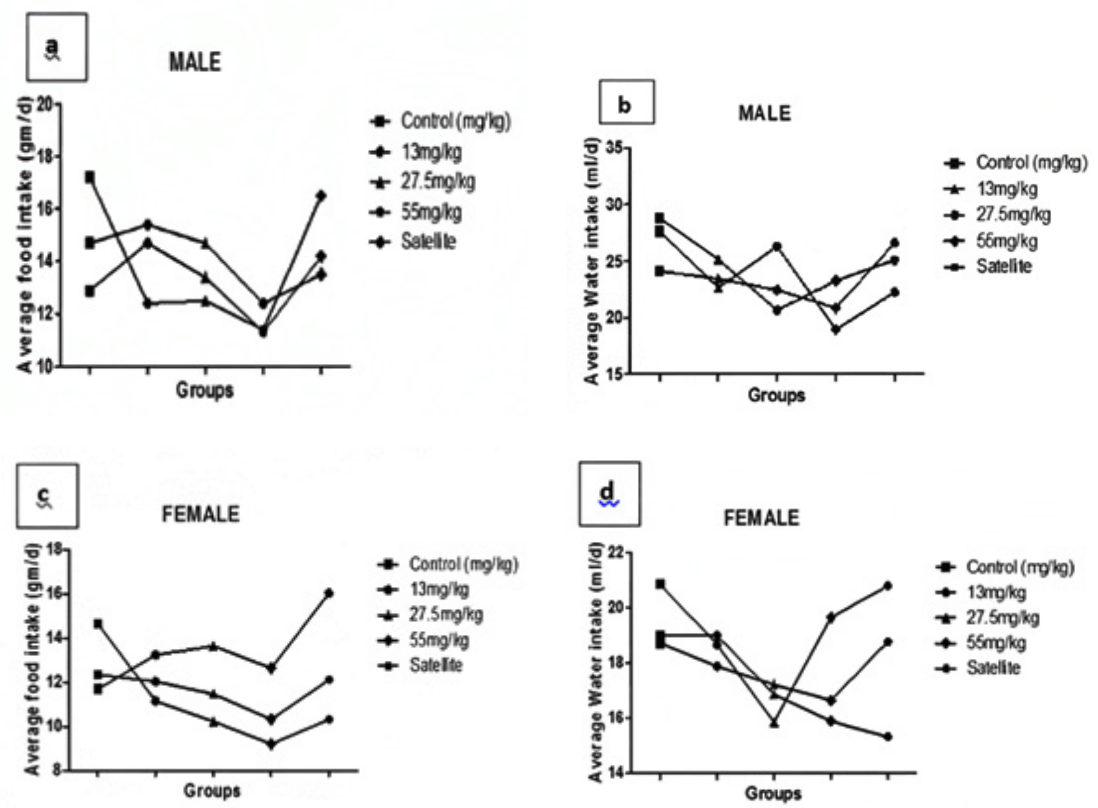

Fig. 2. (a, b) illustrate food and water intake of male, fig. (c, d) illustrate food and water intake of female animals of control and treated groups with Gramine at the doses of 13, 27.5 and 55 mg/kg. (Mean ± SEM). (n = 5).

no mortality after 14 days of surveillance, but at doses of 550mg/kg, mortality was recorded. Gross necropsy findings after the 550mg/kg dose revealed that all of the test animals' essential organs were normal, with smooth and firm consistency, as well as upon opening and checking their abdomen and thoracic areas. (See Figure 1. a and b.)

Sub-Acute (28 Days) Oral Toxicity effects Gramine on Male and Female Wistar Rats

For evaluating the sub-acute toxicity test of Gramine, the OECD guideline (OECD 407) was utilised. In the sub-acute toxicity study, neither male nor female rats treated with 13 mg/kg, 27.5 mg/kg, or 55 mg/kg exhibited indications of any toxicity, but at doses of 55 mg/kg, animals exhibited weight loss. Other identified toxic signs included lethargy and a little fur alteration. During the 28-day trial period, neither group showed any morality or changes in respiration rate. No significant changes were observed in food and water intake, organ weight, serum electrolytes, biochemical parameters, hemological parameters, gross necropsy, or Histopathological analysis when comparing all Gramine-treated animals with the normal control group.

Effect of Gramine on animal food, water intake and body weight

In Table 2 and Fig. 2, respectively, the effects of the Gramine on food and water intakes throughout subacute toxicity study treatment are

Table 3. The effects of the Gramine on the body weight (Male)

Male	Body Wight (gm)						
	wk 0	wk 1	wk 2	wk 3	wk 4	wk 5	wk 6
Control	183.0±1.53	185.3±2.53	192.3±0.94	192.0±1.24	200.3±2.67	-	-
13mg/kg	184.2±0.87	185.5±1.41	183.8±2.11	176.2±2.05*	175.5±2.42**	-	-
27.5mg/kg	186.6±2.27	187.2±1.99	184.6±3.00	175.2±1.85**	172.6±1.76**	-	-
55mg/kg	183.6±3.34	185.3±2.49	184.6±2.49	173.3±1.77**	169.0±4.34***	-	-
Satellite	188.0±2.02	188.6±2.02	186.6±2.15	178.3±1.46**	173.0±1.58***	177.6±1.57	186.3±2.27

Noted: wk = Week

Table 4. The effects of the Gramine on the body weight (Female)

Female	Body Wight (gm)						
	wk 0	wk 1	wk 2	wk 3	wk 4	wk 5	wk 6
Control	169.6±5.07	173.3±3.02	175.6±2.67	177.0±2.88	180.6±3.59	-	-
13mg/kg	169.9±5.17	169.3±3.73	167.9±2.87	166.9±2.29*	164.3±2.47*	-	-
27.5mg/kg	171.6±4.34	170.3±3.19	168.6±2.58	167.6± 0.12*	165.6±2.67**	-	-
55mg/kg	172.7±3.98	172.0±2.87	169.7±1.89	165.7±1.08**	162.0±1.55**	-	-
Satellite	171.4±3.82	170.0±2.81	167.7±2.48	164.4±0.34**	160.4±2.70**	162.4±2.02	167.7±2.10

Noted: wk = Week

Table 3 and 4. Weight Variation in animals of male and female during subacute studies of control and treated with Gramine at the doses of (13, 27.5, and 55 mg/kg). (Mean ± SEM). (n = 5) significant alteration (*p > 0.05)

Table 5. The Effect of Gramine on Organs Weights (male)

Animal (Male)	Control(mg/kg)	Ga Groups			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Liver (g)	7.63± 0.10	7.22± 0.06	7.08± 0.08	7.01± 0.06	7.11± 0.05
Heart (g)	0.86± 0.01	0.82± 0.005	0.80±0.01	0.82± 0.02	0.83± 0.01
Lungs(g)	1.83± 0.03	1.79± 0.02	1.65± 0.06	1.67± 0.04	1.70± 0.03
Brain (g)	1.76± 0.01	1.71± 0.032	1.68± 0.02	1.71± 0.02	1.71± 0.03
Pancreas (g)	0.56± 0.02	0.51± 0.02	0.52± 0.02	0.58± 0.02	0.56± 0.04
Kidney(g)	0.91± 0.02	0.85± 0.02	0.88± 0.00	0.88± 0.01	0.89± 0.01

Table 5:- Male rat organs weight (g) in the Gramine subacute toxicity study. Data expressed as mean ± S.E.M.N= 5. Significantly different from the control, *p< 0.05

illustrated fig. 2. (a, b, c, d). Compared to the control group, the daily oral administration of the Gramine at study dosages (13, 27.5, and 55 mg/kg) and satellite groups for 28 days did not significantly affect food and water intakes (pe"0.05).

Effect of Gramine on the body weights of rats

For 28 days, rats in all groups were given daily doses of Gramine (13, 27.5, and 55 mg/kg and satellite group), which revealed that the first two weeks did not show any significant changes in animal weight, but the third and fourth weeks observed significant changes in animal body (where p e" 0.05). The satellite group does not exhibit significant variations from the control group. (Tables 3 and 4).

Effect of Gramine on Organs Weights

After 28 days of treatment, the organ weights of male and female rats given 13, 27.5, and 55 mg/kg and the Gramine satellite group were not significantly lower than the organ weights of

Table 6. The Effect of Gramine on Organs Weights (female)

Animal (Female)	Control (mg/kg)	Ga Groups			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Liver (g)	5.37± 0.04	5.29± 0.12	5.34± 0.07	5.18± 0.05	5.37± 0.04
Heart (g)	0.57±0.01	0.59± 0.01	0.56± 0.01	0.52± 0.01	0.52± 0.01
Lungs (g)	1.36 ± 0.01	1.37± 0.02	1.33± 0.02	1.38± 0.04	1.37± 0.03
Brain(g)	1.72± 0.02	1.62± 0.11	1.65± 0.06	1.72± 0.02	1.72± 0.05
Pancreas (g)	0.50±0.01	0.41± 0.02	0.44± 0.03	0.48± 0.03	0.48± 0.03
Kidney (g)	0.55±0.01	0.52± 0.01	0.52± 0.01	0.53± 0.02	0.53± 0.00

Table: 6 Female rat organs weight (g) in the Gramine subacute toxicity study. Data expressed as mean ± S.E.M.N= 5. Significantly different from the control, *p< 0.05

Table 7. The Effect of Gramine on Electrolytes Levels (Male).

Animal (Male)	Control (mg/kg)	Ga Groups			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Na (mmol/L)	144.3±2.02	143.4± 1.93	141.8± 0.80	138.5±0.92*	143.6±1.09
K (mmol/L)	4.47± 0.06	5.07± 0.08	4.93± 0.11	6.13 ± 0.13*	5.79± 0.09
Cl (mmol/L)	103.9± 0.66	102.9± 0.35	102.6± 0.63	102.6± 0.90	103.8±0.80
sCr (mg/dl)	0.84 ± 0.03	0.71± 0.02	0.733±0.02	0.66± 0.03	0.72± 0.01
Urea (mmol/L)	6.20± 0.17	5.80±0.26	5.83± 0.29	5.80± 0.17*	5.90± 0.11

Table 7:- Male rats serum electrolytes values in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * p < 0.05. Na: Sodium, K: Potassium, Cl: Chlorine, sCr: Serum Creatinine

Table 8. The Effect of Gramine on Electrolytes Levels (female)

Animal (Female)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Na (mmol/L)	139.0±0.92	138.3±0.82	140.2±0.44	140.0±0.69	140.7± 0.89
K (mmol/L)	5.33±0.02	5.04±5.04	4.93±0.20	5.12±0.17	5.11±0.11
Cl (mmol/L)	99.67±0.46	102.0±0.84	101.6±0.70	101.7±0.99	103.0±0.45
sCr (mg/dl)	0.76±0.03	0.66±0.02	0.72±0.01	0.83±0.02*	0.76±0.02
Urea (mmol/L)	6.96±0.12	6.96±0.12	6.66±0.13	6.86±0.18	6.93±0.08

Table 8:- Female rats serum electrolytes values in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * p < 0.05. Na: Sodium, K: Potassium, Cl: Chlorine, sCr: Serum Creatinine.

control animals (Tables 5 and 6). Group Satellite does not indicate any significant alteration ($p > 0.05$).

Effect of Gramine on Electrolytes Levels

Serum electrolyte analysis reveals that in Gramine-treated males (Table 7), sodium, potassium, and Urea levels were increased at a dose of 55 mg/kg in the kidney function test (*$p < 0.05$). The amounts of sodium, potassium, Chloride, urea, and creatinine in the treated groups (13 and 27.5 mg/kg) did not differ substantially from those in the control group. Females treated with gramine showed only elevated levels of creatinine and a significant change (*$p < 0.05$) in serum electrolyte levels compared with the control group. Other electrolytes were observed as normal. There was a significantly elevated level of sodium, potassium, urea, and creatinine, all of which were brought back down to normal in the satellite groups.

Effect of Gramine on Biochemical Parameters

In the liver function test, Alanine transaminase levels decreased significantly in the

Table 9. The Effect of Gramine on Biochemical Parameters (male)

Animal (Male)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
TP (g/dl)	7.93±0.06	8.01± 0.23	7.79± 0.11	7.80± 0.02	8.08± 0.10
Albumin(g/dl)	3.86±0.12	4.00± 0.11	4.03± 0.12	4.03± 0.12	4.03± 0.17
ALP (IU/L)	86.43±1.71	71.11±5.71	84.33±3.792	89.76± 4.51	88.03±1.208
AST (IU/L)	194.7±4.09	179.0±7.27	186.9±5.31	187.6±7.27	188.3± 5.71
ALT (IU/L)	86.00±5.68	87.67±5.20	68.17± 5.93	63.33±4.70*	82.33± 5.36
Bilirubin (mol/L)	2.59±0.22	2.06±0.22	2.57±1.02	2.55±0.45	2.49±0.63

Table 9:-Male rats biochemical parameters in the serum in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

Table 10. The Effect of Gramine on Biochemical Parameters (female)

Animal (Female)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
TP (g/dl)	8.55± 0.31	8.01± 0.29	7.99± 0.15	8.12± 0.07	8.02± 0.14
Albumin(g/dl)	4.03± 0.12	3.90± 0.15	4.40± 0.26	4.16± 0.20	4.10± 0.26
ALP (IU/L)	78.20± 9.30	75.43± 3.61	79.87± 6.15	81.90± 8.04	81.00±3.00
AST (IU/L)	203.0 ±3.48	186.9± 6.51	189.7± 6.23	192.0± 5.28	197.1±4.20
ALT (IU/L)	86.42± 2.39	85.33± 6.11	83.82± 4.12	83.29± 2.03	84.28±3.22
Bilirubin(mol/L)	2.42±0.22	2.11±0.13	2.18±1.26	2.19±0.68	2.2±0.78

Table 10:- Female rats biochemical parameters in the serum in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

Table 11. The Effect of Gramine on Serum Lipid Profile and Glucose (male)

Animal (Male)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
TC (mmol/L)	52.43±2.63	63.18±2.36	45.34±6.47	87.59±2.637	86.05±4.63
TG (mmol/L)	128.3±7.83	97.98±1.44	92.12±1.16	89.78±0.950	101.9±2.41
HDL(mmol/L)	48.50± 2.80	45.64±4.63	42.18±0.15*	41.60±0.62*	48.37±2.16
Glucose	98.07±2.41	90.43±12.06	90.97±8.78	90.20±10.72	86.90±10.28

Table 11:- Male rats plasma glucose levels and lipid profiles in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

male group treated with 55mg/kg Gramine (* $p < 0.05$), but other TP, ALP, AST, Bilirubin, and Albumin levels did not alter in other treated groups in male as well as female animals when compared to the Control Group. Alanine transaminase (ALT) levels decreased and then recovered to normal in satellite groups (Tables 9 and 10).

Table 12. The Effect of Gramine on Serum Lipid Profile and Glucose (female)

Animal (Female)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
TC (mmol/L)	60.34±3.37	69.74±14.30	119.6±9.97	129.2±16.11	89.14±0.77
TG (mmol/L)	132.2±4.38	137.8±8.23	149.1±7.24	170.4±12.40	130.3±6.96
HDL(mmol/L)	50.13± 2.76	40.59±0.58	38.56±3.00*	34.50±2.96*	45.84±0.42
Glucose	99.02±2.64	93.40± 6.97	96.40±5.87	95.47±4.99	96.33±3.42

Table 12:- Female rats plasma glucose levels and lipid profiles in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

Table 13. Effect of Gramine on Plasma Haematological Parameters (male)

Animal (Male)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Hb (g/dl)	14.03±0.49	14.34±0.63	13.65±0.35	13.94±0.46	14.18±0.16
RBC(mill/cu.mm)	7.96±0.35	7.56±0.29	7.38±0.16	7.66±0.29	7.67±0.09
Haematocrite(%)	39.73±1.12	37.00±2.81	34.83±34.83	38.00±1.19	37.87±1.37
MCV (fL)	71.25±11.85	69.84±10.18	64.22±15.56	61.21±12.68	70.96±10.44
MCHC (%)	35.28±1.47	35.06±1.19	34.76±2.19	34.80±0.63	36.92±0.16
WBC(×103/mm3)	8.73±0.46	9.18±0.81	7.87±1.02	8.27±0.89	7.93±0.71
NEUTRO (%)	34.00±6.65	28.67±5.69	23.00±4.72	24.00±3.46	21.67±1.45
LYMPH (%)	61.00 ±8.02	70.00 ±1.15	71.33±1.45	69.67±1.45	69.00±1.00
MONOS (%)	4.66±0.33	5.66±0.33	4.33±0.33	4.00±0.0	5.00±0.57
EOSINO (%)	2.33±0.33	2.33±0.33	1.66±0.33	2.00±0.57	2.33±0.33
PLT (×103/mm3)	386.0±74.72	469.0±50.85	526.0±49.22	581.7±104.4	461.7±29.48

Table 13:- Male rats Serum haematological values in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

Table 14. Effect of Gramine on Plasma Haematological Parameters (female)

Animal (Female)	Control (mg/kg)	Ga Group			
		13mg/kg	27.5mg/kg	55mg/kg	Satellite
Hb (g/dl)	13.33±0.25	13.34±0.26	12.65±0.22	12.93±0.11	13.51±0.26
RBC(mill/cu.mm)	6.05±0.53	6.59±0.27	5.97±0.43	7.00±0.10	6.09±0.41
Haematocrite(%)	38.32±0.43	40.05±0.24	39.17±0.56	37.16±0.76	38.38±0.43
MCV (fL)	67.57±12.03	64.64±8.75	58.99±13.12	58.78±12.05	67.86±8.93
MCHC (%)	32.88±1.73	33.49±0.94	32.49±1.32	33.06±0.45	33.75±0.90
WBC(×103/mm3)	7.69±0.98	7.68±0.37	7.35±0.81	7.26±0.70	7.32±0.51
NEUTRO (%)	31.67±7.26	26.33±4.48	24.67±4.66	24.33±4.91	20.33±0.88
LYMPH (%)	61.33±6.88	69.00±1.52	70.00±1.15	68.33±0.88	68.67±0.88
MONOS (%)	4.33±0.33	4.66±0.33	3.66±0.33	3.66±0.33	4.00±0.57
EOSINO (%)	2.33±0.332	2.33±0.33	2.66±0.33	2.00±0.57	2.33±0.33
PLT (×103/mm3)	441.7±46.56	451.7±59.48	507.7±56.26	567.7±110.2	498.0±21.96

Table 14:- Female rats Serum haematological values in the sub-acute toxicity study of Gramine. Data expressed as the mean ± S.E.M. N= 5. Significantly different from the control, * $p < 0.05$.

Effect of Gramine on Serum Lipid Profile and Glucose

Male and Female animals treated with Gramine 55mg /kg showed decreases in HDL (high density lipoproteins) when compared to the corresponding control groups. However, in neither the male nor female groups tested, the levels of total cholesterol (TC), triglycerides (TG), nor glucose differed significantly from the control group. Furthermore, decreased levels of HDL were observed in the satellite group.

Effect of Gramine on Plasma Haematological Parameters

Tables 13 and 14 summarise the results of all haematological test parameters in rats after subacute treatment. This hemological treatment included haemoglobin count, Erythrocyte cell count, hemocrite count, Mean corpuscular volume count, mean corpuscular haemoglobin concentration (MCHC), differential leukocyte count (WBC), and platelet count (PLT), which were observed in the normal range when compared with the control group. There were no toxicologically significant differences ($P > 0.05$) between different gramine-treated groups, the satellite group, and the normal control group.

Histopathology

Effect of Gramine on histological parameters

During this histological analysis, we took into consideration different organs like the brain, kidneys, and liver of rats. Which revealed that there were no significant changes observed at doses (13, 27.5, and 55mg/kg) and satellite groups compared with the control group, as shown in Figs. 3, 4, and 5.

Detail studies

Brain: In the brain cells of any treated group of rats with Gramine, no noticeable damage (such as neuronal ischemia, neural cell death, or undamaged glial cells) was seen. The brain cells' architecture displayed normal.

Kidney: No significant alteration occurred in the architecture of the kidneys of any group of rats administered Gramine. Under a microscope, the medullary and cortical parts of the kidney, such as the glomeruli and renal tubules, looked

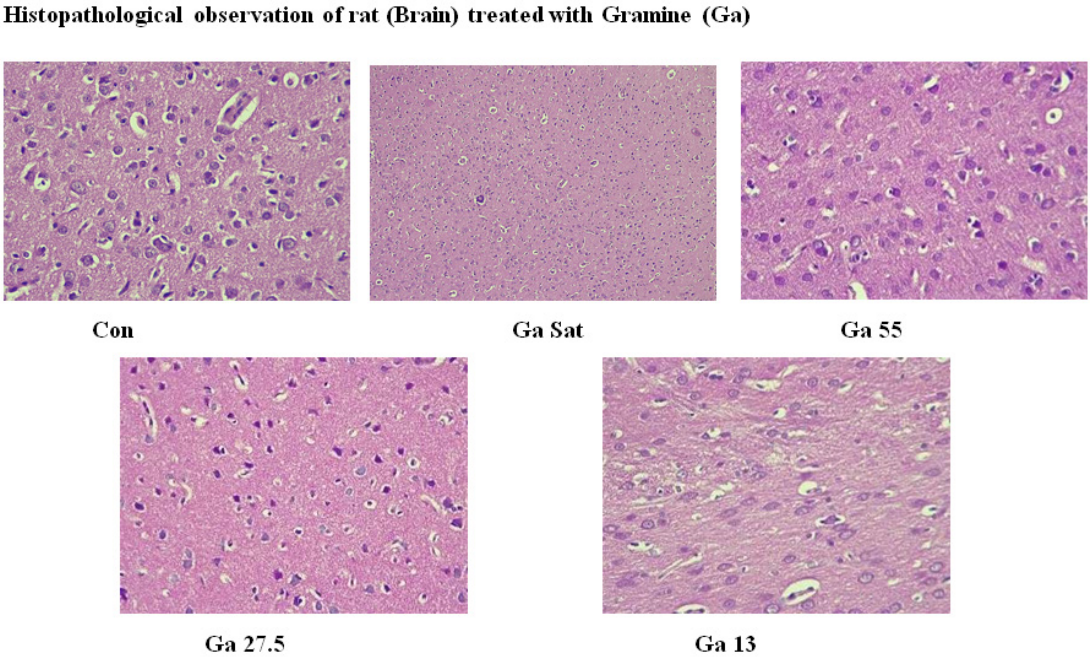

Fig. 3. The histopath evaluation of brain from the different treated group of rats shows no significant damage (ex. neuronal ischemia, neural cell death or undamaged glial cells) was observed in the brain cells when compare with control group rats. The architecture of brain cells is normal. Where Con: Normal control group; Ga sat: Ga satelite group; Ga 55: Ga group with dose (55 mg/kg/p.o); Ga 27.5: Ga group with dose (27.5 mg/kg/p.o); Ga 13: Ga group with dose (13 mg/kg/p.o)

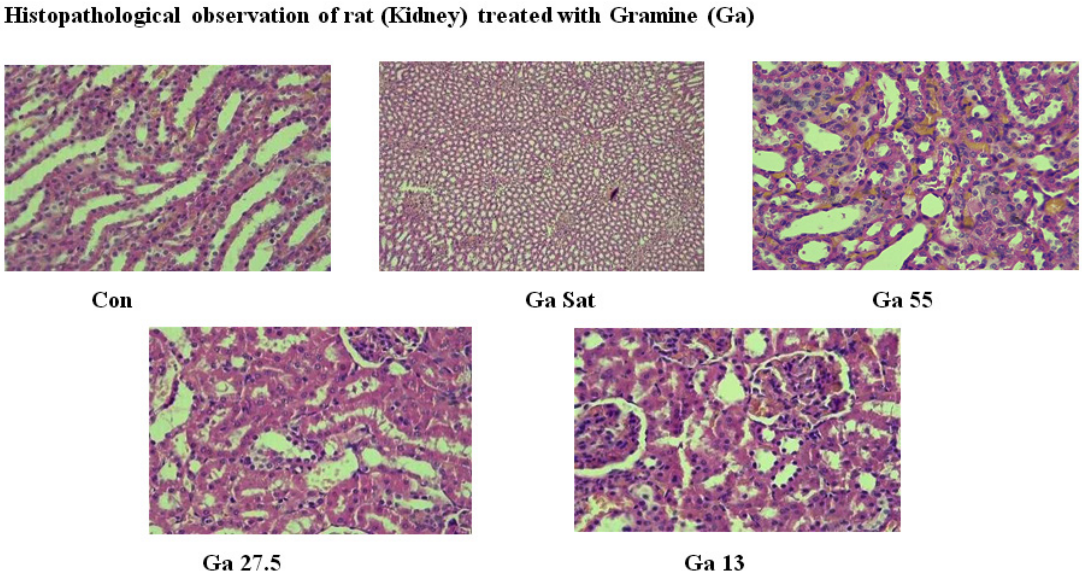

Fig. 4. The histopath evaluation of kidney from the different treated group of rats shows no significant damage. In all treatment groups, microscopic examination of the kidney revealed normal architecture of the medullary and cortical structures, including glomeruli and renal tubules. When compared to a normal control group, no histological abnormalities were identified in the kidney. Where Con: Normal control group; Ga sat: Ga satelite group; Ga 55: Ga group with dose (55 mg/kg/p.o); Ga 27.5: Ga group with dose (27.5 mg/kg/p.o); Ga 13: Ga group with dose (13 mg/kg/p.o)

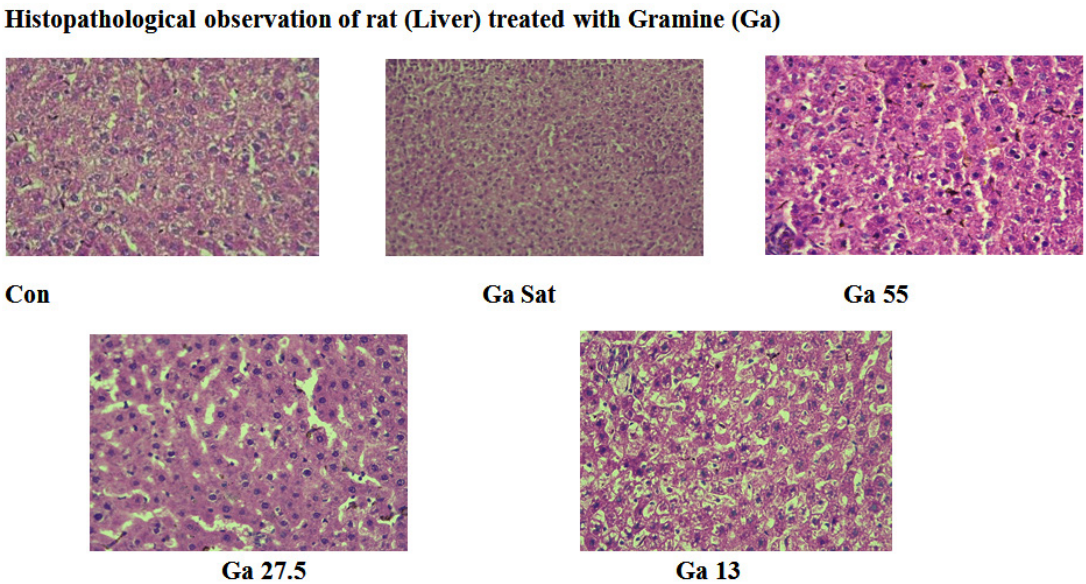

Fig. 5. The histopath evaluation of Liver from the different treated group of rats shows no significant damage. The tissue displayed intact hepatic hepatocytes and central vein. No significant steatosis, hepatic fibrosis and lobular and portal inflammation was observed in the liver hepatocytes, when compared to normal control group. Where Con: Normal control group; Ga sat: Ga satelite group; Ga 55: Ga group with dose (55 mg/kg/p.o); Ga 27.5: Ga group with dose (27.5 mg/kg/p.o); Ga 13: Ga group with dose (13 mg/kg/p.o)

normal in all of the treatment groups. There were no pathological problems found in the kidney.

Liver: The liver sections revealed normal hepatic hepatocytes and a normal central vein. In the liver tissue (hepatocytes), no significant observation of steatosis, hepatic fibrosis, or lobular and portal inflammation was observed when treated with Gramine in rats.

DISCUSSION

Traditional medicines are now very popular for treating a variety of diseases and disorders all over the world, especially in developing countries. A few reasons behind this are cost effectiveness and natural ease of access. Oral route of administration of those medicines or phytoconstiuents is more frequently used, but dose-correlated safety studies and a lack of sufficient scientific research attract attention towards their toxicological study[13]. Bioactive constituents play a vital role in the treatment of chronic diseases, so they may be a good alternative to allopathic medicine[14]. If we are using those active phytoconstituents for a longer duration of time for chronic problems, it may produce undesirable and toxic effects that we can't ignore. There is no published specific acute and subacute study data on Gramine, so this article puts emphasis on it.

Gramine possesses many more pharmacological properties like antibacterial activity, antineoplastic activity, antiviral activity, protein kinase inhibitory effect, regulation of bronchial smooth muscle activity, vasodilator activity acting on the 5-HT2A receptor, maintaining blood pressure, and controlling mitochondrial energy metabolism[15].

This Current acute toxicity study provides preliminary information about the toxic approach of the drug Gramine, which will be helpful in the next approach for the selection of dose in the next toxicological studies. In this article, acute oral toxicity was performed following OECD Guidelines 425. As per the available guidelines of the OECD and literature, it is observed that gramine is toxic in nature for insects, and because very little safety data is available related to gramine, we decided to perform the main test of the OECD 425 guideline. Gramine shows mortality at a dose of 550 mg/kg, followed by software (AOT425StatPgm), but no mortality or significant toxicity signs or Symptoms at doses of 1.75, 55, and 175mg/kg. Furthermore, no internal organ alterations were detected following the acute toxicity study period (14 days) compared to the control group. So the drugs are safe at a limited dose. Which can be used for the next subacute toxicity study.

Animals were given oral dosages of Gramine (13, 27.5, and 55 mg/kg), along with a satellite group, for 28 days in this subacute toxicity study. There was no animal mortality, no change in water consumption, and no change in organ weight, but particular significant changes in food consumption and animal weight were noticed when compared to the control group (tables 2, 3, and 4). Animal weight also explains good health in animals[16]. Organ weights are indicators of an animal's metabolic and pathological health status, and they are also indicators of toxicity in animals as evaluated by toxicity testing[17, 18, 19]. Administration of active constituents by oral route may sometimes be toxic to vital organs of the body like the liver, kidney, heart, brain, etc., which play an important role in normal physiological conditions[20]. This toxicity study found no significant changes in organ weight in the test group or satellite group when compared with the control group (tables 5 and 6). This study suggested that Gramine is nontoxic to animals at a given dose. Longer use of drugs shows a reduction in body weight. After completing the sub-acute toxicity study, animals show a rise in body weight (Tables 3 and 4).

Serum Electrolyte level after treatment of Gramine Illustrations: male (table 7) and female (table 8), sodium and urea levels decrease significantly at dose 55 mg/kg, potassium levels increase at the same dose in males, and creatinine levels increase in females when compared with the control group. It shows a slight alteration during treatment with Gramine. Other electrolytes do not show any significant changes in all other groups. The satellite group shows a normal level of all electrolytes after the completion of the experiment.

In this study, our Gramine drug showed an effect (tables 9 and 10) on biochemical parameters, where serum alanine levels significantly increased at doses of 55 mg/kg when compared with the control group in males. Other side effects, such as total protein (TP), Albumin, ALP, AST, ALT,

and bilirubin, were observed as normal in female animal groups. So this drug could be normal for liver functions[21].

Serum lipid profile and glucose level revealed after complete treatment of 28 days, where high density lipoprotein in serum levels was significantly decreased when compared with the normal control group. No significant changes were observed in TC, TG, or glucose levels (tables 11 and 12).

An evaluation of hemological parameters plays a vital role in studying the good health status of animals. Our hemological parameters do not show any remarkable changes in Hb, RBC, hemocrite, MCV, or MCHC. Red blood cell morphology, haemorrhage, leukaemia, erythropoiesis, and osmotic frugality were not significantly altered by Gramine, indicating that it has no effect on red blood cell morphology, haemorrhage, leukaemia, erythropoiesis, or osmotic frugality[22]. Because no significant changes were seen in the values of the WBC, NEUTRO, LYMPH, MONOS, and EOSINO levels, it was clear that gramine was a safe medicine because changes in the WBCs were associated with tissue damage, infections, and inflammatory responses. Platelet (PLT) count increased during the study, but no significance was observed when compared with the control group. (Tables 13 and 14).

Histopathological features of the brain, liver, and Kidney were displayed in Figs. 3, 4, and 5. No specific architectural or Morphological changes were observed in the brain, kidney, or Liver tissues. Glial cells were observed intact in the brain, with no sign of necrosis. Glomerular and renal cells were observed as normal. Hepatocyte cells and central veins are observed as normal when all are compared with the control group. The satellite group also exhibited no morphological or structural changes[23]. All the above discussions indicate that Gramine is a safe and nontoxic drug in these selected doses. If some changes were observed in electrolytes, serum was recovered after washing.

CONCLUSION

In our acute oral toxicity study, there were no signs of toxicity symptoms produced by Gamine at a 175mg/kg dose, but 550mg/kg showed a lethal effect on animals. 550mg/kg is considered the LD50 of Gamine. A subacute study showed weight reduction in animals at all doses selected for study but not any specific toxic effect not observed in a haematological or histopathological study during or after 28 days of treatment. So it is considered that Gramine is safe to use in a selected range for further preclinical and clinical study.

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Conflict of Interests/Competing Interests

"Conflict of interest: The authors declare no conflict of interest."

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