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Physicochemical, Phytochemical Investigations, Toxicity Studies of Herbal Drug Mono Ammonium Glycyrrhizinate to Assess its Antiobesity Activity in Wistar Rats

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ABSTRACT

Background: Mono Ammonium Glycyrrhizinate a standardized herbal powder is rich in antioxidants derived from the *Glycyrrhiza glabra* plant. The herbal powder has strong free radical scavenging activity, anti-inflammatory activity, and has an anti-obesity effect**. Objective:** The prominent aim of this study was to assess the safety standard of herbal powder by acute and sub-acute toxicity study experimental design in Wistar rats as well as to determine its phytochemical and physicochemical properties. **Methods:** In acute oral toxicity single limit dose of 2000 mg/kg was administered to a Wistar rat and was observed for 4 hours strictly for any sign of mortality and morbidity. After no sign of mortality was observed, Wistar rats were divided into 5 groups. Group 1 was given vehicle and rest treatment groups were administered doses of 300 mg/kg, 500 mg/kg, 700 mg/kg, and 1000 mg/kg body once a day. After 48 hours when no sign of morbidity and mortality was observed, test groups were given a higher dose of 500 mg/kg, 700 mg/ kg, 1000 mg/kg, and 2000 mg/kg body weight respectively and animals were kept under observation for 14 days. In subacute oral toxicity, animals were divided into one control and four treatment groups. Each group was administered with a dose of 300 mg/kg, 700 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight once a day for 28 days. Animals were observed for the sign of mortality and morbidity for 28 days. **Results:** In the acute oral toxicity study, no sign of mortality was observed in test animals, no changes in biochemical parameters were observed. No loss in weight and no behavioral uneasiness was noted. In the subacute oral toxicity study, no signs of lesions were observed on animal vital organs during the histopathological study. Loss in body weight was observed in test groups up to an extent. **Conclusion:** The complete study hereby shows that herbal powder has no toxic effects on animals. Hence, it is safe to administer animals for a longer duration up to one month.

INTRODUCTION

Obesity is a complicated disease nowadays, has spread across the world population. Obesity is a chronic disorder and is highly complex in terms of etiology. The term is classified by Body Mass Index (BMI). Overweight along with obesity has become a leading threat to human health and life.[1] The prevalence rate of obesity is proceeding across the world at a high rate. Obesity is associated with many other disorders like hypertension, gallbladder diseases, bone disorders osteoarthritis, and coronary disorders.[2] Obesity is indicated as a non-communicable disorder as per World Health (WHO). The metabolic disorder is the consequence of several factors including genetics, lifestyle, and dietary pattern, and working pattern.[3] According to WHO, obesity, as well as overweight, is defined as "odd and exaggerated aggregation of fat which influences health".

A person is said to be obese if assessing BMI rate 30 or above it. The excessive energy accumulation specifically in adipocytes accounts increment in the progression of the lipolysis phenomenon, as per its consequence, leukocytes infiltration proceeds cytokines secretion, macrophages produces adipocytes inflammation, leading to a state of pro-inflammation, dysfunction of endothelium, and insulin resistance.[4] The frequency of disease prevalence has been doubled in the world population since up to 13% among whom 15% of women and 11% of men comprise the total blood population. The enormous expansion in body fat has serious constrains of serious metabolic syndromes and disorders.[5] The first and foremost treatment for obese patients is an alteration in lifestyle along with a restricted diet.[6] Herbal formulations are highly effective and possess almost negligible side effects. The active chemicals present in these plants are responsible for the therapeutic effects with a defined mechanism of action on the human body.[7]

Glycyrrhiza glabra is well known for its beneficial medicinal property. In north India, *Glycyrrhiza glabra* is commonly known as Mulaithi. The herb is obtained from *Glycyrrhiza glabra* Linn, belongs to the kingdom Plantae, family Leguminosae and the class is Dicotyledoneae. The plant is perennial, approx. 1m heights having the pinnate type of leaves, leaflets are 9 to 17 in number and length is approx. 7 to 15 cm.[8] The active constituents present in this herb are saponin, amino acids, mineral salts, mucilage, gums, polysaccharides, sterols, and the herb contains a salt of magnesium and calcium of glycyrrhizic acid.[9] Pharmacologically the herb act as antimicrobial, antitussive, antimicrobial, expectorant, antiviral, antioxidant and anti-inflammatory, antimutagenic,

hepatoprotective, antidiabetic, and antiulcer.[10] Mono-ammonium glycyrrhizinate chemically called 20 β- carboxy- 11- oxo – 30 – norolean –12 – en - 3β- yl- 2- O – β- glucopyranuronosyl – α- D – glucopyranusiduronic ammonium salt. Also known as glycyrrhizic acid. It is obtained from *Glycyrrhiza glabra L*, which is light yellow, having a molecular weight of 840 and pH 4.5.[11]

MATERIALS AND METHODS

Collection and Identification of Plant Material

Glycyrrhiza glabra roots were purchased from the local market and authentication has been done by Christ Church College, Kanpur, Botany Lab by (PSIT/SPECIMEN-376 /2019-20).

Extraction Procedure

Glycyrrhiza *glabra* roots were washed and were shed dried for 2 days. The dried licorice roots were crushed into small pieces into mortar pestle. The obtained crushed licorice roots were converted into fine powder through an electric mixture grinder. The obtained powder was sieved and macerated with the solvent mixture of acetone and dilute nitric acid for 2 hours. The contents filtered and an additional 20 ml of acetone was added to the marc and warmed gently. The contents were filtered using what man's filter paper and the filtrate was obtained. To the filtrate sufficient volume of dilute ammonia solution was added until precipitation of ammonium glycyrrhizinate is completed. The precipitate was collected and washed with 5 ml of acetone, dried, and collected.[12]

Pharmacognostic Evaluation

Determination of Foreign Matter

The foreign matter was inspected by following standard procedures. Approx. 100 g of MAG was spread out forming a thin layer. The foreign matters were detected by using a lens (10 X). The foreign matter was separated, weighed and the percentage of foreign matter was calculated.[13]

Organoleptic Evaluation

The evaluation of color, texture, odor, and taste is termed as Organoleptic Evaluation. The powder was evaluated with the naked eye (Figure 1). [14]

Physicochemical Analysis

Physicochemical characteristics of the powder were carried out as per Ayurvedic Pharmacopoeia of Indian. pH determination, Total Ash Value, Acid Insoluble Ash Value, and Water-Soluble Ash Value were determined (Figure 2).[15]

Determination of Physical Characteristics of the Powder

Physical properties of powder including the angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio were determined.[16- 18]

Phytochemical Investigation

Several qualitative phytochemical tests for alkaloids, tannins, steroids, glycoside, flavonoids, and terpenoids were performed (Figure 3).[19]

pH Determination

The pH of the sample was determined by using pH meter MAG was dissolved in 10 ml of distilled water and pH was recorded.[13]

Spectral Analysis

Ultraviolet -Visible Spectroscopy

The absorbance of herbal powder has been taken on 252 λ max. The stock solution of the herbal powder was prepared by using a solvent mixture of methanol and water in the ratio 70:30.

IR Spectroscopy

IR spectroscopy of the herbal powder was done and interpretation was done to identify the functional groups.

Toxicity Study

Experimental Animals

Male and female Wistar rats were procured by Animal House PSIT, Kanpur. All the animals were kept in clean and dry polypropylene cages. Temperature was maintained at 22± 2ºC. 12-

hour light and 12-hour dark atmosphere was maintained. Animals were fed with a normal chow diet and water was given *ad libitum.* Animals were acclimatized for 5 days.

Approval from the Animal Ethics Committee

The approval of the Animal Ethical Committee of PSIT, Kanpur was taken, thereafter the study was conducted. University Reference Number: **CPCSEA/AC/1273.**

Acute Oral Toxicity

The study was designed as per the Organization for Economic Co-operation and Development (OECD) revised draft guidelines no. 423 (acute oral toxicity). The study was done following standard procedures step by step. At each dose level 3 animals were utilized. First dose administered was of 300 mg/kg body weight. Further, as per the sign regarding the presence of mortality and morbidity related compound in test substance, the dose was either elevated to a higher level or minimized to determine the LD_{50} value. The next step was being decided on the basis of mortality and morbidity observed in animals. Next steps involved –

- No testing required further
- Same dose administered to 3 additional animals
- Dosing 3 animals with the next higher dose level or lower dose level

Selection of Animals

All the requirements of the selection of animal species, housing and feeding conditions, preparation, and administration of doses were followed as per the OECD Guidelines. Male and female Wistar Rats of weight 120± 10 g having age 10- 13 weeks were selected.

Housing and Feeding Conditions

The temperature was maintained at 22 ± 2ºC. 12-hour dark and 12-hour lightning was provided and animals were provided food and water adequately.

Preparation of Animals

Animals were selected randomly and were marked and kept in a clean polypropylene cage. Animals were further acclimatized for 1 week.

Preparation of Doses

The standardized herbal drug was dissolved in distilled water and filtered to obtained aqueous extract of Mono Ammonium Glycyrrhizinate (MAG).

Administration of Doses

Animals fasted overnight, were weighed and test substance was administered through oral gavage in a single dose. Animals were kept deprived of food for 4 hours after dose administration.

At each dose level, 3 animals were taken at a particular time in each test group. The first dose given was of 300 mg/ kg body weight and after no morbidity and mortality observed, the maximum dose given was up to 2000 mg/kg in a successive manner. Further LD_{50} value was determined as per observations made.

Observation

Animals were kept in strict observation for the first 30 to 60 minutes. Further were kept under observation for 4 hours. Further were observed for 24 hours routinely for 14 days.

Sub-Acute Toxicity

Before dose administration, all the procedures were followed similarly as in acute oral toxicity**.** Male Wistar rats were divided into 5 groups (Group I – IV). The Group I was considered as control, the only vehicle was given a single time in a day for 28 days. Group II was given dose 300 mg /kg body weight orally, Group III was given 700 mg/kg body weight, Group IV was given 1000 mg/kg body weight, and Group V was given 2000 mg/kg body weight of aqueous extract of Mono ammonium glycyrrhizinate single time in a day for 28 days. The animals were kept under observation for the sign of morbidity and mortality for 28 days. (Figure 4,5)

Foreign Matter

The foreign matter was weighed and the percentage was calculated. Result obtained was 0.15 %, hence the herbal formulation was pure as the value obtained was within the range concerning reference value given in Ayurvedic Pharmacopeia of India (API). The total ash of herbal powder was determined as per standard procedure and the percentage was calculated. The result obtained was within the range of reference value as in API. The result for acid-

insoluble and water-soluble ash value was obtained within the normal range as per API. Tabulated in Table 1.

Organoleptic Evaluation

Organoleptic evaluation of herbal powder indicated that the color of the powder is pale yellow, the odor is pleasantly sweet, the texture of the powder is very fine and the taste is sweet followed by mucilaginous taste. Observations are tabulated in Table 2.

Rheological Study

The rheological study was done by calculating Hausner's ratio, the car's compressibility index determination, and angle of repose determination. For calculating Hausner's ratio and Carr's compressibility index, bulk density (ρb) was calculated using formula ((ρb) = M / Vo), and tapped density of herbal powder was determined using formula ρtap = M / Vf. The obtained value from the angle of repose was 26 which indicated that the relative followability of herbal powder was excellent. Similarly, the value of car's compressibility index was calculated using formula (Carr's index = [(ρtap - ρb) / ρtap] / ×100). Result obtained was 0.377 which again indicates the relative followability of powder was excellent. Hausner's ratio was calculated using the formula (Hausner's Ratio= ρtap/ ρb). All these rheological parameters indicated that the relative followability of the herbal powder was excellent. The results are tabulated in Table 3.

Phytochemical Test

The phytochemical test had been done to acknowledge the presence of different active chemical constituents. Test for alkaloids, flavonoids, saponins, proteins, phytosterols, and glycosides had been done. The observations made had indicated the presence of flavonoids, saponins, phytosterols, and glycosides. Listed in Table 4.

Spectral Analysis

A small amount of powder sample was projected to IR spectroscopy and function groups peaks were recorded and interpreted. The graph showed the presence of carbonyl group (CO), hydroxide(OH), amino group (NH), presence of alkene (C=C), and presence of alkane group (C- C). Figure 6 represents the IR spectra of Mono ammonium glycyrrhizinate powder.

pH value

An aqueous solution of mono ammonium glycyrrhizinate was prepared. pH meter was calibrated and pH was recorded three times. The mean value was calculated. 5.41 ± 0.01. This stated that mono ammonium glycyrrhizinate is acidic.

RESULTS:

No symptoms of morbidity and mortality were recorded after the completion of study. No sign of lesions was observed on the vital organs during histopathology. (Figure 5)

No sign of morbidity and mortality was observed in test groups at the completion of the study. When a higher dose of 2000 mg/kg body weight was given no toxicity parameters were observed to be elevated. The drug was safe up to the maximum dose level.

Acute Oral Toxicity Result

The result of acute oral toxicity was obtained based on behavioral parameter observation. The animals were observed for 14 days at a maximum dose of 2000 mg/kg body weight. No morbidity was observed. Result tabulated in Table 5 and Table 6.

Behavioral parameters were observed and were found normal no changes in skin fur, color and texture were observed. Color and movement of eyes were normal, respiration was normal, urination frequency was normal, no itching, convulsions, restlessness was observed. All the parameter results have been tabulated in Table 6.

Sub-acute Oral Toxicity Result

Bodyweight

Bodyweight of normal control and treated animals were recorded and observation in weight variation between treatment and control group has been analyzed. During the sub-acute toxicity study changes in body weight of the control and all the treatment groups were recorded. The weight of control was seen to be gradually increasing between days 1 to day 28. The increase in weight of control animals was recorded from day 7 to day 28. Among treatment groups changes in weight were observed gradually with the passage of the day. At the completion of the study, the weight of treatment groups was found to fall in comparison with their weight at the initial week of study as shown in Figure 6. Treatment groups receiving a dose of 300 and 700 mg/ kg body weight didn't show a major difference in

weight. However, the treatment group receiving a dose of 1000 and 2000 mg/kg body weight showed a significant fall in weight. Graphically represented in Figure 7.

Individual Organ Weight

The weight of vital organs like heart, kidney, liver, spleen and lungs was recorded. There was no sign of toxic effects of aqueous MAG extract was seen on the vital organs of the treatment group. A significant variation in weights of the treatment group was observed as compared to the control group animal's vital organ weights as it is represented by the graphical representation in Figure 8.

Liver Function

Liver enzymes SGOT, SGPT, and Serum Phosphatase of control and treatment groups were estimated and were found in the normal range. No significant changes were observed. The blood serum enzymes of the treatment groups receiving dose 300 mg/kg and 700 mg/ kg body weight showed a minor drop in SGPT in comparison to the control and treatment group receiving 300 mg/kg body weight dose shown a minor rise in SGOT in level. However, the treatment group receiving dose 700mg/kg, 1000 mg/kg, and 2000 mg/kg body weight had shown a fall in SGPT and SGPT level in comparison to the control. Graphically represented in Figure 9.

Kidney Profile

Serum urea of the control and treatment group were estimated. The concentration of serum urea of control and treatment groups was compared and the treatment group receiving dose 300 mg/kg and /kg had shown a minor rise in serum urea in comparison to the control group. However, the treatment group receiving dose 1000 mg/kg and 2000 mg/kg body weight had shown a fall in serum urea concentration in comparison to the control group. Graphically represented in Figure 10.

Serum creatinine of control and treatment groups were estimated. The concentration of serum creatinine of treatment groups receiving dose 300 mg/kg, 700 mg/kg, 1000 mg/ kg, and 2000 mg/kg body weight was found to be slightly less in comparison with the control group. However, no major variation had been recorded. Hence, the sign of toxicity was not seen on the creatinine level of the treatment groups. The graphical representation is given in Figure 11.

Hematological Parameters

All the Hematological Parameters of normal control and treatment groups were estimated. hemoglobin, total leucocyte count, lymphocytes, monocytes, eosinophils, platelet count, total RBC, packed cell volume, mean cell volume, mean haemoglobin, and mean cell haemoglobin were estimated for control and treatment groups. No sign of toxicity was observed on the blood parameters. No major differences had been observed in the values of the treatment group and the control group. The data is tabulated in Table 7.

Thyroid Hormone Function

The effect of aqueous MAG was observed on thyroid hormones of treatment groups. The concentration of Free Iodo Thyronine, Free Thyronine, and Thyroid Stimulating Hormone was estimated and values of treatment groups and control groups were compared. No sign of toxicity was observed as the concentration of thyroid hormones was in normal range concerning the control group. The variation in the control and treatment group observed were minor, no significant variations were observed.

Blood Sugar

The random blood sugar level of control and treatment groups were estimated. The blood sugar of treatment groups was relatively lower than the control group. The treatment group receiving a dose of 300 mg/kg had not shown major variation in random blood sugar in comparison to the control. Whereas the treatment groups receiving dose 700 mg/kg, 1000 mg/kg, and 2000 mg/kg had shown a significant fall in blood sugar level in comparison to the control group animal's blood sugar. Graphically represented in Figure 12.

Heart Function

Serum Cholesterol, VLDL Cholesterol, and LDL were estimated for the control and treatment group. The concentration of serum cholesterol, VLDL, and LDL in treatment groups receiving doses 300 mg/kg, 700 mg/kg, 900 mg/kg, and 2000 mg/kg body weight were lower in comparison to the control group. There was no sign of toxicity observed as the concentration of blood serum of the treatment groups were in the normal range. A graphical representation is given in Figure 13.

Histopathology

Tissue specimens of heart, liver, kidney, and stomach of control and treatment groups were isolated from the organ and were fixed in 10 % formalin solution after animals were sacrificed. The samples of the tissues were subjected to alcohol xylene protocol and were embedded in paraffin. The tissues were then sectioned and viewed under the light microscope and images were taken (Figure 14).

DISCUSSION

In order to proceed with any pharmacological study on animals, it is very important to evaluate the test substance or herbal formulation. It is a very important and crucial step to perform standardization of herbal formulation by analyzing its physicochemical, phytochemical, safety parameters analysis to know the toxicity and effectiveness potential of the herbal formulation. The testing and utilization of herbal formulation are widely appreciated and forwarded. As it is most frequently and widely being accepted that herbal formulations are effective with minimum and almost negligible toxicity. So the demand for evaluation of herbal preparations is at peak [6-7]. Safety of herbal powder was evaluated by acute and subacute oral toxicity after standardizing it as per standard procedures. There was no sign of toxicity was observed at a maximum dose of 2000 mg /kg, at this dose the drug didn't show any sign of toxicity in terms of morbidity and mortality. All the observations depicted that the herbal powder is safe and has antioxidant, antiobesity, fat reducing potential. It can be helpful in treatment and pharmacological study on animals for various purposes.

CONCLUSION

Standardization of herbal powder was done according to standard procedures and all the procedures adopted was according to Ayurvedic Pharmacopeia of India. The safety standard of herbal powder Mono Ammonium Glycyrrhizinate was established by conducting acute and subacute oral toxicity study on Wistar rats in accordance of OECD guideline. During and at the completion of the study no symptoms of toxicity was observed at maximum dose of 2000 mg/kg. Therefore, the herbal drug can further withstand to chronic toxicity study and can be utilized in various pharmacological studies for future.

Ethics Approval and Consent to Participate

The reported research work has been done after seeking approval from Institutional Ethical Committee of Department of Pharmacy **CPCSEA/AC/1273,** PSIT Kanpur, India.

Human and Animal Right

No utilization of humans was done in this study. All the procedures have been performed using animals under the strict OECD guidelines.

Conflict of Interest

The author declares no conflict of interest.

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Table No. 1: Result of foreign matter, total ash, acid insoluble and water soluble ash determination

S. No	Test Parameters	Observed Value (%)	Reference value (API) (%)
1	Foreign matter determination	0.15	0.5- 2
2	Total ash	0.032	Not greater than 10
3	Acid- insoluble ash	0.006	Not greater than 2.5
4	Water soluble ash	0.29	Not greater than 20

Table No. 2: Organoleptic evaluation of Mono Ammonium Glycyrrhizinate powder

S. No.	Test parameters	Observations
1	Color	Pale yellow
2	Odor	Pleasantly sweet
3	Texture	Very fine
4	Taste	Sweet

Table No. 3: Determination of flow property values

S. No.	Test Parameters	Observed Value (%)
1	Hausner's ratio	1.606
2	Car's compressibility index	0.377
3	Angle of Repose	26

Table No. 4: Phytochemical investigation of herbal powder

SI. No	Test name	Observations
		Mono ammonium glycyrrhizinate powder
1.	**Test for Alkaloids**	
	Mayer's test	-
	Dragon droff's test	-
	Hager's test	-
	Wagner's test	-
2.	**Test for Flavonoids**	
	Lead acetate test	+
	Shinoda test	+
	Ferric chloride test	+
	Zinc hydrochloride test	+
3.	**Test for Saponins**	
	Forth Forming Test	+
	Foam Test	+
4.	**Test for Phytosterols**	
	Salkowaski Test	+
5.	**Test for Glycosides**	
	Kedd's test	-
	Baljet test	-
	Test for hydroxyl glycosides	-
	Schotenten's test	-
6.	**Test for Proteins**	+
7.	**Test for**	-

(+) sign = Presence of Compound

(-) sign = Absence of Compound

Table No. 5: Acute oral toxicity dose level and morbidity assessment

Test Solution	First Phase		Second Phase	
Mono ammonium glycyrrhizinate aqueous preparation	Dose (mg/kg) bd. Wt.	Mortality observed	Dose (mg/kg) bd. Wt.	Mortality observed
	300	0/4	500	0/4
	500	0/4	700	0/4
	700	0/4	1000	0/4
	1000	0/4	2000	0/4

Key: (mg/kg) bd. Wt. = Milligram per kilogram body weight.

Table No. 6: Behavioral pattern observation of normal control and Mono Ammonium Glycyrrhizinate treated rat

Behavioral Parameters	Observation of control group and treated groups													
	At 30 Minutes		At 4 Hours		At 8 Hours		At 24 Hours		At 48 Hours		On 7th Day		On 14th Day	
	NC	TG	NC	TG	NC	TG	NC	TG	NC	TG	NC	TG	NC	TG
Fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	+	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urine color	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Faeces texture	N	+	N	+	N	+	N	+	N	+	N	+	N	+
Alertness	N	+	N	+	N	N	N	N	N	N	N	N	N	N

Restlessness	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sleep	N	+	N	+	N	N	N	N	N	N	N	N	N	N
Coma	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Convulsions	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Itching	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations used: NC = Normal Control; TG = Treatment Group; N = Normal; NS = Not Seen; (+) = Slight increased; (-) = absent

Table No. 7: Biochemical analysis of control and treatment groups

Parameters	Normal Control	TG (300) mg/kg)	TG (700) mg/kg	TG (1000) mg/kg	TG (2000) mg/kg
Haemoglobin (g/100ml)	12.3 ± 0.081	13 ± 0.054	12.6 ± 0.471	12.4 ± 0.230	12.0 ± 0.115
Total Leucocyte Count (cells/cumm)	7695 ± 4.921	9098 ± 2.054	8202 ± 4.6427	8999 ± 0.4714	8999 ± 1.527
Lymphocytes (%)	75.70 ± 0.225	74.98 ± 0.012	69.69 ± 0.433	62.24 ± 0.394	61.88 ± 0.172
Monocytes (%)	2.97 ± 0.030	4.05 ± 0.105	2.05 ± 0.106	2.03 ± 0.065	2.03 ± 0.04
Eosinophils (%)	2.00 ± 0.038	1.97 ± 0.731	3.67 ± 0.469	4.69 ± 0.484	4.66 ± 0.585
Platelet Count (%)	3.15 ± 0.053	4.09 ± 0.038	3.99 ± 0.445	4.26 ± 0.184	4.30 ± 0.110
Total RBCs Count (million/µl)	6.13 ± 0.027	6.42 ± 0.016	6.54 ± 0.016	6.66 ± 0.121	6.74 ± 0.050
Packed Cell Volume (%)	45.84 ± 0.012	48.12 ± 0.089	49.40 ± 0.070	49.62± 0.555	49.84 ± 0.321
Mean cell Volume (fl)	59.02 ± 0.012	60.29 ± 0.899	62.25 ± 0.070	62.95 ± 0.055	63.00 ± 0.032
Mean cell Haemoglobin (pg)	15.63 ± 0.447	17.00 ± 0.077	17.47 ± 0.035	17.54 ± 0.016	17.70 ± 0.083
Mean cell HaemoglobinConc (g/dl)	29.96 ± 0.065	32.01 ± 0.016	33.03 ± 0.092	32.23 ± 0.495	31.36 ± 0.550

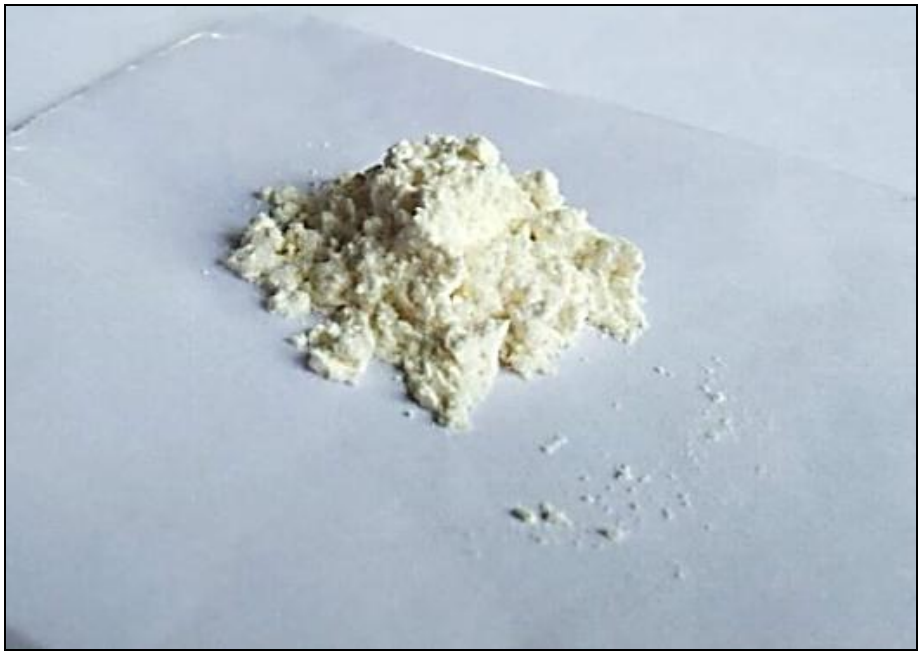


Figure No. 1: Mono Ammonium Glycyrrhizinate Powder

Figure No. 2: Physicochemical analysis of herbal powder

Figure No. 3: Phytochemical investigation of herbal powder

Figure No. 4: Dissection of animal after sub-acute oral toxicity study

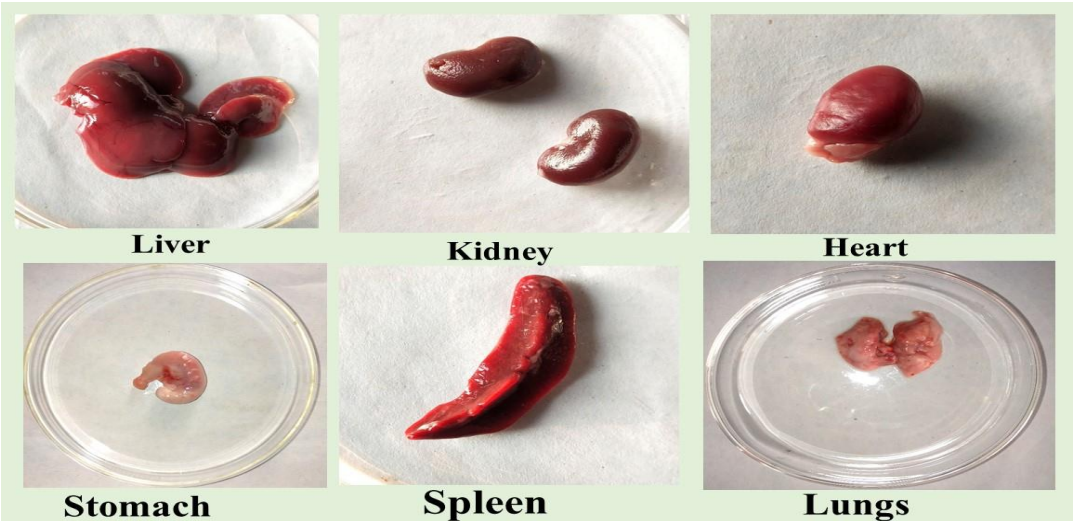


Figure No. 5: Vital organ of animal isolated after dissection of animals

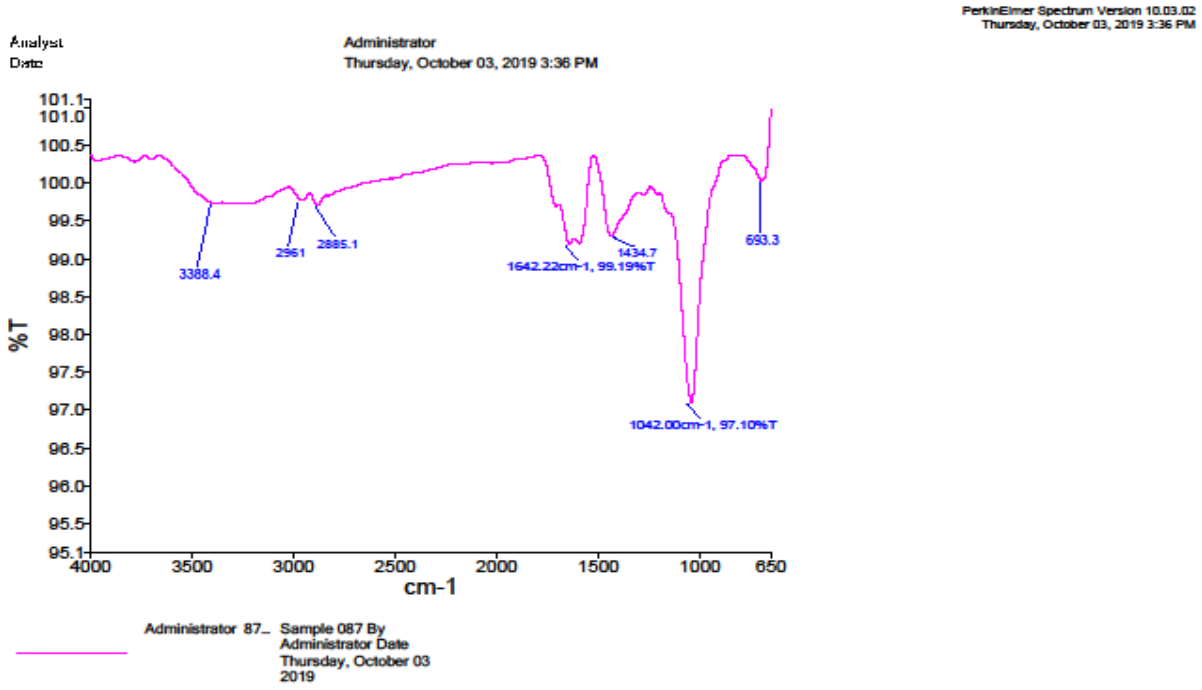


Figure No. 6: IR Spectra of Mono Ammonium Glycyrrhizinate powder

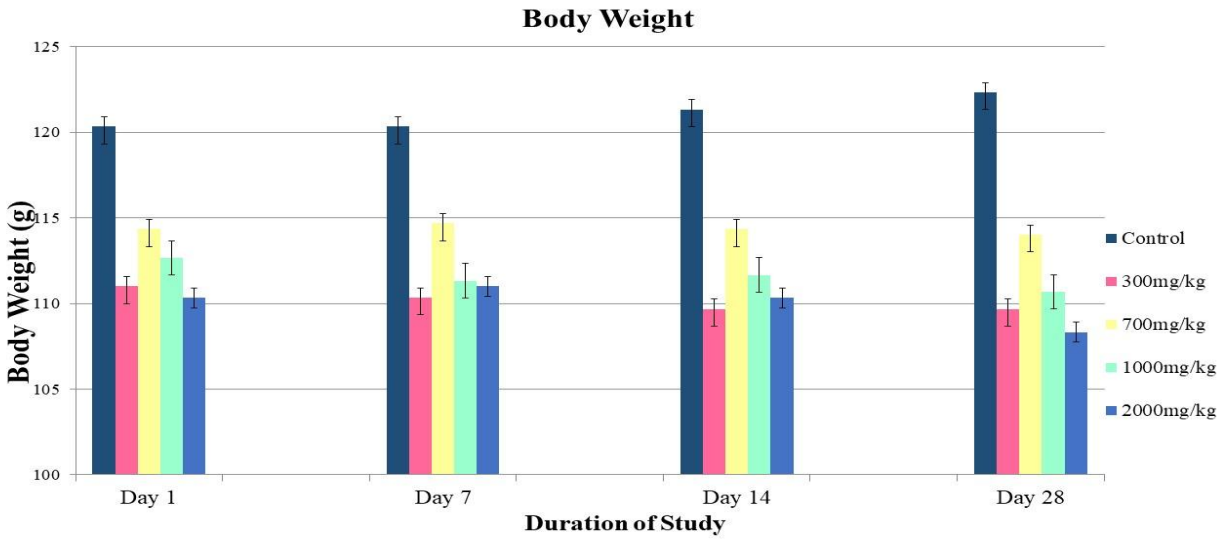


Figure No. 7: Graphical representation of body weight changes during sub-acute oral toxicity study

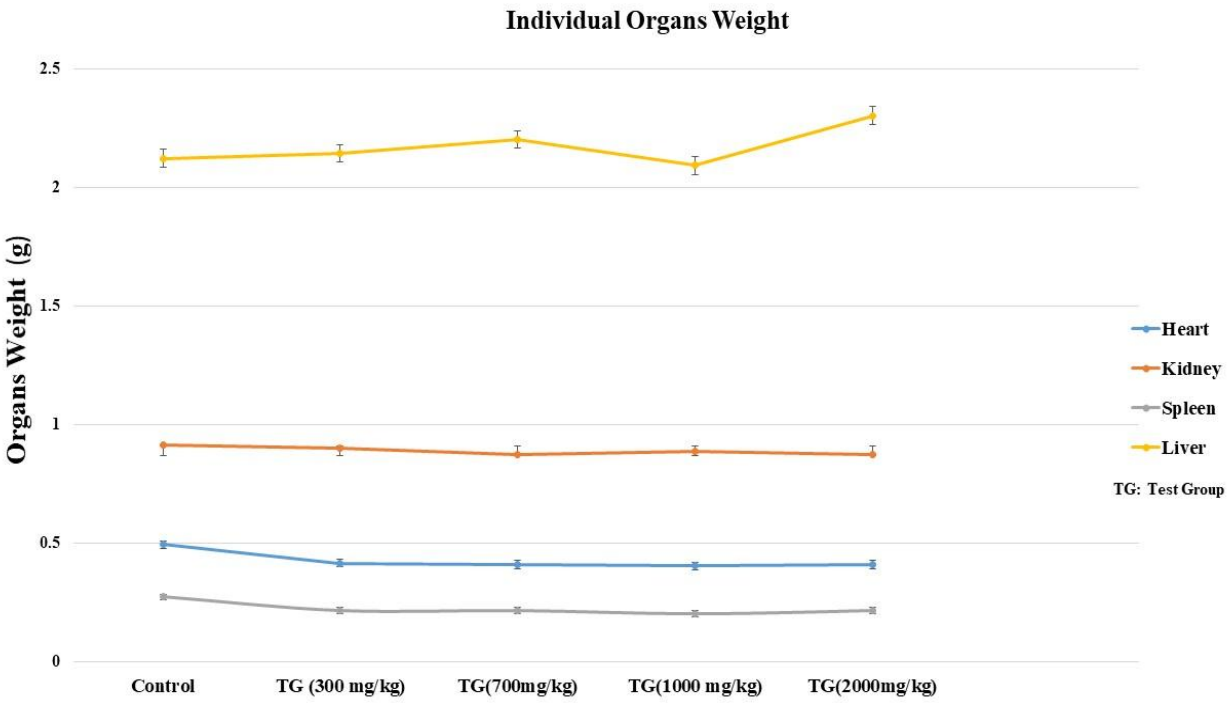


Figure No. 8: Graphical representation of individual organ weight of control and treatment groups

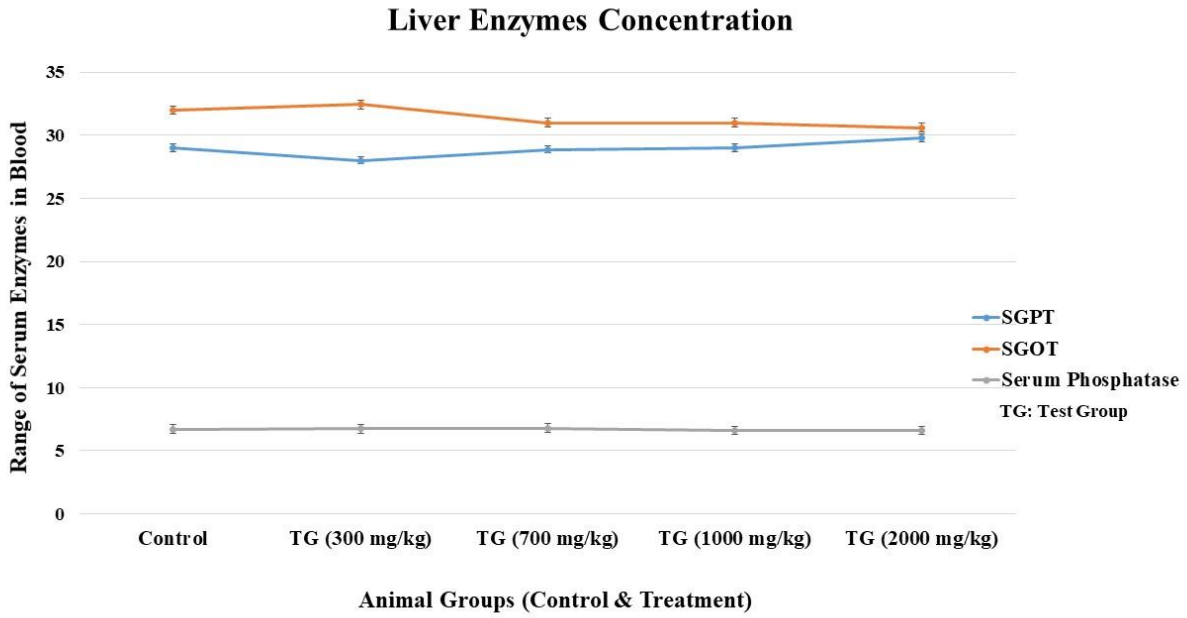


Figure No. 9: Graphical representation of liver enzymes concentration changes in control and the treatment group

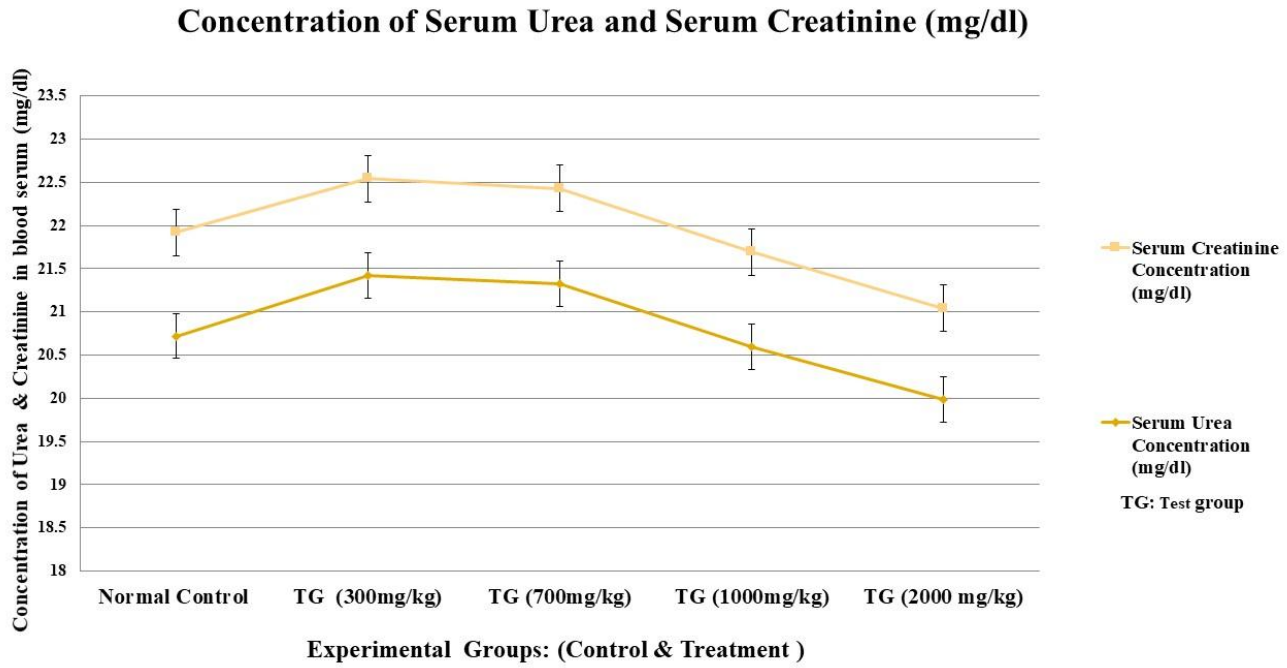


Figure No. 10: Graphically representation of serum urea concentration of control and treatment group

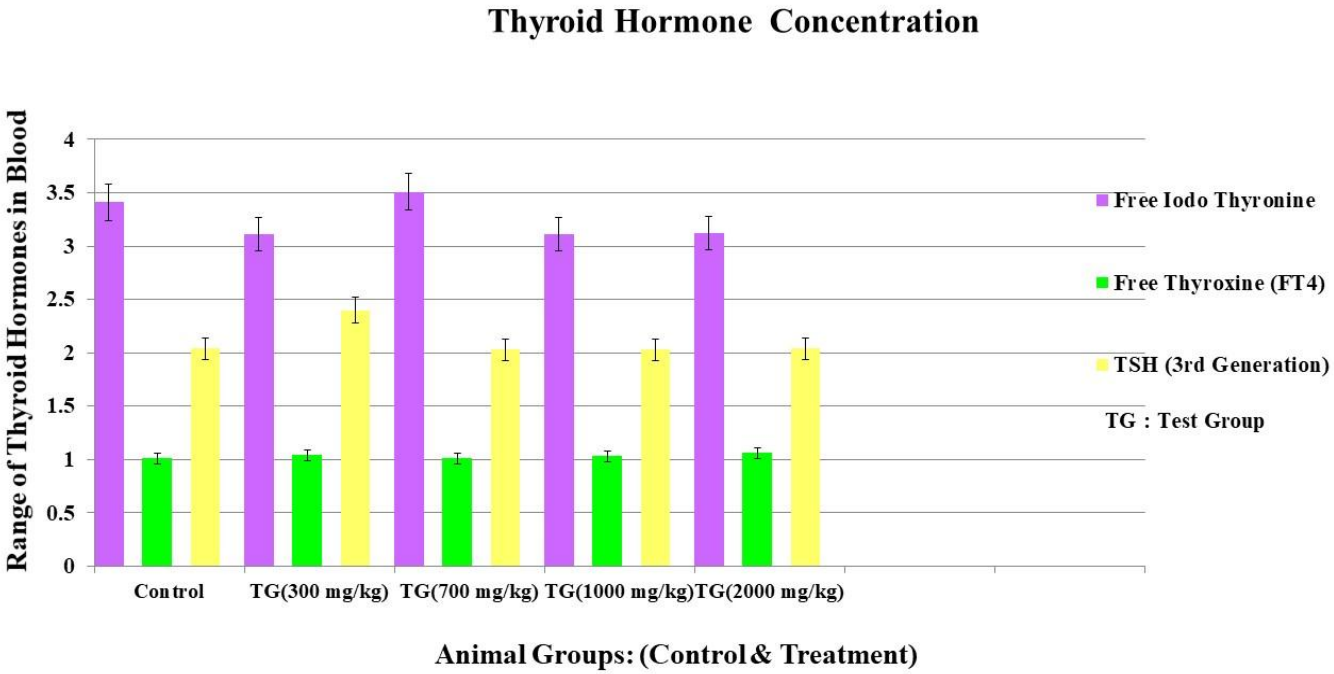


Figure No. 11: Graphical representation of thyroid hormone concentration in blood of control and treatment group

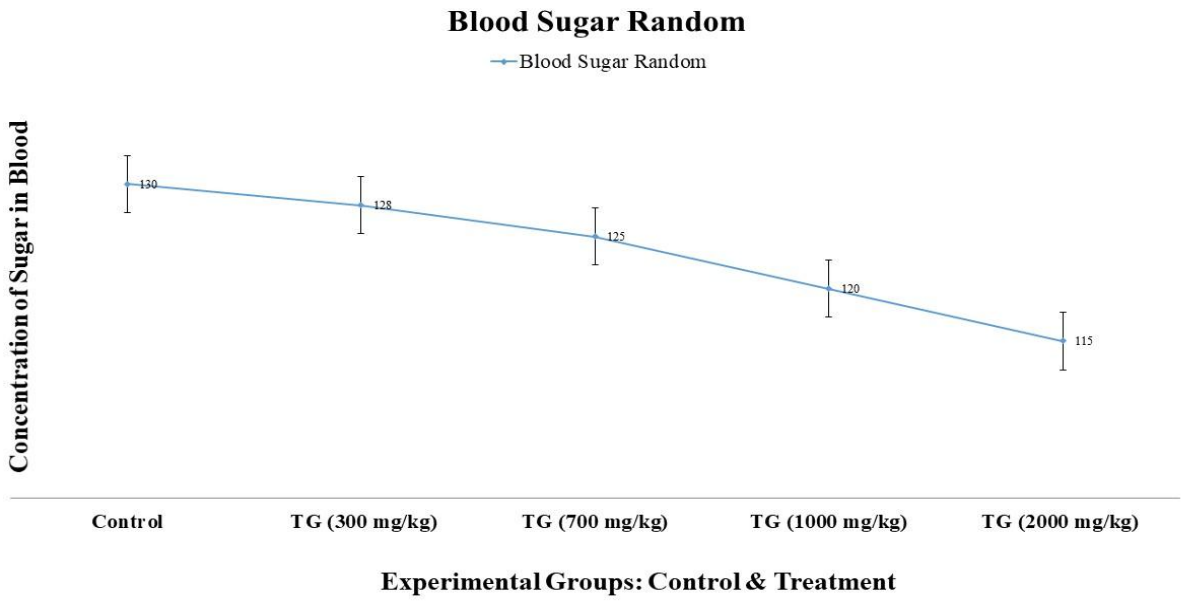


Figure No. 12: Graphical representation of random blood sugar concentration of control and treatment groups

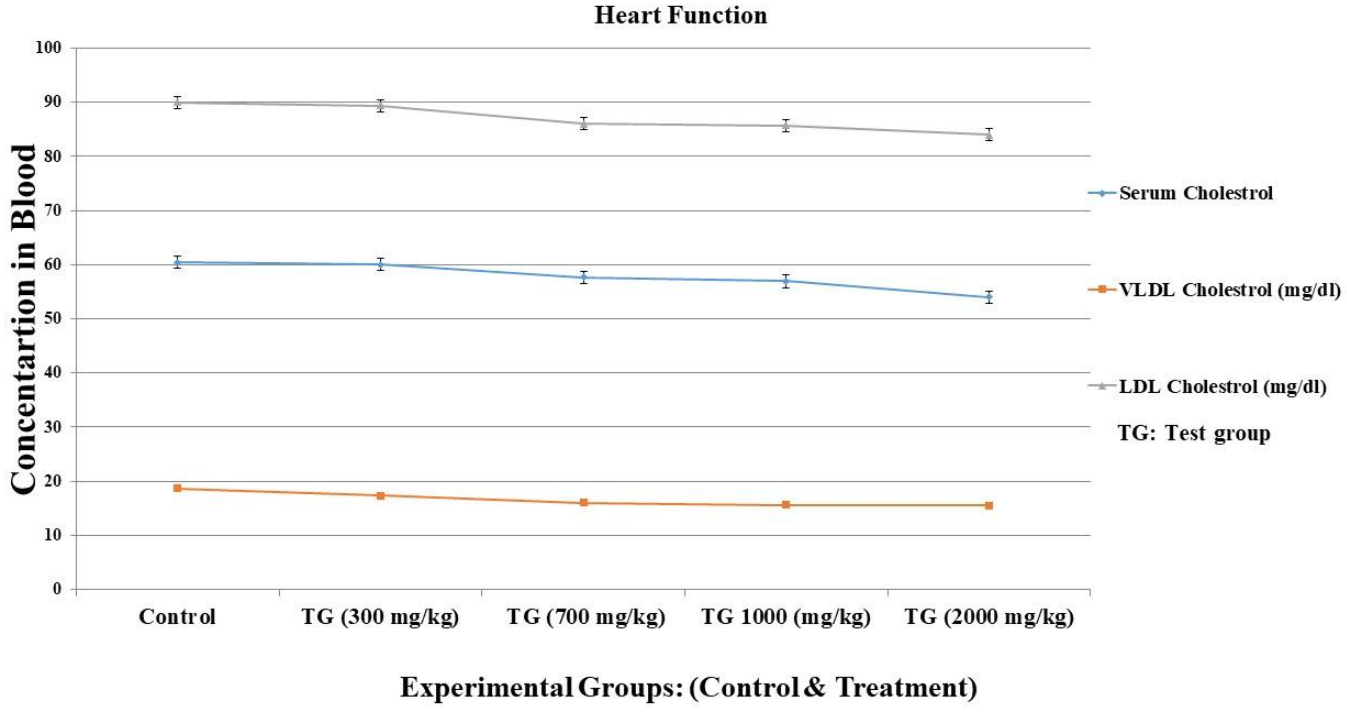


Figure No. 13: Graphical representation of effect of test drug on heart of the control and the treatment group

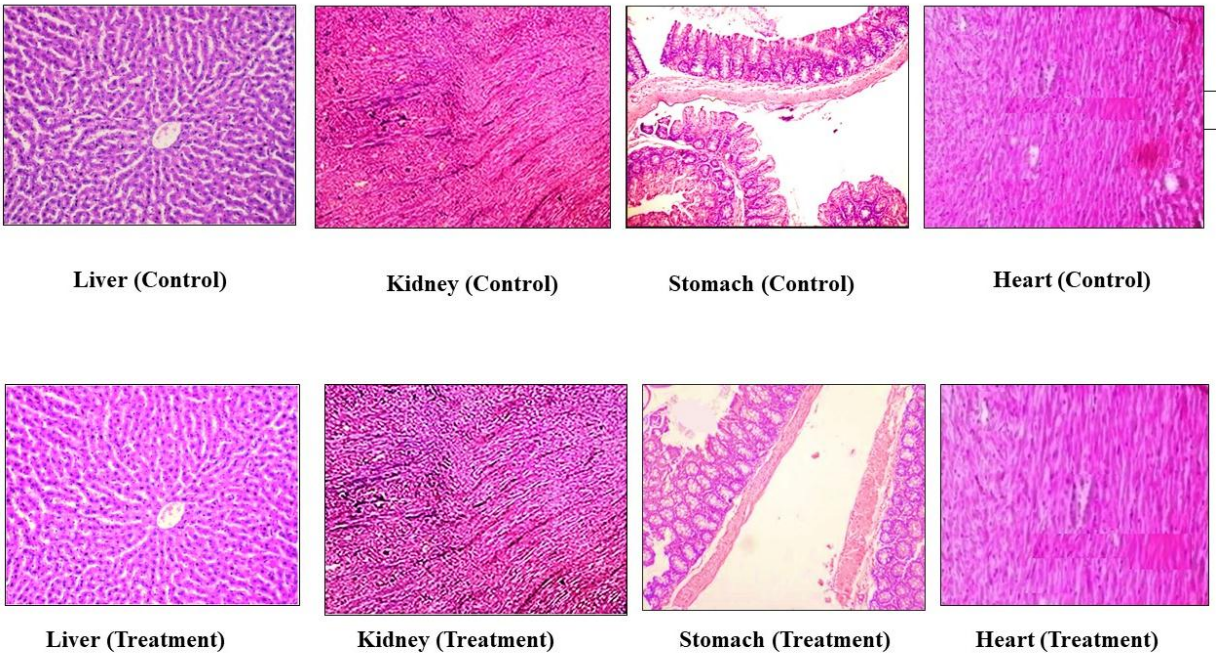
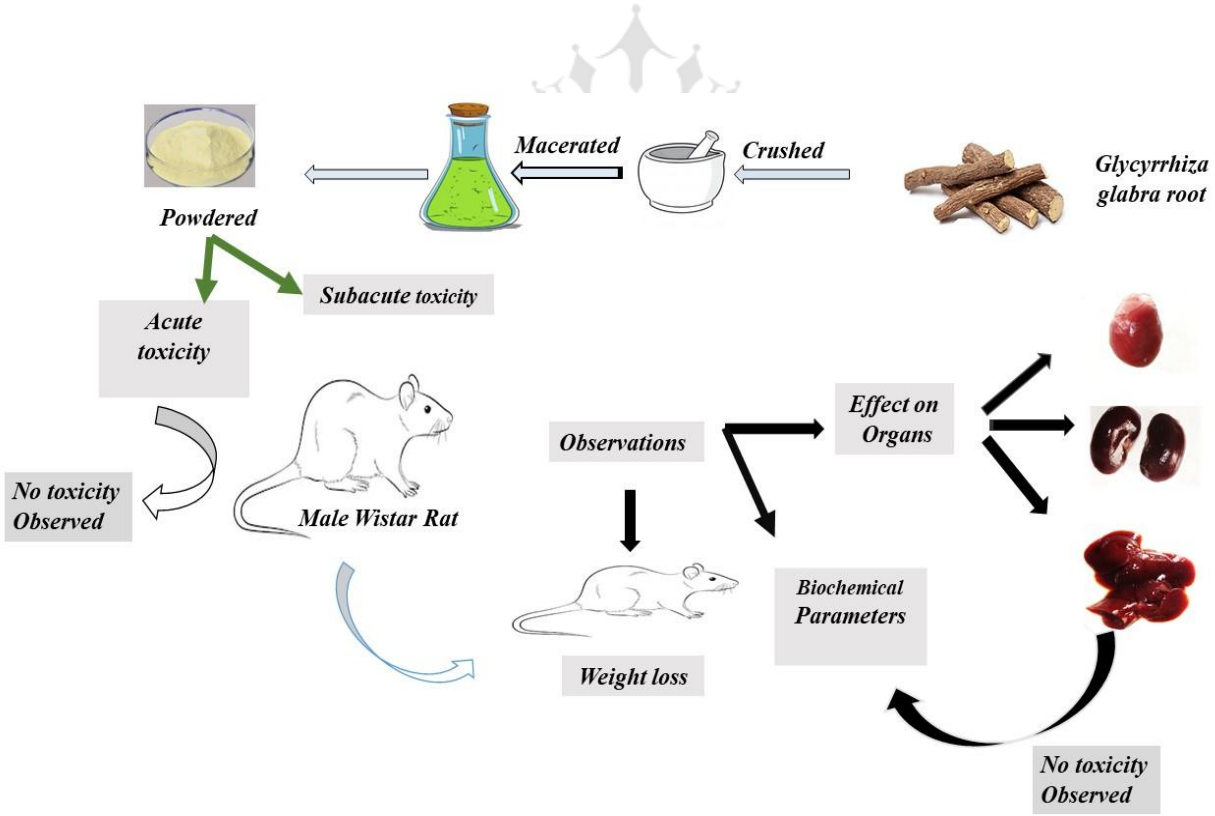


Figure No. 14: Histopathology of vital organs of control and treatment animal



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