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Acute and Sub-Acute Oral Toxicity Effects ofaqueous ethanolic Extract of *Boswellia serrata* (Boswegex®) on Biochemical, Hematological and, Histopathological Parameters in Wistar Rats

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Abstract:
Background:*Boswellia serrata gum resin extracts have shown promise in reducing inflammation. Although it is used frequently, there is no scientific proof of its safety. So, the present study evaluated the acute and sub-acute toxicity of B. serrata (Boswegex®) aqueous ethanolic extract in Wistar rats.*
Materials and methods:*In acute toxicity studies, healthy rats were given 2000mg/kg b.w of* B. serrata *(Boswegex®) extract for 14 days. In a sub-acute toxicity experiment, healthy rats were divided into four groups: group I received carboxymethylcellulose; groups II, III, and IV were given 250, 500, and 1000 mg/kg b.w. p.o. of B. serrata(Boswegex®) for 28 days. The amount of bodyweight, feed consumption, and water intake were recorded on a daily basis, and the data was expressed as a 7-day cumulative value. On the 29th day of the experiment, blood samples were collected for hematological, histopathological, and biochemical analysis.*
Results:*The test group at a single oral dose of 2000 mg/kg b.w. did not cause death or clinical symptoms in rats observed over a period of 14 days.*
Conclusion:*It can be concluded that B. serrata(Boswegex®) was safe for acute oral administration at a dose of 2000mg/kg b.w. There were no significant changes observed in the biochemical, hematological, and histopathological results of B. serrata(Boswegex®) treated groups when compared to the control group. According to these results, B. serrata (Boswegex®) was safe when administered orally for 28 days. All of these findings support the safety and nontoxicity of B. serrata (Boswegex®) for all of its therapeutic benefits.*
Key word: *B. serrata (Boswegex®), Acute oral toxicity, Sub-acute oral toxicity, Histopathology, Hematology, Biochemical analysis*

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I. Introduction

Despite the belief that natural remedies are safe and free of any possible toxicities, harmful substances continue to be a significant obstacle that restricts their application[1].Hepatotoxicity, nephrotoxicity, neurotoxicity, pulmonary toxicity, cardiac toxicity, convulsions, and acute eosinophilic pneumonia are among the prevalent toxicities[2].The presence of naturally occurring toxic secondary metabolites, the method used to prepare the herbal product, variations in the active or toxic ingredients due to growth conditions and soil chemistry, wrong identification of herbs during harvest, contamination by pathogenic fungi during storage and transport, and adulteration are potential sources of toxicity[3,4]. The World Health Organisation (WHO) therefore recommends that herbal treatments must undergo rigorous scientific research for both efficacy and safety in order to prevent individuals from exposure to harmful phytochemicals.

One of the oldest and most esteemed herbs in the Ayurvedic medical system is *Boswellia serrata*[5]. In the dry areas of India, Africa, and the Middle East, *B. serrata* trees can be found in abundance. Traditional uses for resin, which is found under the tree's bark, include incense in a variety of religious and social rituals as well as several therapeutic benefits[6,7]. The recognised chemical constituents of the exudate include resin, gum, essential oils, and terpenoids[8]. 25–35% of the resin is made up of the medicinally significant terpenoids boswellic acid[9–13]. It has been reported that *B. serrata* extract (BSE) has analgesic and psychopharmacological effects from the non-phenolic extract, and the alcohol-extracted component is beneficial in managing inflammatory diseases, arthritis, and hyperlipidaemia[14]. The six major boswellic acids, 11-keto-β-boswellic acid, 3-O-acetyl-11- keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, and 3-O-acetyl-β-boswellic acid, have been suggested to be the primary contributors to the *B. serrata* extract's curative properties Figure (1). Furthermore, β-boswellic acid, 11-keto-β-boswellic acid, and acetyl-11-keto-β-boswellic

acid have been developed to promote apoptosis in tumour cells primarily afflicted by leukaemia or colon cancer. *B. serrata* has been the centre of numerous studies on its effectiveness for a variety of purposes; however, there are very few or inadequate studies on the extract's toxicity. Additionally, *B. serrata* extract should be tested for any potential cytotoxic, genotoxic, or mutagenic action because it is usually intended for long-term consumption. In addition, as multiple investigations demonstrate a connection between mutagenicity and carcinogenicity, mutagenicity tests assist in reducing the risks associated with human genetic or carcinogenetic mutations[15]. Even though several studies on experimental animals that investigated the safety profile of *B. serrata* have been published, they are insufficient to explain the systemic effect.

Biochemical, hematological, and histopathological characteristics at dosages up to 1000 mg/kg b.w. of *B. serrata* did not show any adverse effects during toxicity studies in rats. Boswellic acids have been shown to be safe in acute, subacute, and chronic conditions. The toxicity and serious adverse effects of *B. serrata* are unknown. The safety of *B. serrata*: investigations demonstrate that *B. serrata* extract does not have harmful side effects at higher doses[16-18]. These suggest that, based on available data, the active ingredient in *B. serrata* (AKBA) is safe. Therefore, an essential aspect of evaluating *B. serrata* for potential hazardous effects is the necessity for additional studies into the toxicological effects of the organism intended for use in either humans or animals.

To the best of our knowledge, this is the first comprehensive collection of data evaluating *B. serrata*'s safety and efficacy. As a result, the preclinical and clinical use of *B. serrata*will be enhanced through this research investigation. The influence of this *B. serrata* on organ toxicity has not been evaluated. Consequently, a repeated dosage, 28-day oral toxicity study in rats was carried out to evaluate the safety of a standardised extract of *B. serrata* oleo gum resin containing a known quantity of boswellic acids. This study was carried out to determine the process by which *B. serrata* affected the biochemical, hematological, and histopathological parameters in Wistar albino rats after receiving the extracts daily for 28 days through oral administration. This study was carried out to determine the process by which *B. serrata* affected the biochemical, hematological, and histopathological parameters in Wistar albino rats after receiving the extracts daily for 28 days through oral administration.

Our Boswegex® is standardised with 65% boswellic acids based on their hydrophobicity. This product is more bioavailable because of its quick dissolving and long-lasting solubility. These research findings are intended to boost confidence in *B. serrata's* (Boswegex®) safety for use in treating a range of human diseases.

II. Material and methods

Preparation of Boswegex® with Regular boswellic acids: An aqueous ethanolic extract of the gum resin of *Boswellia serrata* was used for manufacturing Boswegex®, which contains Regular boswellic acids Figure (2). Boswegex® with Regular boswellic acids were standardised to contain 65% by HPLC, in order to maintain quality and batch-to-batch consistency.

Dried gum was ground into a coarse powder and then extracted using aqueous ethanol. The extraction process was repeated two to three times. The mixed extracts were then filtered under vacuum, and the filtrate was then heated in a rotary evaporator to between 50-60^{o}C to create a viscous extract. The resinous portion of the extract was then removed by washing, and after that, the organic acids were selectively precipitated by treating with alkaline water and then acidification. A semi-dried cake was then produced by decolorizing, filtering, and washing in water with a neutral pH. To make powder, this was further dried at 50–60°C in an oven with a vacuum. By using HPLC, this product was further standardised to 65% boswellic acids.

Characterization of boswellic acids (Boswegex®)by HPLC: Extraction and purification processes were standardized for Boswegex®. The analytical method was standardized based on HPLC. Briefly, HPLC analysis was performed using a Phenomenex Luna C18, 250 × 4.6 mm, 5 µl column with a flow rate of 1 ml/min in a Shimadzu liquid chromatography equipped with a UV/Vis Detector at a wavelength of 210nm and 254nm to detect the peaks. The sample was eluted by injecting 20 µl of mobile phase (Acetonitrile: Water: Acetic acid (90:10:0.1)) with a run time of 35 min. The peaks 1 to 6 represented 11-keto-β-boswellic acid (KBA), 3-O-acetyl-11-keto-β-boswellic acid, α- boswellic acid, β-boswellic acid, 3-O-acetyl- α- boswellic acid, and 3-O-acetyl-β-boswellic acid, respectively Figure (3).

Experimental Design

Acute oral toxicity study

Experimental animals

Female Wistar rats (180-200 g), 8–12 weeks old, were purchased from the animal facility at Radiant Research Services Pvt. Ltd. in Karnataka, India. Three similar animals were housed in typical wooden cages (15 ×21 × 29 cm) wrapped with sterile rice husks. The animals were provided with unlimited access to food and water, and their cage bedding was replaced every day. The animals were acclimatised for two weeks, adapting to their environment. The Institutional Animal Ethics Committee (IAEC) of Radiant Research Services Pvt. Ltd.

(Regd No. 1803/PO/RcBi/S/2015/CPCSEA), which was established in accordance with the guidelines of the CPCSEA, Government of India, reviewed and approved all the experimental procedures and protocols used in this study.

Acute Oral Toxicity Study

The single-dose acute oral toxicity study was evaluated following the recommendations of the OECD 423 Guidelines. An acute oral toxicity study was performed on overnight fasted female Wistar rats aged 8–12 weeks old. Three animals received a single dose of Boswegex® by oral administration of 2000 mg/kg b.w. After that, all rats were given free access to food and water, and they were all kept under close observation for 24 hours, with particular consideration given to the first 4 hours and once daily for 14 days for any possible signs of acute toxicity. The various changes in physical appearance or behaviour were conducted once daily for 14 days. All the rats were observed at least twice daily with the purpose of recording any signs, symptoms, or behavioural changes. A limit test was conducted using six animals at 2000mg/kg body weight and observed for a period of 14 days. The organs, namely the liver, heart, spleen, and kidney, were carefully excised and weighed. For histopathological evaluation, these organs were preserved in a medium containing 10% buffered neutral formalin. The rats were further observed once a day for up to 14 days following treatment for behavioural changes, signs of toxicity and/or death, and the latency of death. According to El Allaoui[19]the LD50 value was calculated using the Dragstedt and Lang method. Considering a safer dosage, the clinical signs and symptoms of the medications were evaluated.

Sub-acute oral toxicity study

Experimental animals

Male and Female Wistar rats (180–220 g) about 8–10 weeks' old were purchased from the animal facility at Radiant Research Services Pvt. Ltd., Karnataka, India. The CPCSEA Registration Number: 1803/PO/RcBi/S/2015/CPCSEA demonstrates that animal experiments were carried out in compliance with the committee's policies for controlling and supervising animal experimentation. Animals were housed at a temperature of 23±3^{o}C, relative humidity of 30–70%, and a 12-hour light and 12-hour dark cycle. Picric acid was used to mark each animal, and each animal acquired a unique number. To differentiate among the group, each cage received an individual number. A single animal was housed in a standard polypropylene cage with a stainless steel top grill, pelleted food facilities, and water bottles for drinking. Sterile paddy husk (Source: Shree Balaji Rice Industries, Bangalore) was used as bedding material and changed every day. Water was freely available when Aqua Guard was at work. Water that is fresh, potable, and uncontaminated should be available to animals at all times. Every procedure involving animals was carried out with compassion and under the supervision of skilled or knowledgeable persons. Before the study was allowed to begin, the protocol was reviewed and approved by Radiant Research Services Pvt. Ltd.'s Institutional Animal Ethical Committee (IAEC).

Sub-acute oral toxicity study

The Organisation for Economic Co-operation and Development (OECD) recommendation 407 was followed for conducting a sub-acute oral toxicity study. Fourty rats of both sexes were used in this experiment. The animals were divided into four groups. Group I received 0.5% CMC vehicle orally at a dose volume of 10 ml/kg b.w. and served as a control group. Whereas group II, group III, and group IV received Boswegex® at 250 mg/kg b.w., 500 mg/kg b.w., and 1000 mg/kg b.w., p.o. respectively, for 28 days. From the first day of the study until its end, all rat groups were subjected to twice-daily mortality and morbidity assessments. Daily clinical manifestations and investigations were carried out on all the animals. The body weights of the animals in each group were measured three times: once before dosing started, once a week while receiving treatment, and finally on the day of sacrifice. Every day, the quantity of food consumed was noted, and the data was given as a cumulative value during the seven days prior to the experiment. Retro-orbital bleeding was used to collect blood samples from overnight-starved animals on the 29th day of the experiment for haematological and biochemical investigations. Rats were then euthanized after blood collection, and the internal organs (Liver, Kidneys, Adrenals, Testes, Ovaries, Uterus, Epididymis, Spleen, brain, and Heart) were removed and weighed to determine the relative organ weights and observed for any gross lesions. For histological analysis, the internal organs were preserved in a 10% buffered neutral formaldehyde solution[20].

Body Weight

Before the study started, once a week, on the day of sacrifice, the body weight of each rat was carefully recorded.

Mortality and Toxic Signs

Throughout the course of 28 days, visual observations of mortality, various physical changes, behaviour (such as sleepiness, salivation, and lethargy), and any injuries or illnesses were performed, especially after treatment and up to 4 hours following medication[21].

Relative Organ weight

The internal organs (Liver, Kidneys, Adrenals, Testes, Ovaries, Uterus, Epididymis, Spleen, brain, and Heart) were removed, weighed to calculate the relative organ weights, and observed for any gross lesions. The internal organs were preserved in a 10% neutral buffered formaldehyde solution for histopathological study. The relative organ weight was recorded using the formula:

Relative organ weight = (organ weight (g)/body weight of the animal on sacrifice day (g)) × 100

Hematological parameters

Abacus (DiatronMesstechnik, Austria) Automated Haematology Analyzer was used to evaluate several haematology parameters after blood was collected and punctured into EDTA-containing tubes. The haematological parameters, like white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), Lymphocytes (Ly), myocardial infarction (MI) and Granulocytes (GR) were estimated[22].

Biochemical analysis

Blood was drawn into non-heparinized tubes, which were then centrifuged at 10,000 rpm for 10 minutes. The serum separated was analysed using fully Automated Clinical Chemistry Analyser EM360, Transasia Bio-medicals Ltd, for various parameters such as Total bilirubin, Direct Bilirubin, Alkaline phosphatase (ALP), Albumin, Total protein, Aspartate aminotransferase (AST), Alanine aminotransferase(ALT), Urea, Creatinine, Total Cholesterol, Triglycerides, HDL, LDL, Calcium, Phosphorous and Glucose.

Histopathology study

On the 28th day, the Liver, Kidneys, Adrenals, Testes, Ovaries, Uterus, Epididymis, Spleen, Brain and Heart excised from the sets administered with the Boswegex® and the control groups were collected and weighed and quickly set in 10% neutral buffered formalin at pH 7.4 and developed for histological studies. All the weighable tissues that could be removed from the control and Boswegex® treated groups, along with all the lesions identified during gross pathology, were processed for histopathology analysis.The tissues were dehydrated in progressively stronger alcohols, cleared in xylene, embedded in paraffin wax, and sliced into 5 micron sections.A standard microscope with magnifications of X 100 and X 400 was used to view the Segments[23].

Statistical analysis

Graph-Pad Prism Software, version 5.01, was used to statistically analyse all data, including body weight, feed intake, clinical chemistry, haematology, electrolytes, and organ weights. All values were determined as mean ± SEM. One-way ANOVA in addition to Dennett's test was used to assess the difference between the treatment and control groups that was statistically significant. The statistical analysis's complete outcomes have been given in different tables. In each case, $P<0.05$ was used to determine whether the data were statistically significant.

III. Result

Acute oral toxicity

The present study, conducted as per OECD Guidelines 423, revealed that Boswegex® did not produce any mortality throughout the study period of 14 days, even when the limit dose was maintained at 2000mg/kg b.w. The test group at a single oral dose of 2000 mg/kg b.w. did not cause death or clinical symptoms in rats observed over a period of 14 days (Table no 1). Throughout the duration of the experiment, each animal maintained good health. After the dose was administered, behavioural changes were carefully recorded. During the course of the examination, none of the animals displayed any aberrant symptoms (Table no 2). All surviving animals had gained body weight by the 3rd, 7th, and14th days as compared to day 0 (Table no 3; Figures 4 and5). Increased body weight in animals during the study was observed in all the animals, and it was a normal pattern in healthy animals. All surviving animals were sacrificed at the end of the experiment and discarded after gross macroscopic pathological changes were observed and recorded (Table no 4; Figure 6). No organs or tissues were retained. So the median lethal dose of Boswegex® was determined to be more than 2000 mg/kg b.w.

Table no 1: Clinical and Behavioural Signs/Symptoms

CODES	OBSERVATIONS	SIGNS/SYMPTOMS
01	NAD	02-40codesobservationsare notseen
02	Accidentaldeath	---
03	PartialCannibalism	Ananimalof a speciesconsumingpartofanother animalofthesame species
04	TotalCannibalism	Ananimalofa speciesconsumingthe majororgansof anotheranimalofthesamespecies
05	Dead	Irreversiblecessationofallbodyfunctions,manifested byabsenceof spontaneousbreathingand total lossofcardiovascularand cerebral functions
06	Moribundcondition	Approachingdeathanimalwillnotbeavailablefor examinationfornextday
07	Weakness	Aweak bodilystate asexpressed bydifficultyin rising, ashuffling,disinclinationtomove,eatingslowlyandadroopingposture
08	Lethargy	Alevelofconsciousnesscharacterizedbydecreasedinteractionwithobjectsintheenvironment , sluggishness,abnormaldrowsiness
09	Salivation	Flowofsaliva,Drooling (Abnormallyabundantflowof saliva)
10	Lacrimation	Flowoftears
11	Discharge	Abnormaldischarge
12	Snuffling (Unusual respiratorypattern)	Abubblingsound from thenasalcavities
13	Bronchialrales	Anabnormal respiratorysound(crackles) in auscultationoflungs
14	Cough	Aforcefulreleaseofair fromthelungs
15	Dyspnea(Unusual respiratorypattern)	Shortnessof breath
16	Corneal opacity	Opaquewhitespoton thecornea
17	Cataract	Opacityofthecrystalline lensoftheeye
18	Diarrhea	Diarrheaisthefrequentpassageofloose,watery, soft stools.
19	Hematuria	Presence ofblood inthe urine
20	Piloerection	Erectionofhair
21	Response tohandling	Normalresponse toapproach.
22	Convulsions	Violentinvoluntarycontractionofamuscle ormuscles
23	RepetitiveCircling	Continuouscircling
24	Headtiltedononeside	Headfacingtowardssome other directionother than straight
25	Ataxia	Inabilityto control voluntarymuscle movement
26	Dermatitis	Inflammationofthe skin
27	Blister	Alocal swellingof theskin thatcontainswateryfluid
28	Urticaria	An itchyskineruption characterized byweal'swith paleinteriorsandwell-definedredmargins
29	Necrosis	Deathofa portionoftissuedifferentiallyaffectedby localinjury
30	Erythema	Rednessof theskin
31	Edema	Aswellingfrom effusion ofwateryfluid inthe cellular tissuebeneaththe skinormucousmembrane
32	Cyanosis	Bluishdiscoloration oftheskinandmucous membranes
33	Paralysis	Lossof sensationoveraregionofthebody.
34	Edema	Anexcessiveaccumulationofserousfluidintissue spacesor a bodycavity.
35	Crepitation	Adry, cracklingsound orsensation

36	Dehydration	Lossofwaterandsalts.Theskinturnspale andcold,the mucousmembraneslininglosetheirnatural moisture
37	Dull	Lackingresponsivenessoralertness
38	Posture	Position of thebodyorof bodyparts
39	Epistaxis	Bleedingfrom the nose
40	Urine dribbling	Leakingof urine

Table no 2: Clinical signs and behavioral observations of Boswegex® during the period of study in rats

OBSERVATION															
	AfterTreatment*	**Daysofposttreatmentexamination**													
AnimalId	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
RAT 01	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT 02	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT 03	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT04	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT05	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT06	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT07	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT08	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RAT09	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01

AnimalID	**Dose**	**Treatment**			
		Before		**After**	
		Day0	**Day3**	**Day7**	**Day14**
RAT01 RAT02 RAT03	2000 (mg/kgb.w.)	189.3±0.9	192.0±0.6	195.7±0.7	204.7±0.7
RAT04 RAT05 RAT06 RAT07	Limit test(2000mg/kgb.w.)	186.3±0.8	188.8±1.0	192.7±1.1	201.8±1.0

*****Observation of the first four hours after treatment: 01: No abnormality detected (NAD) 02-40 code observations are not seen.

Table no3: The body weight of rats treated with a single dose of Boswegex® (2000mg/kg b.w.) for 0–14 days Valueswere expressedasMean±SEM, $p < 0.05$

RAT08 RAT09					

Table no 4: Resultofgrosspathologicalexaminations of Boswegex®

AnimalID	Dose	Macroscopiclesions
RAT01 RAT02 RAT03	2000 (mg/kg b.w)	No macroscopic alteration occurredNomacroscopicalterationoccurred Nomacroscopicalterationoccurred
RAT 04 RAT05 RAT06 RAT07 RAT08 RAT09	Limit test(2000mg/kg b.w)	No macroscopic alteration occurredNo macroscopic alteration occurredNo macroscopic alteration occurredNo macroscopic alteration occurredNomacroscopicalterationoccurred Nomacroscopicalterationoccurred

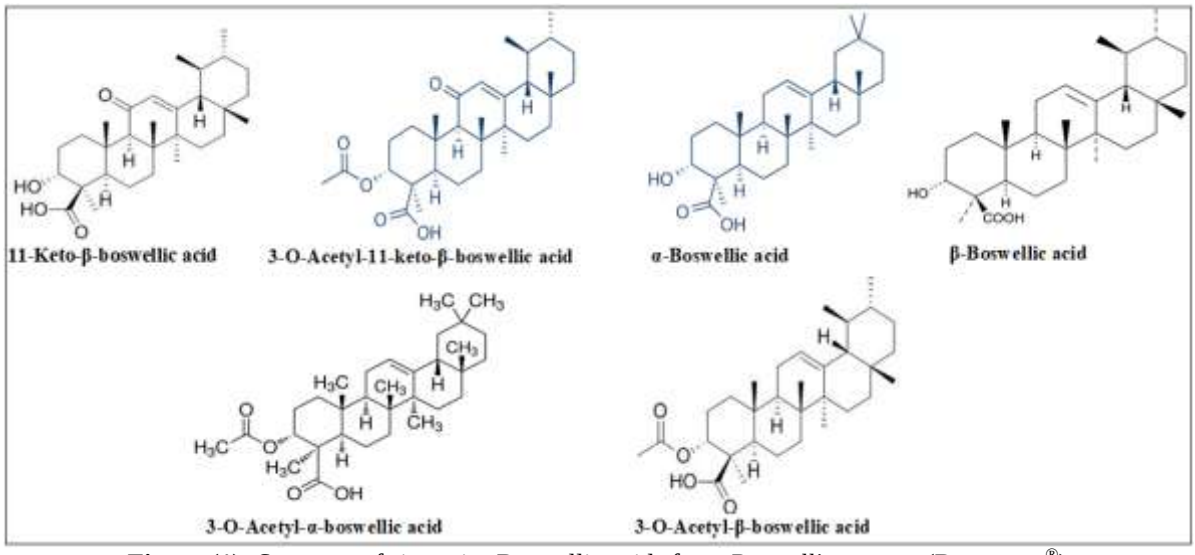


Figure (1): Structure of six major Boswellic acids from *Boswellia serrata* (Boswegex®)

Figure (2): *Boswellia serrata* (Boswegex®) with Regular boswellic acids Extract

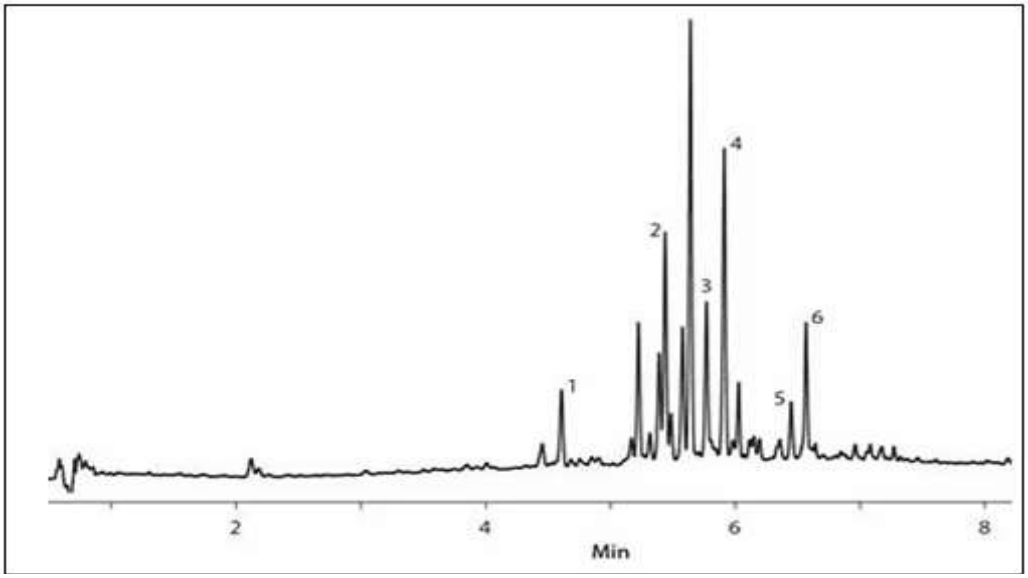
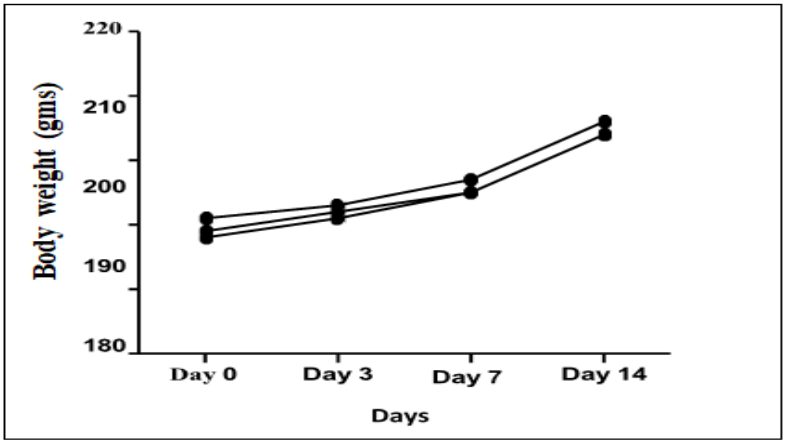
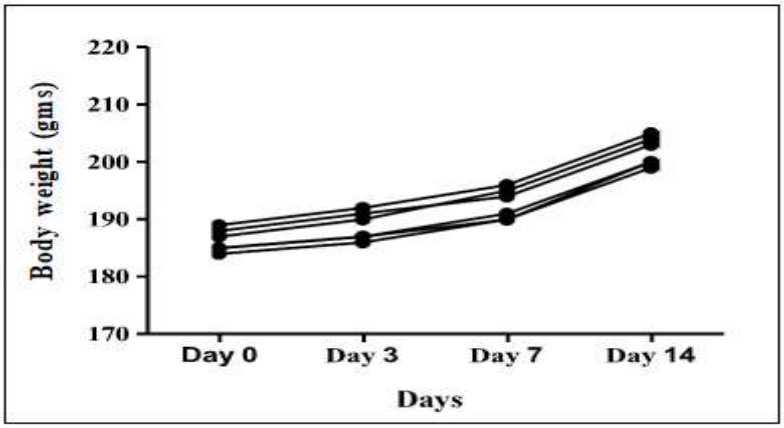


Figure (3): A representative high-performance liquid chromatography image shows the elution profile of the major boswellic acids in Boswegex® at 210 nm. The peaks 1 to 6 represent 11-keto-β-boswellic acid, 3-O-acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, and 3-O-acetyl-β-boswellic acid, respectively.



Figure(4): The body weight of rats treated with a single dose of Boswegex® (2000mg/kg b.w.) for 0–14 days



Figure(5): The body weight of rats treated with a limit dose of Boswegex® (2000mg/kg b.w.) for 0–14 days

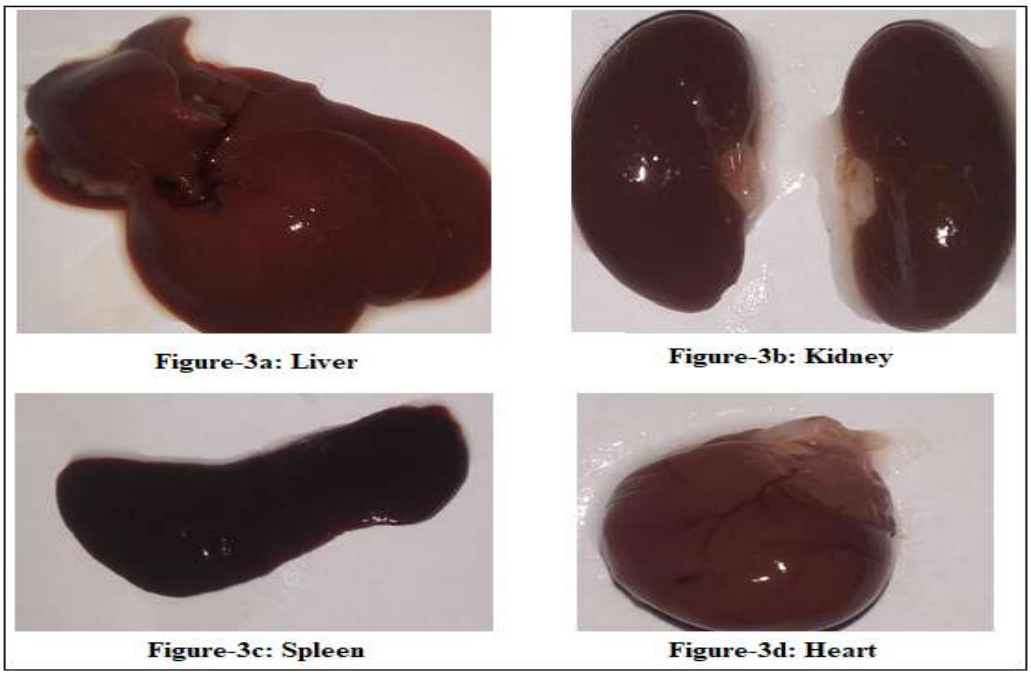


Figure (6): Histopathological examination of various organs of the rat liver, kidney, spleen, and heart (a, b, c, d) in an acute oral toxicity study

Sub-acute oral toxicity

During 28 days of treatment, all of the treatment rats of both sexes at 250, 500, and 1000 mg/kg b.w. did not produce any death or hazardous signs such as sleepiness, salivation, lethargy, or other physiological activities as compared to the control animals. No visible toxicity symptoms were observed in the rats treated with Boswegex® compared to the control group.

Effect of Boswegex® on body weight and food intake in rats

During the study, no major changes in the animal's body weight were observed (Table 5; Figure 7). The food intake was also not affected after 28 days of oral administration of Boswegex®. So, the Boswegex® indicated that there were no significant appetite changes and no adverse impact on animal growth. In comparison with the control group, no significant variations were found in the rats' physiological and metabolic activity. The effect of Boswegex® on food intake is given in Table 6 and Figures 8.

Table no 5: Effect of body weight assessment Boswegex® in sub-acute toxicity study of treated rats

Groups	BODY WEIGHT (gms)									
	MALE					FEMALE				
	Basal	Week-01	Week-02	Week-03	Week-04	Basal	Week-01	Week-02	Week-03	Week-04
Group I Normal control	196.4±0.50	206.2±0.58	215.4±0.50	226±0.63	235.8±0.66	186.4±0.67	193.2±0.37	200.6±0.40	208.2±0.37	219±0.44
Group II Boswegex® (250 mg/kg b.w.)	196.6±0.4	206±0.44	216.8±0.37	226.6±0.40	236.8±0.48	186.2±0.58	193.8±0.37	200.8±0.48	208.6±0.50	218.8±0.58
Group III Boswegex® (500 mg/kg b.w.)	196.4±0.50	206.6±0.40	216.4±0.40	226.4±0.50	236.2±0.58	186.2±0.58	193.4±0.92	201±1.00	209.4±0.97	219.2±0.73
Group IV Boswegex® (1000 mg/kg b.w.)	196.6±0.74	207.2±0.79	217.2±0.86	226.8±1.15	236±1.04	186.2±0.91	193.2±0.58	200.4±0.50	208.2±1.06	218.6±1.00

Values are given in Mean ± SEM, n=5 animals/group, $p < 0.05$

Table no 6: Boswegex® effect on rat food intake during 28 days of treatment

Groups	FEED CONSUMPTION (gms)							
	MALE				FEMALE			
	Week-01	Week-02	Week-03	Week-04	Week-01	Week-02	Week-03	Week-04
Group I Normal control	104.71±1.74	106.28±1.66	109.42±0.84	116.57±0.75	86.14±2.16	89.28±2.54	91±1.35	98.71±0.89
Group II Boswegex® (250 mg/kg b.w.)	105.42±1.80	107.42±2.78	110.42±1.54	116.71±1.19	86.28±1.73	88±0.72	91.57±1.04	97.42±0.69
Group III Boswegex® (500 mg/kg b.w.)	105.42±2.39	107.57±2.90	110.42±1.49	116.85±0.51	86.71±3.47	88.71±2.49	91.28±0.92	97.57±1.13
Group IV Boswegex® (1000 mg/kg b.w.)	105.28±2.33	107.14±1.08	110.14±1.10	117.85±0.63	87.14±2.64	89.57±1.77	92.28±1.30	98.71±1.02

Values are given in Mean ± SEM, n=5 animals/group, $p < 0.05$

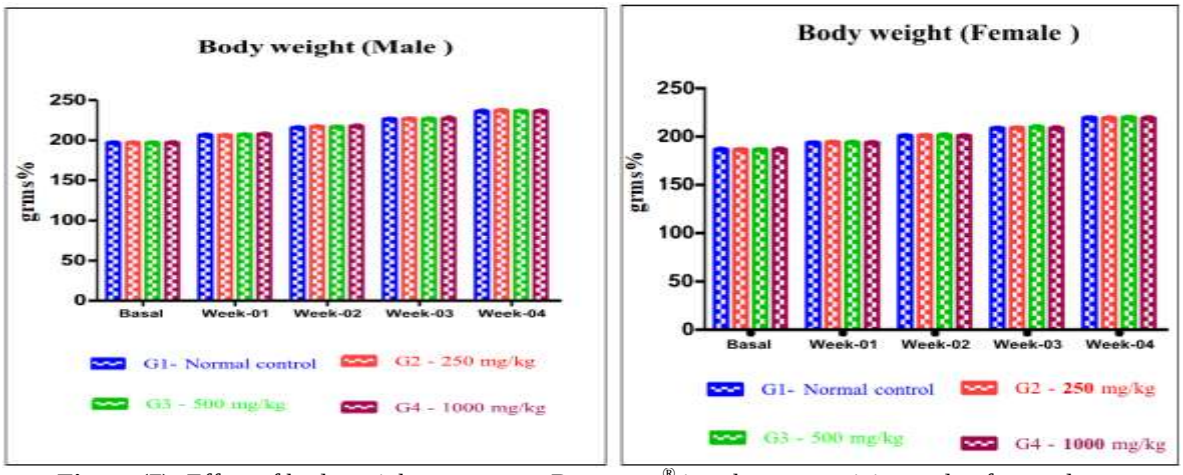


Figure (7): Effect of body weight assessment Boswegex® in sub-acute toxicity study of treated rats

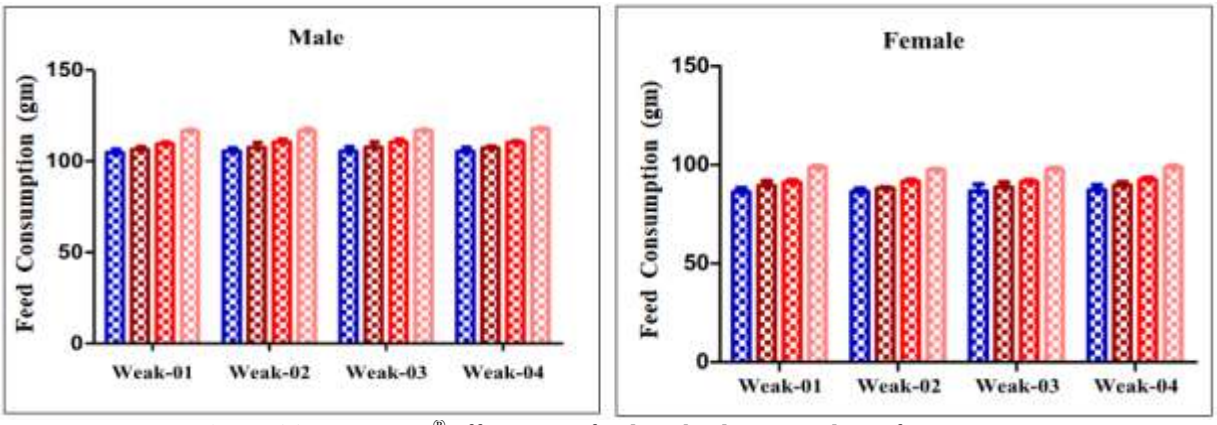


Figure (8): Boswegex® effect on rat food intake during 28 days of treatment

Mortality and Toxic Signs

The maximum dose examined, 1000 mg/kg b.w., of Boswegex® administered orally daily for 28 days, did not cause any adverse effects in rats. Throughout the investigation, no group had any fatalities or apparent clinical symptoms. The skin, fur, eyes, sleep, salivation, diarrhoea, and behaviour of the rats didn't exhibit any toxicity-related symptoms (Table 7 and Table 8).

Table no 7: Observations for clinical signs and symptoms of Boswegex®

SIGNS/SYMPTOMS	CODES	OBSERVATIONS
02-40codesobservationsarenotseen	01	NAD
	02	Accidentaldeath
Ananimalofa speciesconsumingpart ofanotheranimalofthesame species	03	PartialCannibalism
An animal of a species consuming themajororgansof anotheranimalof the samespecies	04	TotalCannibalism
Irreversible cessation of all bodyfunctions,manifested byabsenceof spontaneous breathing and total loss ofcardiovascularandcerebral functions	05	Dead
Approachingdeathanimalwillnotbe availableforexaminationfornextday	06	Moribundcondition
A weak bodily state as expressed bydifficultyinrising,ashuffling, disinclination to move, eating slowlyand adroopingposture	07	Weakness
A level of consciousness characterizedbydecreasedinteractionwithobjectsin theenvironment,sluggishness,abnormaldrowsiness	08	Lethargy
Flowofsaliva,Drooling (Abnormally abundantflowof saliva)	09	Salivation
Flowoftears	10	Lacrimation
Abnormaldischarge	11	Discharge
Abubblingsound fromthenasal cavities	12	Snuffling
eye		
Diarrheaisthefrequentpassageof loose,watery,softstools.	18	Diarrhea
Presence ofblood inthe urine	19	Hematuria
Erectionofhair	20	Piloerection
Normalresponse toapproach.	21	Response tohandling
Violentinvoluntarycontractionofa muscleormuscles	22	Convulsions
Continuouscircling	23	RepetitiveCircling
Headfacingtowardssome other directionotherthanstraight	24	Headtiltedononeside
Inabilitytocontrol voluntarymuscle movement	25	Ataxia
Inflammationofthe skin	26	Dermatitis
Alocalswellingof theskin that containswateryfluid	27	Blister
An itchy skin eruption characterized by weal'swith paleinteriorsand well-defined redmargins	28	Urticaria
Deathofa portionoftissue differentiallyaffectedbylocalinjury	29	Necrosis
Rednessof theskin	30	Erythema

Aswellingfromeffusionofwatery fluidinthecellulartissuebeneaththeskinor mucousmembrane	31	Oedema
Bluishdiscolorationoftheskinand mucousmembranes	32	Cyanosis
Lossofsensationoveraregionofthe body.	33	Paralysis
Anexcessiveaccumulationofserous fluidintissuespacesorabodycavity.	34	Edema

	ORGANSWEIGHT(mg)

Chestdiscomfort/shortnessofbreath	35	Palpitation
Lossofwaterandsalts.Theskinturns paleandcold,the mucousmembraneslininglosetheirnaturalmoisture	36	Dehydration
Lackingresponsivenessoralertness	37	Dull
Position of thebodyorof bodyparts	38	Posture
Bleedingfrom the nose	39	Epistaxis
Leakingof urine	40	Urine dribbling

Table no 8: Codes for clinical observation during study

CODES OF OBSERVATION														
Groups	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**
Group I Normal control	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group II Boswegex® (250 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group III Boswegex® (500 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group IV Boswegex® (1000 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Groups	**D15**	**D16**	**D17**	**D18**	**D19**	**D20**	**D21**	**D22**	**D23**	**D24**	**D25**	**D26**	**D27**	**D28**
Group I Normal control	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group II Boswegex® (250 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group III Boswegex® (500 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
Group IV Boswegex® (1000 mg/kg b.w.)	01	01	01	01	01	01	01	01	01	01	01	01	01	01
***D indicates Day**														

Relative Organ Weight

Table 9 and Figures9-13 shows the rats' relative organ weights at day 28 after treatment. When compared to the control group, there was no discernible variation in the relative organ weight of each organ in the Boswegex® treatment groups.

Table no 9: The relative organ weight of rats treated with different doses of Boswegex® for 28 days

	MALE			
	Group I Normal control	**Group II** Boswegex® (250mg/kg b.w.)	**Group III** Boswegex® (500mg/kg b.w.)	**Group IV** Boswegex® (1000mg/kg b.w.)
Liver	7849.4±57.85	7855.00±126.53	7858.20±78.93	7839.20±120.23
Heart	844±12.90	841.20±25.84	833.20±19.10	840.80±23.42
Kidney	1402.6±21.32	1401.80±21.96	1424.00±22.52	1404.60±18.87
Brain	1796.2±13.12	1792.20±34.35	1793.00±16.38	1808.40±27.42
Spleen	561.4±8.96	557.40±18.38	558.20±8.00	559.40±7.03
Lungs	1787.6±10.17	1777.20±9.57	1787.00±16.05	1780.00±27.86
Adrenals	70±1.55	68.80±0.58	69.20±1.07	69.00±1.41
Testes	2771.8±34.50	2722.80±17.36	2717.20±11.09	2737.00±22.39
Epididymis	1697±19.71	1698.60±6.48	1697.80±33.98	1698.80±13.62
Organs	**ORGANSWEIGHT(mg)**			
	FEMALE			
	Group I Normal control	**Group II** Boswegex® (250mg/kg b.w.)	**Group III** Boswegex® (500mg/kg b.w.)	**Group IV** Boswegex® (1000mg/kg b.w.)
Liver	6796.40±64.29	6811.80±97.33	6701.80±239.91	6806.40±167.76
Heart	785.80±17.39	787.60±10.55	786.00±6.80	780.00±15.12
Kidney	1381.80±17.99	1381.60±26.45	1371.40±18.62	1389.20±10.81
Brain	1730.60±20.28	1713.00±31.64	1760.80±21.07	1762.40±20.77
Spleen	498.40±27.75	495.20±25.38	491.40±20.25	493.40±26.39
Lungs	1504.60±26.55	1505.80±24.49	1531.80±22.90	1528.60±30.09
Adrenals	64.60±1.78	65.40±1.75	65.00±1.58	65.60±0.81
Uterus	504.80±18.60	508.20±15.85	509.60±26.42	506.80±14.32
Ovaries	105.04±3.77	108.20±2.60	102.00±2.30	102.20±2.11

Values are given in Mean ± SEM, n=5 animals/group, $p < 0.05$

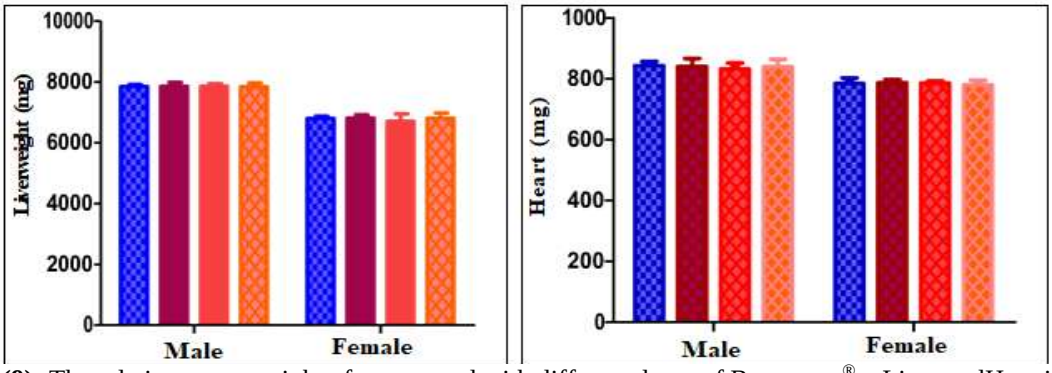


Figure (9): The relative organ weight of rats treated with different doses of Boswegex®onLiver andHeartinrats

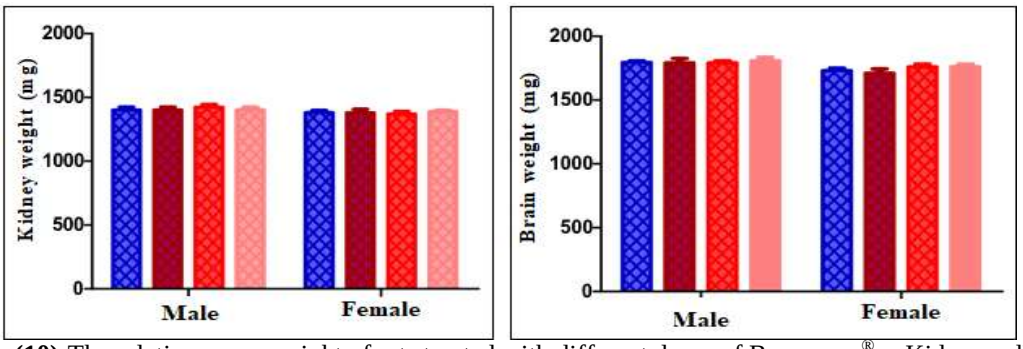


Figure (10): The relative organ weight of rats treated with different doses of Boswegex® onKidneyandBrain inrats

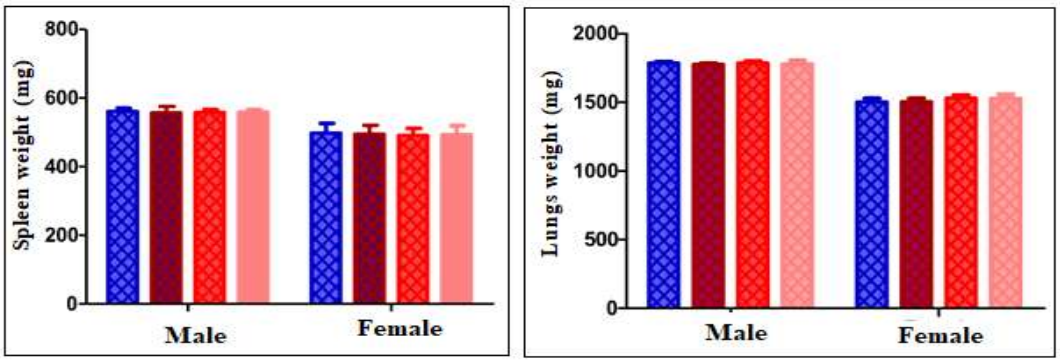


Figure (11): The relative organ weight of rats treated with different doses of Boswegex®onSpleenandLungsinrats

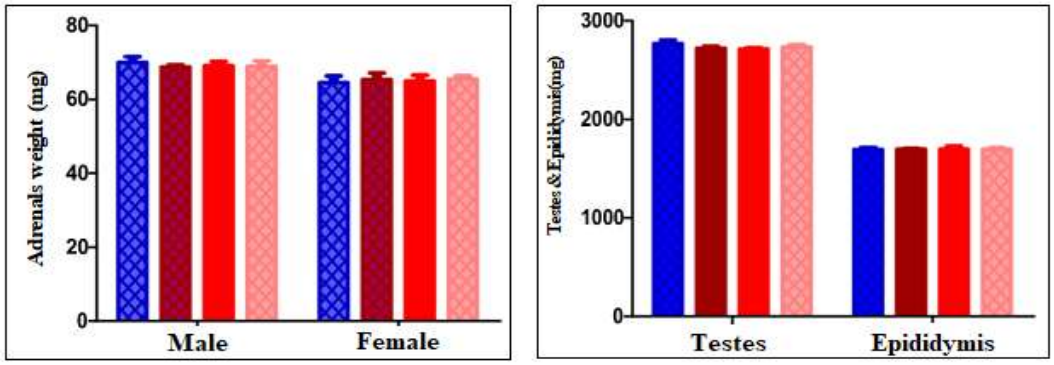


Figure (12): The relative organ weight of rats treated with different doses of Boswegex®on Adrenals, Testes and Epididymis in rats

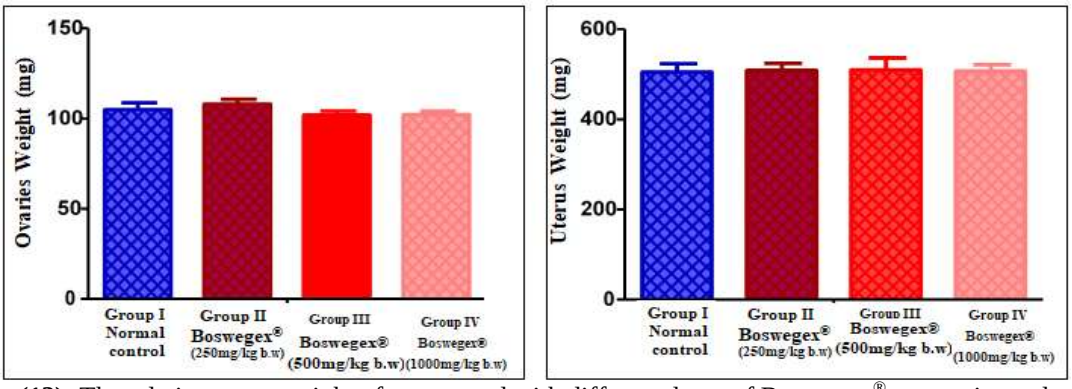


Figure (13): The relative organ weight of rats treated with different doses of Boswegex® on ovaries and uterus in rats

Hematological Parameters

The effects of sub-acute administration of Boswegex on hematological parameters are given in Table 10 and Figure 14. Most hematological parameters, like white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), Lymphocytes (Ly), myocardial infarction (MI), and Granulocytes (GR), in treated rats were not significantly different as compared to the control group.

Table no 10: Effect of Boswegex® on hematological parameters in the sub-acute oral toxicity study

Parameters	HEMATOLOGY							
	MALE				FEMALE			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
WBC	7.74±0.17	7.84±0.30	7.80±0.78	7.70±0.27	6.86±0.27	7.02±0.36	6.78±0.25	6.92±0.41
RBC	9.20±0.21	9.02±0.40	9.26±0.19	9.18±0.14	8.47±0.14	8.51±0.15	8.54±0.16	8.59±0.18
HGB	14.42±0.31	14.24±0.43	14.70±0.55	14.38±0.44	13.36±0.44	13.28±0.15	13.48±0.22	13.36±0.20
HCT	41.06±0.30	41.12±1.40	41.80±0.85	41.46±0.86	38.60±0.86	38.68±0.38	38.30±0.55	38.86±0.63
MCV	48.40±0.51	48.40±0.40	46.20±0.37	48.00±0.32	39.52±0.32	39.16±0.32	39.30±0.85	39.20±0.85
MCH	15.96±0.31	15.60±0.30	15.66±0.38	15.58±0.32	15.32±0.32	15.16±0.27	15.20±0.63	15.60±0.30
MCHC	32.66±0.54	32.56±0.35	31.84±0.36	32.24±0.66	31.94±0.66	31.92±0.39	31.80±0.31	32.00±0.32
PLT	9.75±0.62	9.82±0.39	9.99±0.19	9.98±0.37	9.09±0.37	8.86±0.37	9.06±0.12	9.07±0.23
Ly	68.40±0.88	68.12±1.44	67.64±1.26	67.20±1.55	58.48±1.55	58.54±1.31	58.36±0.92	58.82±0.51
MI	7.20±0.98	7.28±0.80	8.06±0.67	7.50±0.66	8.90±0.66	8.92±0.24	7.50±0.87	7.38±0.80
GR	24.16±1.84	24.60±0.83	25.90±1.30	25.50±1.24	32.14±1.24	32.54±1.19	33.08±0.92	32.80±1.12

Values are given in Mean ± SEM, n=5 animals/group, $p < 0.05$

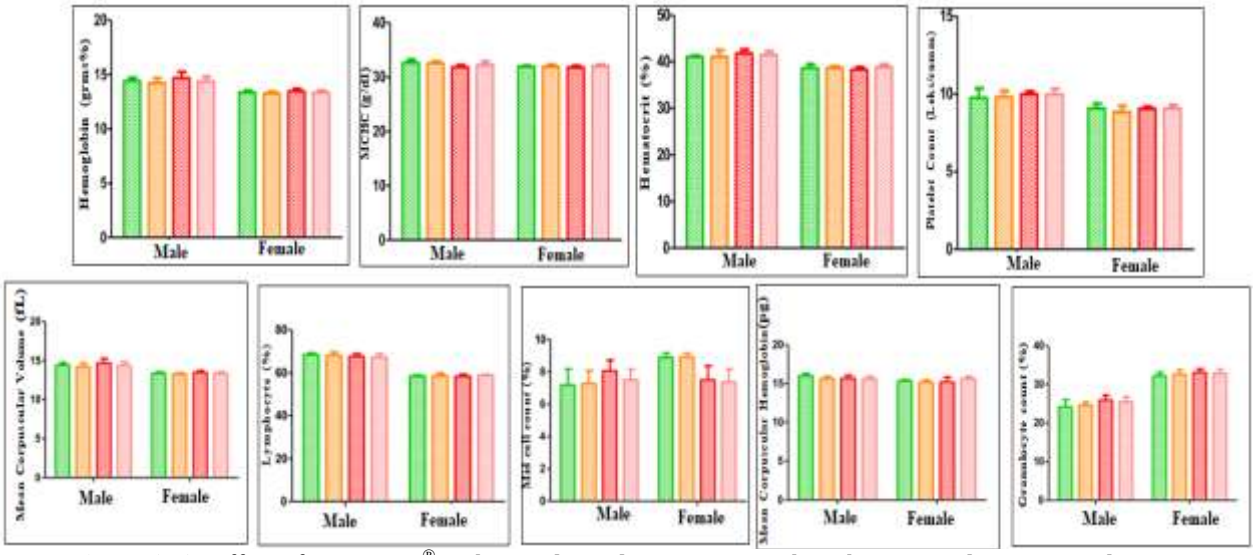


Figure (14): Effect of Boswegex® on hematological parameters in the sub-acute oral toxicity study

Biochemical Analysis

The effects of sub-acute administration of Boswegex® on biochemical parameters are demonstrated in Table 11 and Figures(15-22). Boswegex® had no relevant changes in biochemical parameters such as Total bilirubin, Direct Bilirubin, Alkaline phosphatase (ALP), Albumin, Total protein, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Urea, Creatinine, Total Cholesterol, Triglycerides, HDL, LDL, Calcium, phosphorous, and Glucose.

Table no 11: Effect of Boswegex® on biochemical parameters in the sub-acute oral toxicity study

Parameters	BIOCHEMISTRY							
	MALE				FEMALE			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
Total Bilirubin	0.62±0.04	0.62±0.03	0.65±0.03	0.64±0.03	0.57±0.02	0.55±0.04	0.55±0.02	0.53±0.02
Direct Bilirubin	0.21±0.02	0.21±0.01	0.20±0.01	0.20±0.01	0.18±0.02	0.19±0.01	0.18±0.01	0.19±0.01
Alkaline Phosphate	183.00±3.82	180.60±3.50	179.40±3.76	183.40±2.89	170.20±2.84	170.60±1.72	170.40±1.91	171.40±1.99
Protein	6.82±0.10	6.88±0.12	6.76±0.21	6.78±0.13	6.48±0.21	6.46±0.14	6.54±0.22	6.62±0.13
Albumin	4.40±0.19	4.32±0.21	4.30±0.18	4.28±0.12	4.40±0.24	4.40±0.24	4.26±0.19	4.40±0.19
ALT	82.80±2.54	83.80±3.20	80.80±2.67	83.00±3.96	71.00±2.35	72.20±2.78	71.40±2.36	70.80±2.52
AST	29.40±1.29	30.40±2.27	28.60±1.75	29.20±1.74	21.40±0.68	21.80±0.37	20.80±1.16	21.00±0.55
B.UREA	35.40±0.93	36.20±1.39	35.40±1.17	35.00±1.30	29.20±0.80	29.60±0.81	29.40±1.44	29.20±1.07
S. Creatinine	0.98±0.05	0.96±0.05	0.94±0.02	0.94±0.02	0.82±0.04	0.74±0.05	0.72±0.05	0.80±0.03
Total Cholesterol	83.20±2.75	85.80±2.33	84.20±3.47	82.40±2.87	76.20±3.02	75.80±2.13	77.20±1.88	78.20±0.73
Triglycerides	91.40±4.32	91.60±3.01	90.20±3.60	90.40±1.78	83.00±2.10	82.80±2.75	83.80±2.78	84.60±1.47
HDL	28.80±2.97	27.00±1.30	28.20±0.86	28.20±0.37	27.60±1.69	27.20±0.97	27.60±1.47	27.40±1.03
LDL	41.60±1.21	42.60±2.48	39.60±1.72	40.40±1.21	36.20±1.74	38.60±2.77	36.60±2.38	36.40±2.42
Calcium	7.82±0.17	7.82±0.15	7.80±0.15	7.84±0.19	7.26±0.12	7.26±0.10	7.16±0.10	7.22±0.24
Phosphorous	2.96±0.12	2.94±0.07	2.94±0.12	2.92±0.09	2.70±0.11	2.68±0.04	2.72±0.05	2.66±0.10
Glucose	85.40±2.18	84.80±1.85	86.60±1.63	84.20±3.01	83.80±2.13	82.80±1.77	82.40±1.81	83.20±2.35

Values are given in Mean ± SEM, n=5 animals/group, $p < 0.05$

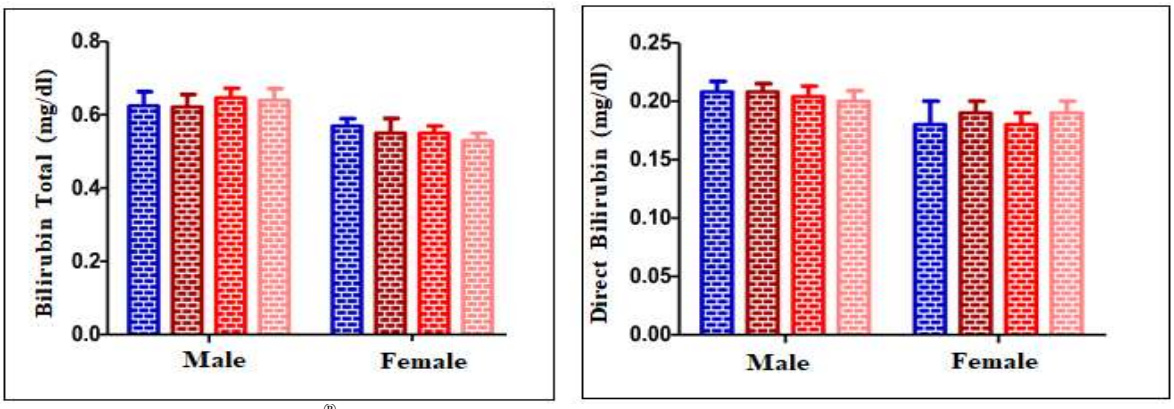


Figure (15): EffectofBoswegex® onBilirubin-TotalandBilirubin-Direct inrats

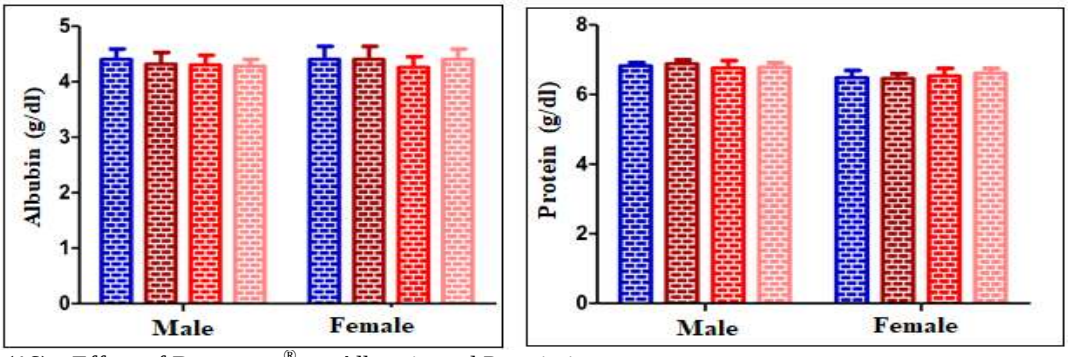


Figure (16): Effect of Boswegex® on Albumin and Protein in rats

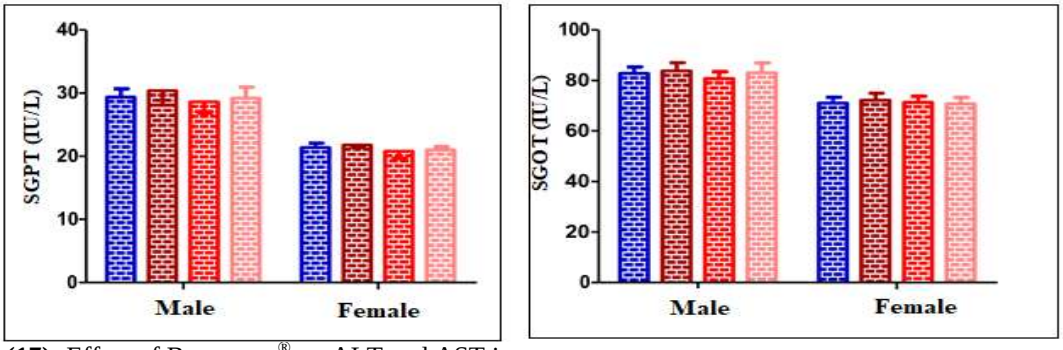


Figure (17): Effect of Boswegex® on ALT and AST in rats

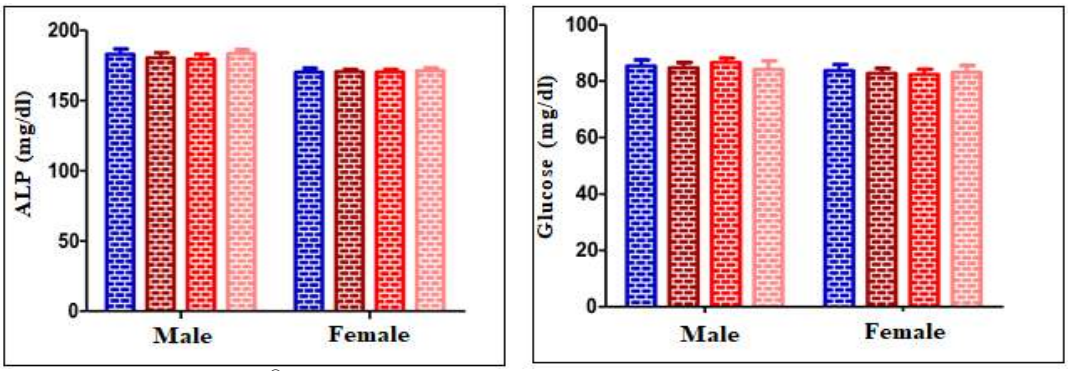


Figure (18): EffectofBoswegex®onalkalinephosphataseandGlucoseinrats

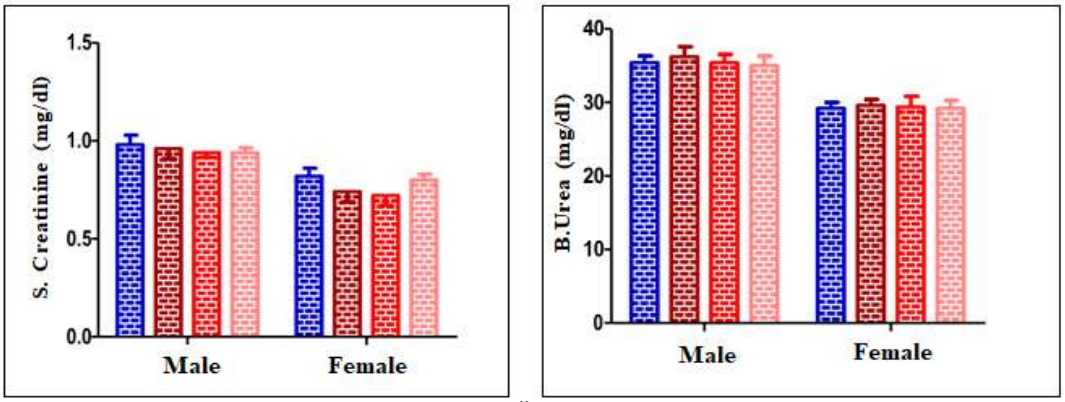


Figure (19): EffectofBoswegex®onS. Creatinine andB. Ureainrats

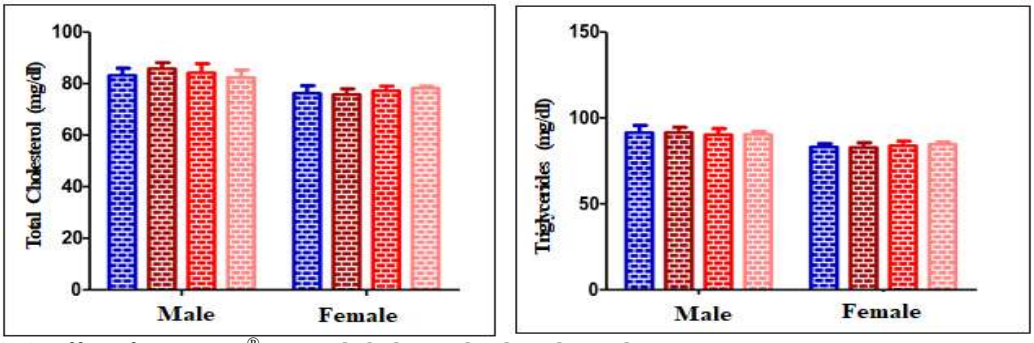


Figure (20): EffectofBoswegex® onTotalCholesterolandTriglyceridesinrat

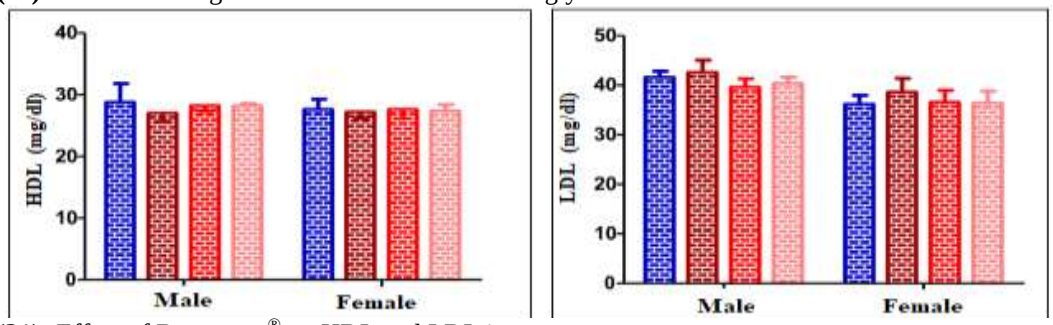


Figure (21): Effect of Boswegex® on HDL and LDL in rats

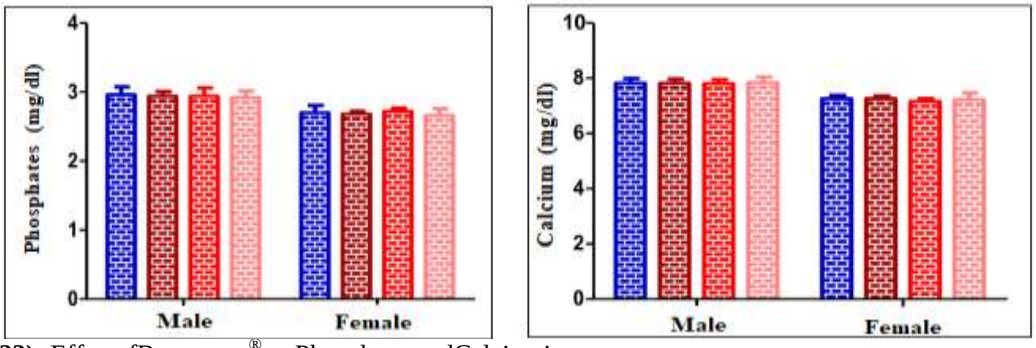


Figure (22): EffectofBoswegex® onPhosphatesandCalciuminrat

Histopathological Study

The liver, kidneys, adrenals, testes, ovaries, uterus, epididymis, spleen, brain, and heart organs of the control and treated groups had normal histology and had no evidence of any obvious pathological abnormalities. Additionally, when compared to the organs of the control group, a macroscopic inspection of the treated rats' organs showed no abnormalities in colour or texture Figures(23-29).

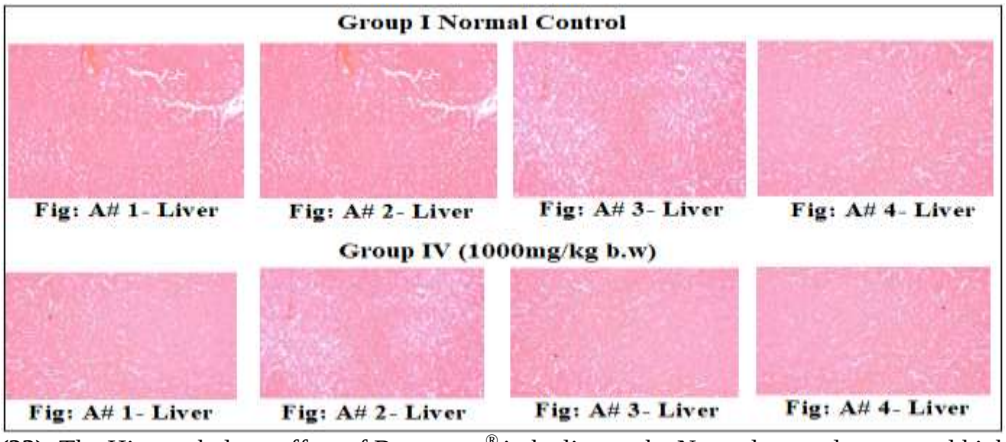


Figure (23): The Histopathology effect of Boswegex® inthe liverat the Normal control group and higher dose

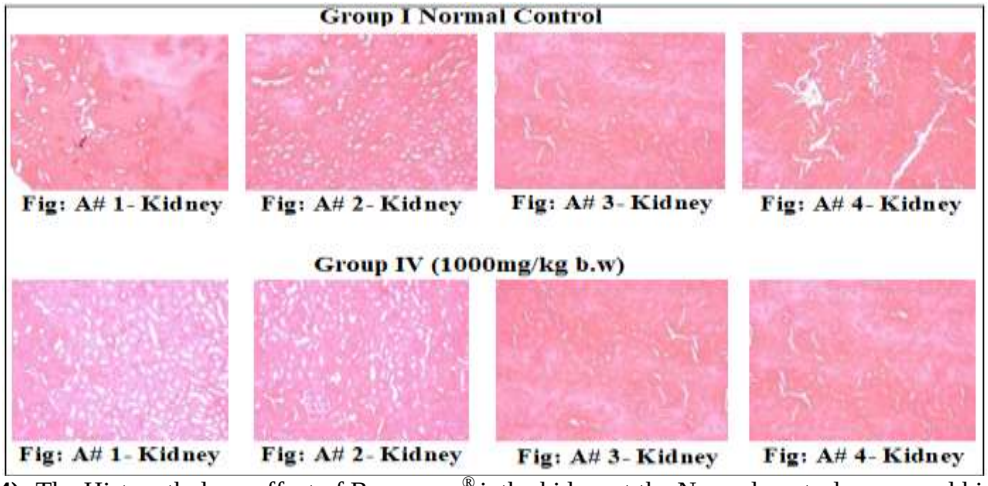


Figure (24): The Histopathology effect of Boswegex® inthe kidneyat the Normal control group and higher dose

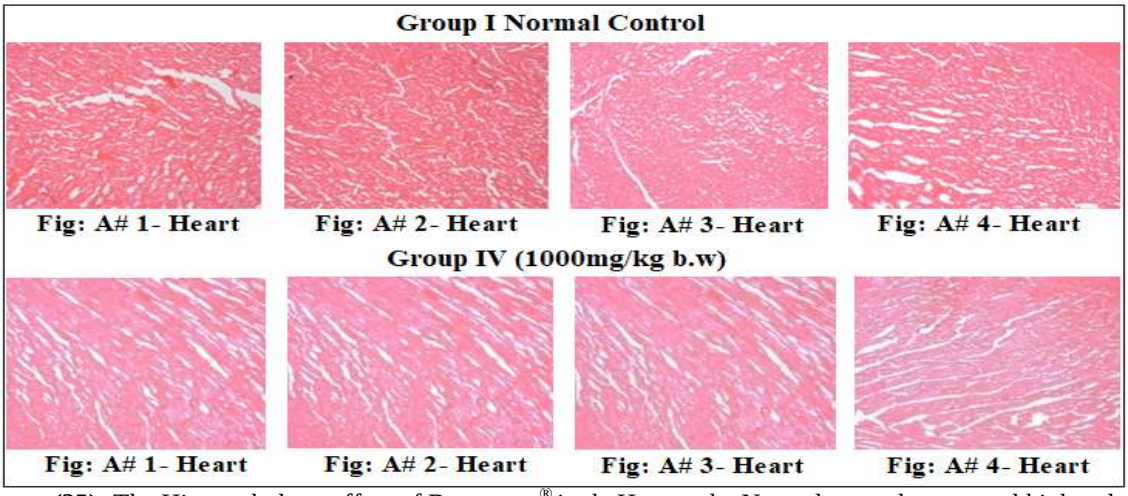


Figure (25): The Histopathology effect of Boswegex® in theHeartat the Normal control group and higher dose

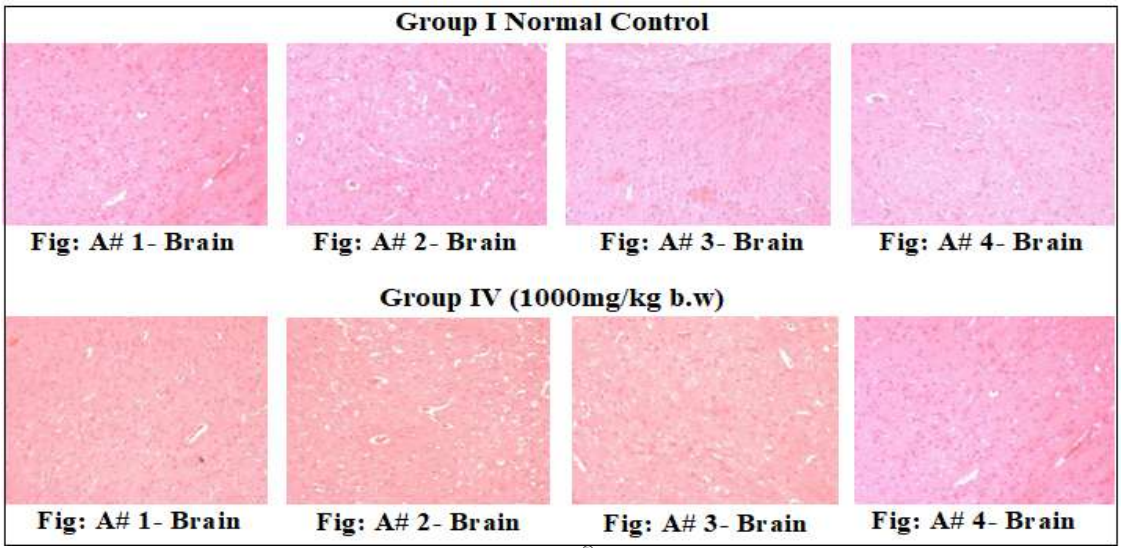


Figure (26): The Histopathology effect of Boswegex® inthe Brainat the Normal control group and higher dose

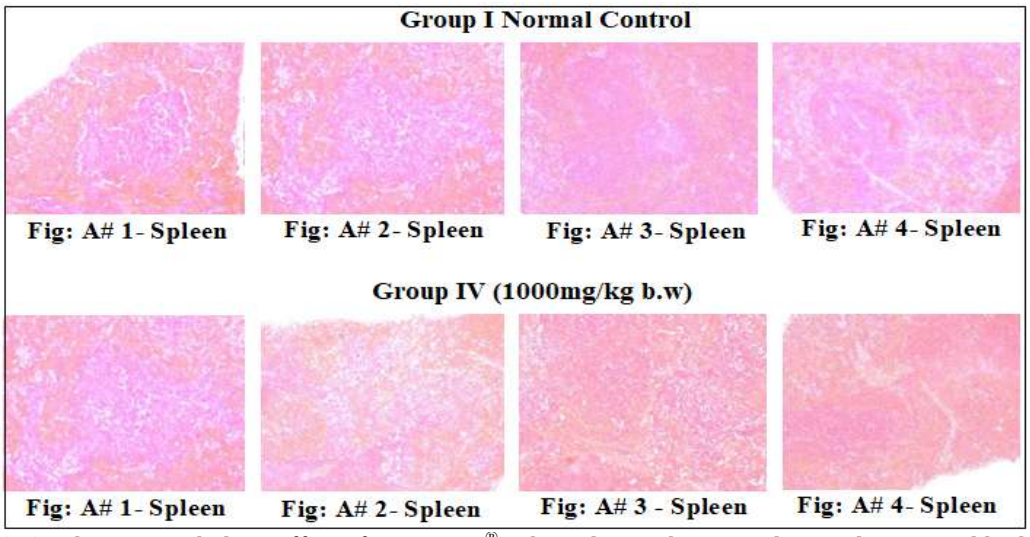


Figure (27): The Histopathology effect of Boswegex® inthe Spleenat the Normal control group and higher dose

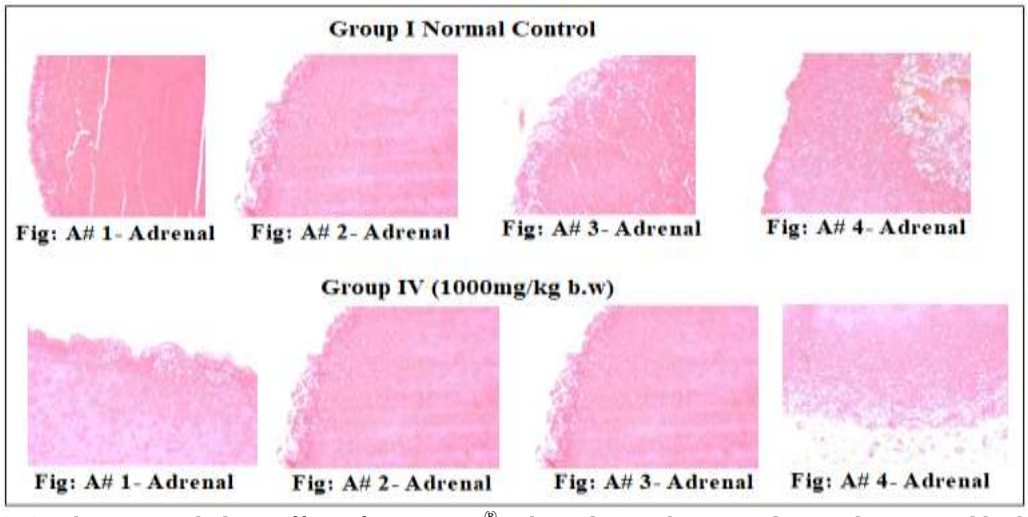


Figure (28): The Histopathology effect of Boswegex® inthe Spleenat the Normal control group and higher dose

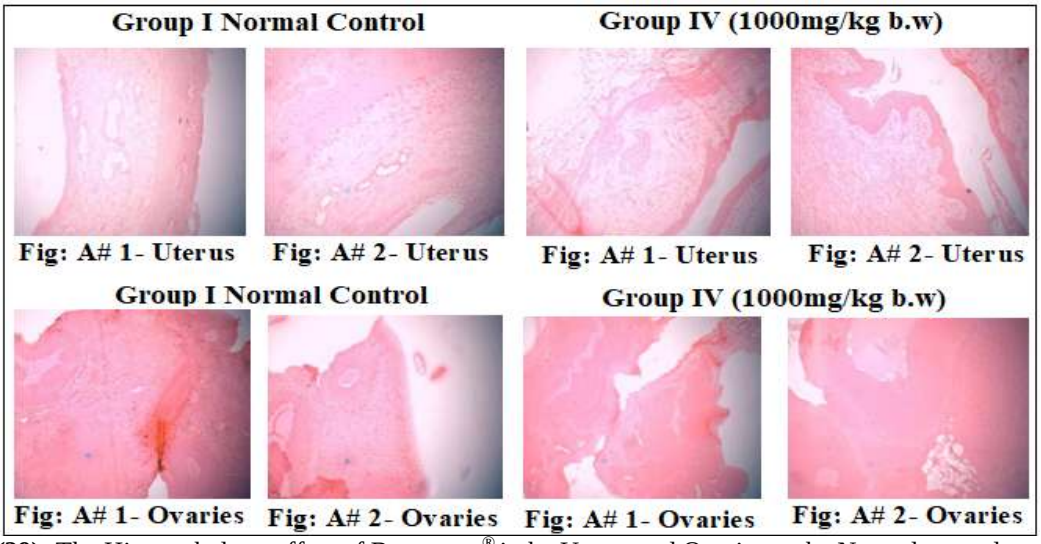


Figure (29): The Histopathology effect of Boswegex® inthe Uterus and Ovaries at the Normal control group and higher dose

IV. Discussion

Currently, the pharmacological properties of therapeutic plants are well known. The potential toxicity of these physiologically active compounds, however, is less well understood[24]. When a product is being tested, the adverse reactions that arise soon after a single dose are examined in an acute toxicity study. These experiments, which are often performed on rats, are carried out early in the development of a new chemical to learn more about its toxicity[25]. The results of this investigation demonstrated that, at a dose of 2000 mg/kg b.w., aqueous ethanolic extracts of *B. serrata* (Boswegex®) did not result in death or behavioural abnormalities in rats. This *B. serrata* (Boswegex®) can be regarded as not causing an imminent risk of acute toxicity in accordance with the OECD 423 Guidelines because the LD_{50} is more than 2000mg/kg b.w.

The average lifespan of experimental animals may be impacted by obvious unfavourable effects that are identified through subacute investigations. These studies also provide information on dosing systems and target organ toxicity. As a result, during 28 days, rats were given dosages of 250, 500, and 1000 mg/kg b.w. of *B. serrata* (Boswegex®) to test its effects. The changes in body weight are a sensitive indicator of an animal's general health status. After receiving therapy with *B. serrata* (Boswegex®) for 28 days, all of the animals showed a typical increase in body weight.The normal metabolism of animals was not hindered by *B. serrata* (Boswegex®), it could be observed. The substantial rise in food intake is thought to be the primary factor in the acceleration of weight gain. Similar results were obtained for the weight of the liver, kidneys, adrenals, testicles, ovaries, uterus, epididymis, spleen, brain, and heart, demonstrating that *B. serrata* (Boswegex®) given at subacute oral doses had no impact on normal growth.In toxicity studies, relative organ weight includes consideration of the organs' capacity to predict toxicity and its good correlation with histopathological alterations. The findings of this investigation demonstrated no significant differences between the treatment and control groups in terms of the relative organ weight, demonstrating that none of the organs were adversely affected or exhibited any indicators of toxicity during the trial.

The body weights of the rats given *B. serrata* (Boswegex®) treatment and control rats did not differ significantly between their initial and final weights. Comparing all treatment groups to the control group, there was no difference in feed consumption. Blood samples taken from every animal on day 29 were used to estimate hematological parameters, and a comparison of the treatment group's results with the control group's results was conducted. The findings showed that hematological parameters like hemoglobin, hematocrit, red blood cells, mean corpuscular haemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cells, mid cell leukocytes, platelet, and leukocyte counts were within the normal range in both the control and *B. serrata* (Boswegex®) treated groups, showing non-significant.

Oral administration of *B. serrata* (Boswegex®) did not significantly alter the biochemical parameters of glucose, urea, creatinine, cholesterol, triglycerides, HDL, LDL, bilirubin-total/direct, ALT, AST, albumin, alkaline phosphate, calcium, or phosphorous when compared to the control animals, according to the data on biochemical parameters in treated and control animals presented in the study. The weight of the animals' organs in the treatment and control groups did not change significantly. When all the animals were sacrificed at the conclusion of the study and their organs were grossly inspected for pathology, there were no pathological differences between the treated groups and the control group.

Based on the results of our examination, we came to the conclusion that *B. serrata* (Boswegex®) was a safer, non-toxic substance that could be utilised effectively for pharmacological and therapeutic purposes.

V. Conclusion

Our study evaluated the acute and subacute safety or toxicity of aqueous ethanolic extracts of *Boswellia serrata* (Boswegex®) after oral administration in rats. The acute toxicity study showed that the LD_{50} value was greater than 2000 mg/kg b.w., and the Boswegex® showed no signs of toxicity during the 14 days of the study. In the subacute toxicity study, no deaths were recorded after oral administration of 1000 mg/kg b.w. of *B. serrata* (Boswegex®) for 28 days. In the light of these findings, we may conclude that *B. serrata* (Boswegex®) is not toxic in all doses studied herein and did not produce any evident symptoms in the acute and subacute oral toxicity studies. The histology examination revealed no remarkable changes in the internal organs, like liver, kidneys, adrenals, testes, ovaries, uterus, epididymis, spleen, brain, and heart of the rats, in both control and treated groups. Furthermore, the data of acute and subacute toxicity studies on *B. serrata* (Boswegex®) were obtained in order to increase the confidence in its safety to humans for the use in the development of pharmaceuticals.

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