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Acute And Subacute Oral Toxicity Of *Saraca Asoca* In Rats

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Abstract:

Ashoka is one of India's most revered and mythical trees. Some information regarding its potential toxicity is available. This study assessed *Saraca asoca's* acute and subacute toxicity in wistar rats. The mice were observed for 28 days after being given three separate doses of the plant orally for the acute and subacute toxicity. Result indicates no significant changes in body temperature, body weight and food and water consumption when compared to control group. No significant changes were observed in the relative organ weights of vital organs such as liver, lung, heart, spleen, kidney, brain and uterus with ovary as compared with control group. Body temperature of the rats of both sexes treated with SA Extract at doses low dose (250mg/kg), Intermediate dose (500mg/kg) and high dose (1000mg/kg) were not significantly different from control. When compared to the control groups, the current toxicity investigations of oral administration of SA bark extract (at dosages of 250, 500, and 1000 mg/kg) showed substantial alterations in physical, hematological, and biochemical parameters. Bark extract of SA administration may be hemolytic.

Keywords: *Saraca asoca*, acute toxicity, subacute toxicity, OECD

1. Introduction:

According to the World Health Organization, because of poverty and lack of access to modern medicine, about 65-80% of the world's population in developing countries depends essentially on plants for primary health care (Calixto, 2000). Many questions concerning the efficacy and safety of herbal medicines have also been raised in light of the enormous growth in the usage of medicinal plants throughout the world. Safety of some herbal ingredients has been recently called into question because of identification of adverse events associated with their use and increase in data generated regarding clinically relevant herb – drug interactions (Lewis and Elvin-Lewis, 1995). The growing interest of society in herbal products and other dietary supplements is due to misconception that "herbal products are always safe", though, their use may cause severe life threatening complications. These medicines may be a part of self-medication bought over-the-counter without prescriptions of a registered medical practitioner (Sunita et al., 2008). However, contrary to the popular belief, many plant formulations are found to be toxic and there is a high risk of adverse effects and interactions with conventional drugs (Nath and Rudra, 2002).

One of the most important plants used since ancient times is S*araca asoca*. The Sanskrit word asoka, sometimes known as Ashoka, meaning "without sorrow" or "that which causes no grief." One of India's most fabled and revered trees is the Ashoka. The Ashoka tree, or *Saraca indica*, is a member of the Caesalpinaceae family and is commonly referred to by its binomial Latin name, *Saraca asoca* (Roxb.) in Indian Ayurvedic pharmacopoeia. India as a whole has it, but the Himalaya, Kerala, Bengal, and the entire south are the best places to find it. Ashoka is considered a sacred plant in Hinduism. It is a special plant for Kamadeva. Every year on December 14th a festival is observed by all Hindus in honor of this plant. (Scartezzini and Speroni, 2000, Hegdea et al., 2007).

Ashoka has been used for a wide range of pharmacological purposes, including the treatment of cancer, anti-menorrhagic, anti-oxytoxic, antibacterial, and homeopathic remedies. It is useful for a variety of conditions, including genito-urinary disorders, CNS disorders, and skin infections. *Saraca asoca* is available as various marketed formulations such as Ashokarishta, Ashokkwath, Seed powder, Flower powder, Ashokghrita, decoction, pill, paste, herbal wine, syrup, capsules etc consists of bark extract as higher concentration of active principles found in bark. (Hegdea et al., 2007). Since this plant has a wide range of applications and diverse pharmacological activities have been conducted using different plant parts and extraction techniques, it is imperative that we employ current science to focus on the plant's safety and efficacy. But there hasn't been a lot of research done on *Saraca asoca's* general toxicity.

The purpose of the current investigation was an examination of the pharmaco-toxicological and behavioral consequences following acute and subacute administration in wistar rats, the current study aims to explore the safety of the extract of *Saraca asoca* bark. .

2. Materials and methods:

2.1. Plant material:

A little, evergreen tree that can grow up to ten meters tall is called *Sarca asoca*. Its wood is reddish-brown and its bark is blackish; its orange to crimson flowers, which in bunches turn deep red, have a potent scent; Seeds 4–8 ellipsoid–oblong, pods tapering at both ends. Asoka flowers from December to May, and it fruits from June to July. Amsar Pvt. Ltd. in Indore provided the Saraca asoca bark extract (SA Extract) (Batch No. 9172, No. F/D–645).

2.2. Evaluation of toxicity following single dose administration

2.2.1. Animals:

Wistar rats, both male and female, aged 8–12 weeks, with weights within ±20% of the average. Women ought to be non-pregnant and nulliparous. The animals were kept in transparent plastic cages and were randomly assigned to three treatment groups (5 rats/sex/group). Throughout the trial, five rats of the same sex were housed in each cage, fed a standard laboratory diet, and allowed unlimited access to tap water. The temperature and relative humidity were controlled at 23±2 ◦C and 40±10%, respectively, with a 12-hour light/dark cycle. The Institutional Animal Ethical Committee (IAEC) approved the experimental protocols and animal care, with permission number (IAEC/10-11/P-05). All of the tests were conducted with careful adherence to ethical norms.

2.2.2. Observations

An oral high dose of SA Extract 2000 mg/kg was administered to the animals. The animals were observed one-on-one for the first thirty minutes following oral administration, then once a day for the next twenty-four hours. During the first four hours, and then every day for the next fourteen days, particular attention was paid to observations such as body weight, food and water consumption, and signs of toxicity or mortality. The animal's behaviour, nervous system, and autonomic characteristics were noted, including alterations to the skin, fur, eyes, mucous membrane, tremor, convulsions, salivation, lethargy, sleepiness, piloerection, lacrimation, excessive grooming, and posture changes. These observations were made continuously for the first four hours following dosage and sporadically for the next 24 and 72 hours in case of any mortality or death. Lastly, the animals were kept for a further 13 days with signs and under observation.

2.3. **Subacute Oral Toxicity Study for 28 days in rats** (OECD 407,2008, Remirez, 2006)

Group (n=10) (5 Males & 5 Females)	**Treatment**	**Duration**
Group I (Control)	Distilled Water (0.1ml/kg p.o.)	28 Days
Group II (Low dose)	SA Extract (250 mg/kg p.o.)	28 Days
Group III (Intermediate dose)	SA Extract (500 mg/kg p.o.)	28 Days
Group IV (High dose)	SA Extract (1000 mg/kg p.o.)	28 Days

2.3.1. Animals

Wistar rats, both male and female, aged 8–12 weeks, with weights within ±20% of the average. Women ought to be non-pregnant and nulliparous. The animals were kept in transparent plastic cages and randomly assigned to three treatment groups. Throughout the trial, five rats of the same sex were housed in each cage, fed a standard laboratory diet, and allowed unlimited access to tap water. The temperature and relative humidity were controlled at 23±2 ◦C and 40±10%, respectively, with a 12-hour light/dark cycle. The Institutional Animal Ethical Committee (IAEC) approved the experimental protocols and animal care, with permission number (IAEC/10-11/P-05). All of the tests were conducted with careful adherence to ethical norms.

2.3.2. Treatment

Aqueous extract of *Saraca asoca* bark at 250, 500, 1000mg/kg of body weight, were administered by oral route. The volume of drug solution (oral) was calculated based upon the body weight of animal and was kept constant throughout the study with respect to their body weights (1ml/100gm for oral route). Throughout the test, the animals had unrestricted access to food and tap water.

2.3.3. Observations

For ten hours following dosage, all animals were watched closely for any indications of toxicity. Animals were observed daily for the remaining 28 days of the study period for indicators of toxicity, including any changes in food and water consumption and mortality. The weight of the animals was recorded both before and after the 28-day period. On day 28, all animals were killed and at the end of the study the number of dead animals was expressed in percentage and, if possible, the LD50 value was established using Probits method (Angelis Pereira et al., 2003).

2.3.3.1. In life evaluations:
For a period of 28 days, observations were conducted every day about toxicological symptoms and mortality. If any symptoms occurred, information about their duration, intensity, and time of occurrence was noted. Throughout the study, the weight of each rat was noted on day 0 and then every week. Water and food intake were recorded.

2.3.3.2.. Analysis of urine, hematological, and biochemical
Urine samples were taken and examined for physical characteristics such as amount, colour, appearance, deposit, and reactivity at the conclusion of the study. Chemical characteristics include urobilinogen, protein, glucose, ketone bodies, and bile pigments. Microscopic examination is done to detect the existence of casts, crystals, RBC, epithelial cells, and pus cells.
Hematological analyses were done for Hemoglobin concentration (Hb), Hematocrit (PCV), Using a blood cell counter, the following counts were made: leukocytes, neutrophils, eosinophils, monocytes, platelets, white blood cells, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin conc. (MCHC), and white blood cell count (WBC).
Blood samples were centrifuged at 3000×g for five minutes in order to collect the plasma for biochemical analyses. An auto analyser was used to analyze the plasma for total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, SGOT, SGPT, creatinine, blood urea nitrogen (BUN), total cholesterol, triglycerides, blood glucose, and creatinine phosphokinase (CPK).

2.3.3.3. Gross necropsy
The organs of the sacrificed animals were removed after the animals were dissected, cleaned with saline, and then gently dried with filter paper. Using an electronic balance, the weight of each treatment group's liver, kidney, testicles, heart, spleen, brain, and ovaries was measured in relation to the overall weight of the subjects for further investigation.
Relative organ weight = Organ weight (gm) / Body weight (gm) X 100

2.3.3.4. Histopathological profiles
Prior to regular processing in paraffin-embedded blocks, the experimental animal's organs were fixed in 10% formalin. Hematoxylin-Eosin (H & E) stain was used to cut and colour sections that were 5µm thick. All tissues underwent microscopic and gross pathological examinations. For histological screening, sections were examined under a microscope and photomicrographs were produced.

2.3.4. **Statistical analysis**
The statistical significance between the tests and controls was ascertained using the Dunnett test and analysis of one-way variance (ANOVA). Only the values with a $p < 0.05$ were considered statistically significant (Yamakoshi et al., 2002; Bidhe and Ghosh, 2004).

3. Results:

3.1. **Subacute Oral Toxicity**
Clinical observations and measures did not show any signs of substance-related toxicity, and no deaths had occurred during the trial. Rats administered with a 2000 mg/kg dose of SA extract did not exhibit any toxicological symptoms, including lethargy and sleep, but test animals did exhibit salivation, diarrhea, piloerection, lacrimation, and tremors. Numerous studies have demonstrated the benefits of plants with flavonoids, saponin, and tannin in treating a variety of CNS illnesses. Because the SA leaf extract has the same phytoconstituents as the bark, it exhibits depressive action. According to Verma et al. (2010), this could be the likely cause of the animals' slumber and lethargic behaviour.

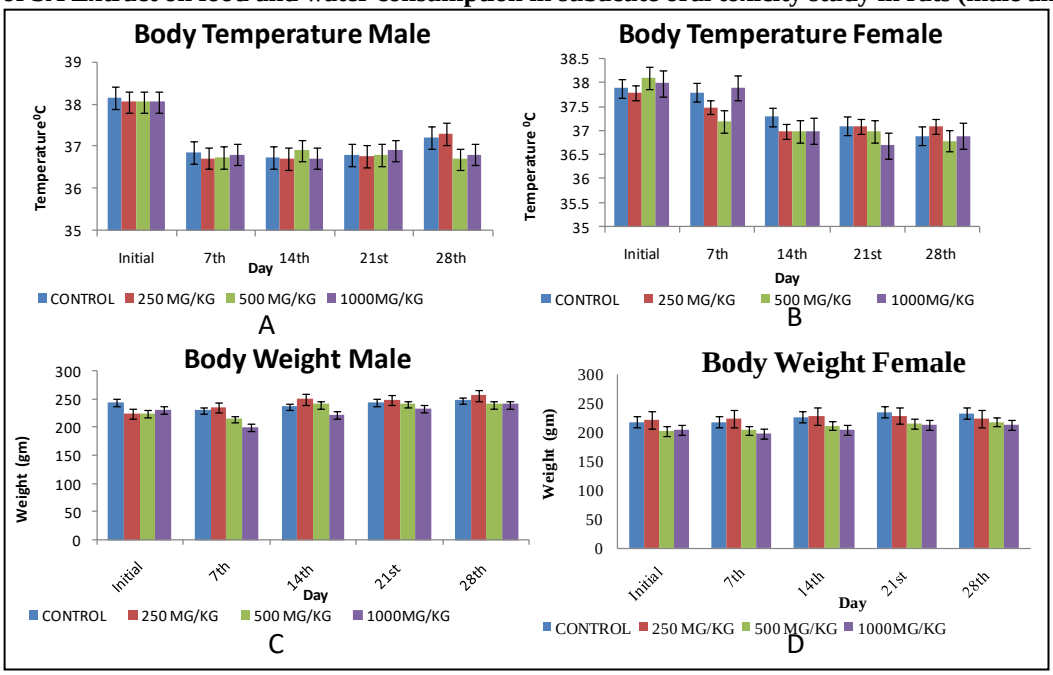
3.2. **Effect of SA Extract on body temperature in subacute oral toxicity study in rats (male and female):**
Body temperature of the rats of both sexes treated with SA Extract at doses low dose (250mg/kg), Intermediate dose (500mg/kg) and High dose (1000mg/kg) were not significantly different from control.

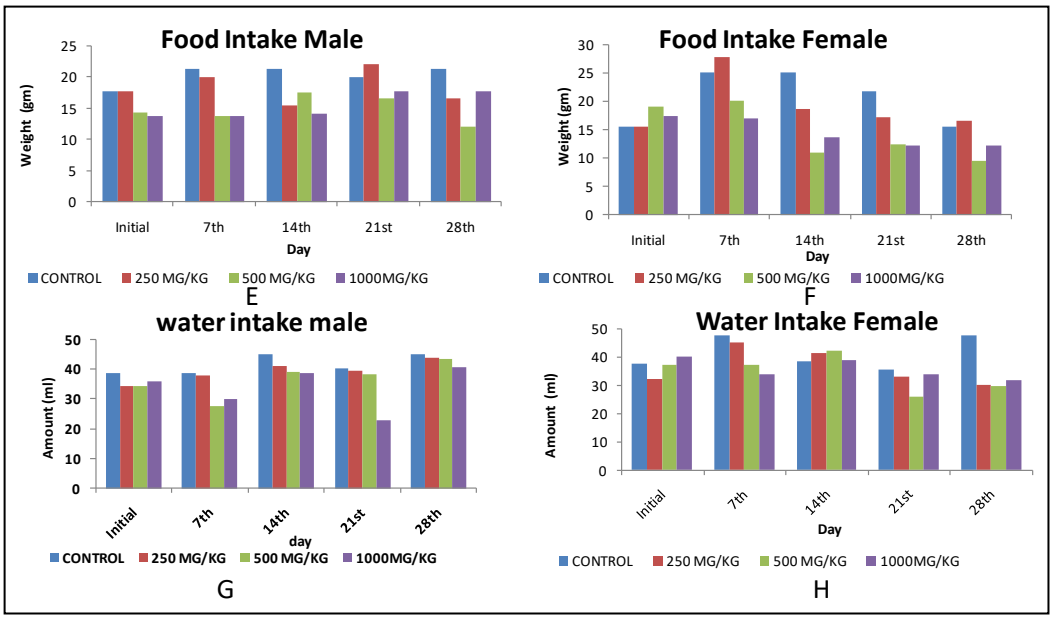
No. of Day	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Male				
Initial	38.16±0.078	38.05±0.12	38.05±0.12	38.05±0.12
7th	36.86±0.11	36.72±0.22	36.74±0.075	36.8±0.28
14th	36.74±0.075	36.7±0.13	36.9±0.11	36.72±0.22
21st	36.8±0.28	36.76±0.14	36.8±0.13	36.9±0.12
28th	37.2±0.17	37.3±0.16	36.7±0.12	36.8±0.16
Female				

Initial	37.9±0.13	37.8±0.13	38.1±0.12	38.0±0.11
7th	37.8±0.13	37.5±0.041	37.2±0.57	37.9±0.13
14th	37.3±0.36	37.0±0.063	37.0±0.11	37.0±0.11
21st	37.1±0.25	37.1±0.19	37.0±0.23	36.7±0.18
28th	36.9±0.23	37.1±0.13	36.8±0.14	36.9±0.13

Data are expressed as mean± S.E.M., n = 5. No statistical difference between control and test groups ($p > 0.05$), analyzed by using one-way ANOVA followed by Dunnett's multiple comparison test.

3.3. **Effect of SA Extract on food and water consumption in subacute oral toxicity study in rats (male and female):**





The results were expressed as mean± S.E.M., n = 5. No statistical difference between control and test group, analyzed by using student t-test.

3.4. **Effect of SA Extract on urine parameters in subacute oral toxicity study in rats (male and female):**
Effect of SA Extract on urine parameters in subacute oral toxicity study in male rats

Parameters	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Physical examination				
Quantity(ml)	10± 0.509	9.5 ±0.583	9.8± 0.583	9.0±0.509
Colour	Yellow	Yellow	Yellow	Yellow
Appearance	Clear	Clear	Clear	Clear
Deposit	Absent	Absent	Absent	Absent
Reaction	Acidic	Acidic	Acidic	Acidic
Chemical examination				
Protein	Absent	Absent	Absent	Absent
Glucose	Absent	Absent	Absent	Absent
Ketone bodies	Absent	Absent	Absent	Absent
Bile pigment	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal	Normal
Microscopic examination				
Pus cells	Occasional	Occasional	Occasional	Occasional
R.B.C.s	Absent	Absent	Absent	Absent
Epithelial cells	Occasional	Occasional	Occasional	Occasional
Crystals	Absent	Absent	Absent	Absent
Casts	Absent	Absent	Absent	Absent

Effect of SA Extract on urine parameters in subacute oral toxicity study in female rats

Parameters	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Physical examination				
Quantity(ml)	8.7± 0.5	8.9 ±0.83	7.9± 0.3	8.5±0.9
Colour	Yellow	Yellow	Yellow	Yellow
Appearance	Clear	Clear	Clear	Clear
Deposit	Absent	Absent	Absent	Absent
Reaction	Acidic	Acidic	Acidic	Acidic
Chemical examination				
Protein	Absent	Absent	Absent	Absent
Glucose	Absent	Absent	Absent	Absent
Ketone bodies	Absent	Absent	Absent	Absent
Bile pigment	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal	Normal
Microscopic examination				
Pus cells	Occasional	Occasional	Occasional	Occasional
R.B.C.s	Absent	Absent	Absent	Absent
Epithelial cells	Occasional	Occasional	Occasional	Occasional
Crystals	Absent	Absent	Absent	Absent
Casts	Absent	Absent	Absent	Absent

The results were expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups.

3.5. **Effect of SA Extract on hematological parameters in subacute oral toxicity study in rats (male and female):**
Effect of SA Extract on hematological parameters in subacute oral toxicity study in male rats

Parameters	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Haematocrit (%)	43.218 ±1.2	45.662±2.00*	48.972± 1.19*	49.82±0.84**
Haemoglobin (g/dl)	7.73±0.087	9.4±2.1053**	9.8± 0.0858**	10.1± 0.184**
RBC ($\times 10^6$ mm $^{-3}$)	6.034±0.14	5.386±0.168	5.24±0.230*	4.686±0.189**

MCH (pg)	17.65± 0.23	16.19± 0.368	16.46± 0.463	24.15± 2.258**
MCHC (g/dl)	19.75±0.49	20.486±0.27	20.268±0.008	18.006± 1.9
MCV (fl)	87.17± 1.1	81.816±2.9	81.202± 2.2	105.684±3.9**
Platelet ($\times10^3$ mm $^{-3}$)	345200± 22637	272650± 5612.5**	227500± 12722**	187000± 4242.6**
WBC ($\times10^3$ mm $^{-3}$)	3512.6±371.2	4312.6±371*	5750±494.9**	8225±318.2**
Lymphocyte (%)	49.9± 4.78	64.5±0.93**	75.64±4.42**	78.26± 1.87**
Neutrophils (%)	34.8±5.42	22.8±0.86**	12.8±0.332**	9.1±1.46**
Eosinophils (%)	7.26±0.35	4.6±0.50**	1.8±0.22**	1.5±0.37**
Monocytes (%)	2.8± 0.58	2.2± 0.40*	1.0± 0.00**	1.0± 0.00**

Effect of SA Extract on hematological parameters in subacute oral toxicity study in female rats

Parameters	**Group I (Control)**	**Group II (Low dose 250mg/kg)**	**Group III (Intermediate dose 500mg/kg)**	**Group IV (High dose 1000mg/kg)**
Haematocrit (%)	38.176±1.17	42.192±3.32*	41.81±0.38*	50.73±0.54**
Haemoglobin (g/dl)	7.7±0.24	9.48±0.08**	9.068±0.16**	10.29±0.11**
RBC ($\times10^6$ mm $^{-3}$)	4.854±0.007	4.126±0.32	3.628±0.42*	2.936±0.024**
MCH (pg)	18.662±0.58	15.192±1.7	19.238±1.61	32.306±0.52**
MCHC (g/dl)	19.75±0.49	20.486±0.28	20.268±0.008	18.006±1.2
MCV (fl)	92.50±2.808	77.67±8.221	87.19±2.727	159.1±2.799**
Platelet ($\times10^3$ mm $^{-3}$)	361200 ±6922	271050 ±1237**	229750 ±3891**	141490 2148**
WBC ($\times10^3$ mm $^{-3}$)	3230±242.2	3386.4±125.9*	3647.6±37.4**	6330±37.41**
Lymphocyte (%)	49.9±4.78	64.5±1.871**	75.64±0.93**	78.26±4.42**
Neutrophils (%)	42.6±4.523	29.2±1.772**	20.6±3.03**	13.8±1.53**
Eosinophils (%)	6.64±0.18	3.36±0.51**	2.6±0.40**	1.6±0.60**
Monocytes (%)	4±0.18	3.66±0.44	1.8±0.37**	1.6±0.4**

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at *($p < 0.05$), ** ($p<0.01$).

3.6. **Effect of SA Extract treatment on biochemical parameters in subacute oral toxicity study in rats (male and female):**

Effect of SA Extract on biochemical parameters in subacute oral toxicity study in male rats

Parameters	**Group I (Control)**	**Group II**	**Group III**	**Group IV**

		(Low dose 250mg/kg)	(Intermediate dose 500mg/kg)	(High dose 1000mg/kg)
Total protein (gm/dl)	7.39± 0.28	7.57±0.16	7.89±0.54	7.61±0.21
Albumin (gm/dl)	3.4±0.47	3.16±0.34	3.1±0.25	3.9±0.21
Globulin (gm/dl)	2.65±0.187	3.756±0.505	3.94±0.299	3.00±0.274
Total Bilirubin (mg/dl)	0.764±0.009	0.904±0.0375*	0.95±0.018**	1.13±0.064**
Direct Bilirubin (mg/dl)	0.40±0.0044	0.408±0.022	0.378±0.0037	0.42±0.0114
Indirect Bilirubin (mg/dl)	0.466±0.013	0.486±0.028	0.546±0.0186	0.77±0.07**
SGPT (IU/L)	103.7±3.367	95.46±2.43	112.4± 1.86	81.5± 1.871**
SGOT (IU/L)	108.8±7.658	105.2±1.497	100.5±0.224	91.5±9.35
Creatinine (mg/dl)	1.56±0.075	1.46±0.075	0.78±0.080**	0.78±0.08**
Blood Urea Level (mg/dl)	37.6±0.748	43.36±3.56	27.5±2.12**	19.6±0.51**
Blood urea nitrogen (mg/dl)	20.26±0.35	17.57±2.59	12.85±0.125**	9.15±0.007**
Cholesterol (mg/dl)	120.7±2.62	118.1±4.12	118.3±5.52	117.86±0.37*
Triglyceride (mg/dl)	98.1±23.65	92.8±2.99	88.4±0.969	58.3±0.66**
Blood Glucose (mg/dl)	121.7±2.44	92.2±2.81**	66±5.81**	25.2±2.72**
CPK (IU/L)	166.56±2.45	182.2±0.86**	193.98±2.7**	259.64±3.1**

Effect of SA Extract on biochemical parameters in subacute oral toxicity study in female rats

Parameters	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Total protein (gm/dl)	6.3±0.3742	6.906±0.108	7.5±0.114	7.084±0.5705
Albumin (gm/dl)	2.6±0.245	3.6±0.245	4.084±0.571	4.5±0.114
Globulin (gm/dl)	3.3±0.3742	4.056±0.025	3.3±0.3	3±0.2739
Total Bilirubin (mg/dl)	0.824±0.03	0.816±0.034	1.09±0.002**	1.27±0.11**
Direct Bilirubin (mg/dl)	0.406±0.02	0.316±0.021	0.429±0.0179	0.354±0.011
Indirect Bilirubin (mg/dl)	0.598±0.03	0.5±0.04472	0.5±0.04278	0.916±0.1**
SGPT (IU/L)	96.6±4.49	96.56±5.03	106.7±5.073	74.4±3.265**
SGOT (IU/L)	95.9±1.122	93±3.742	96.8±2.245	88.96±0.563
Creatinine (mg/dl)	1.366±0.018	1.36±0.080	0.9±0.0374**	0.9 ±0.051**
Blood urea level (mg/dl)	38.56±1.311	37.14±5.130	25.1±0.900**	25.2±1.319**

Blood Urea Nitrogen (mg/dl)	18.02±0.688	15.48±2.396	11.73±0.3**	8.12±0.2**
Cholesterol (mg/dl)	114.3±1.6	101.6±2.62	106.26±4.12	99.1±4.68*
Triglyceride (mg/dl)	94.1±2.619	93.8±1.881	84.2±5.238	55.9±4.864**
Blood Glucose (mg/dl)	107±5.407	78.0±4.30**	51.0±1.497**	27.0±0.400**
CPK (IU/L)	125.94±2.89	208.6±3.9**	223.1±3.19**	258.7±2.08**

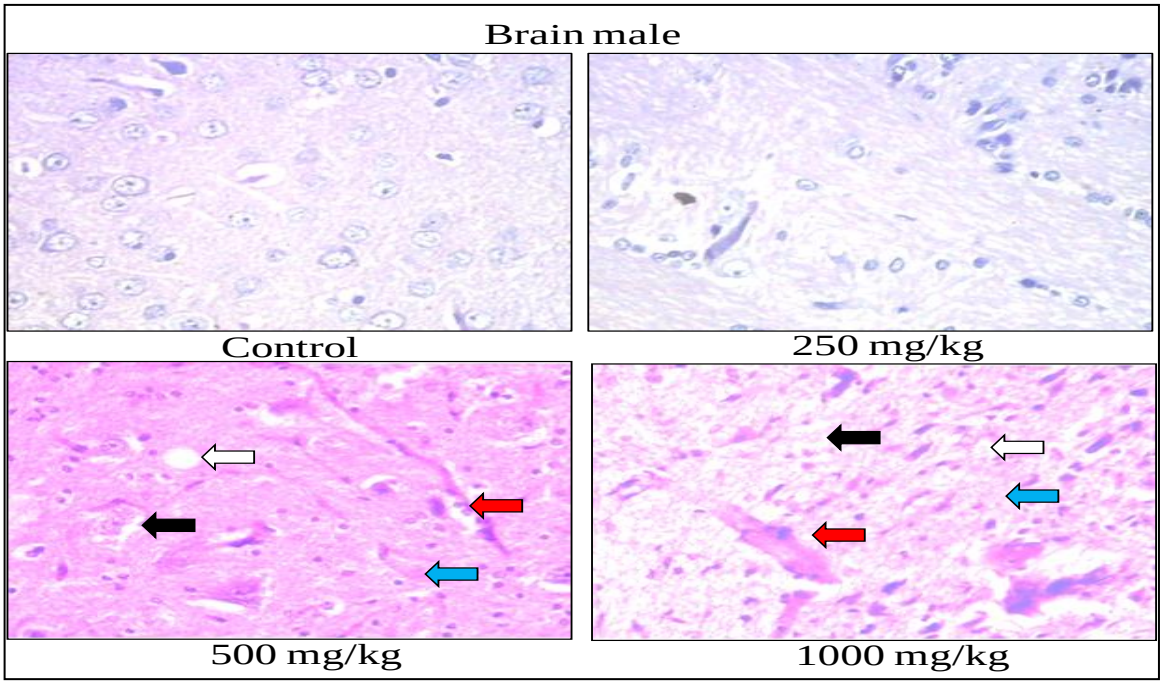
The data are expressed as Mean ± SEM (n=5). The data was analyzed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at *($p < 0.05$), ** ($p<0.01$).

3.7. ***Effect of SA Extract treatment on relative organ weights (g/100g BW) in subacute oral toxicity study in rats (male and female):***

Name of Organ	Group I (Control)	Group II (Low dose 250mg/kg)	Group III (Intermediate dose 500mg/kg)	Group IV (High dose 1000mg/kg)
Male				
Heart	0.658±0.015	0.664±0.012	0.64±0.007	0.646±0.015
Brain	1.182±0.005	1.157±0.018	1.154±0.019	1.168±0.012
Kidney	0.812±0.018	0.648±0.003**	0.648±0.003**	0.618±0.013**
Liver	6.324±0.118	5.36±0.064**	5.29±0.022**	4.76±0.050**
Lungs	1.416±0.036	1.392±0.016	1.356±0.016	1.326±0.009
Spleen	0.65±0.007	0.58±0.005**	0.542±0.003**	0.533±0.005**
Testis	0.744±0.012	0.764±0.011	0.728±0.013	0.758±0.014
Female				
Heart	0.4314±0.01	0.4714±0.019	0.468±0.003	0.455±0.001
Brain	1.24±0.02915	1.364±0.1353	1.416±0.03187	1.12±0.04572
Kidney	0.728±0.011	0.572±0.009**	0.556±0.010**	0.532±0.005**
Liver	5.584±0.127	4.962±0.033**	4.76±0.035**	4.226±0.021**
Lungs	1.277 ±0.022	1.26±0.006	1.232±0.002	1.232±0.017
Spleen	0.774±0.012	0.612±0.006**	0.482±0.005**	0.392±0.003**
Ovary	0.098±0.055	0.095±0.0012	0.0788±0.017	0.077±0.016

The data are expressed as Mean ± SEM (n=5). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. No statistical difference between control and test groups ($p > 0.05$), statistically significant at ** ($p<0.01$).

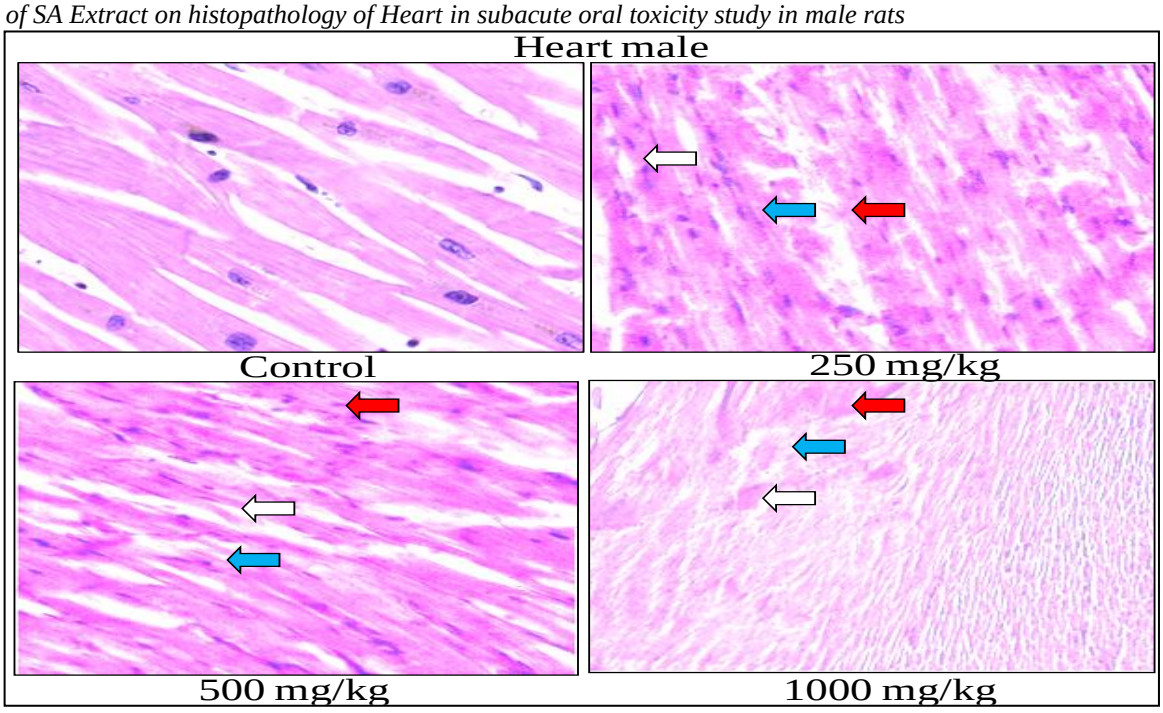
3.8. ***Histopathological study (in subacute oral toxicity study in rats (male and female) :***
Effect of SA Extract on histopathology of Brain in subacute oral toxicity study in male rats



Photograph of H & E staining of brain

➡	Vascular changes	➡	Neuronal degeneration	➡	Microglial infiltration	⇨	Mild hypoxic changes

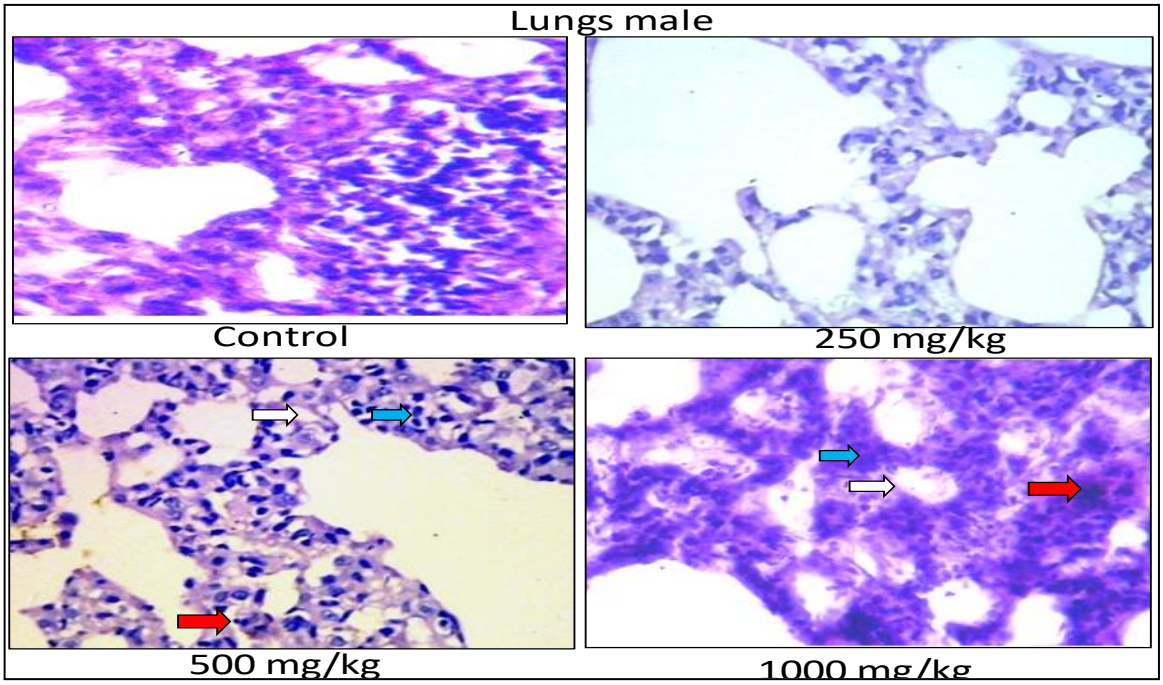
Effect of SA Extract on histopathology of Heart in subacute oral toxicity study in male rats



Photograph of H & E staining of heart

➡	Vascular changes	⇨	Myocardial degeneration	➡	Leucocytic infiltration

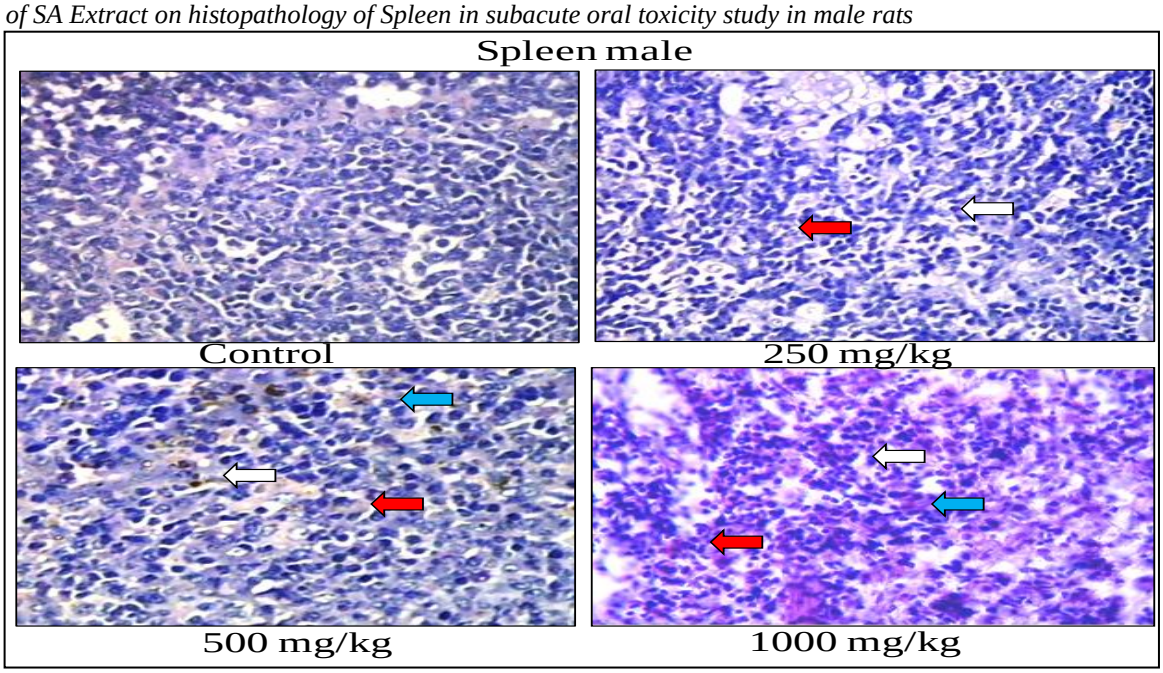
Effect of SA Extract on histopathology of Lungs in subacute oral toxicity study in male rats



Photograph of H & E staining of lung.

➡	Vascular changes	⇨	Emphysema structure	➡	MNC Infiltration

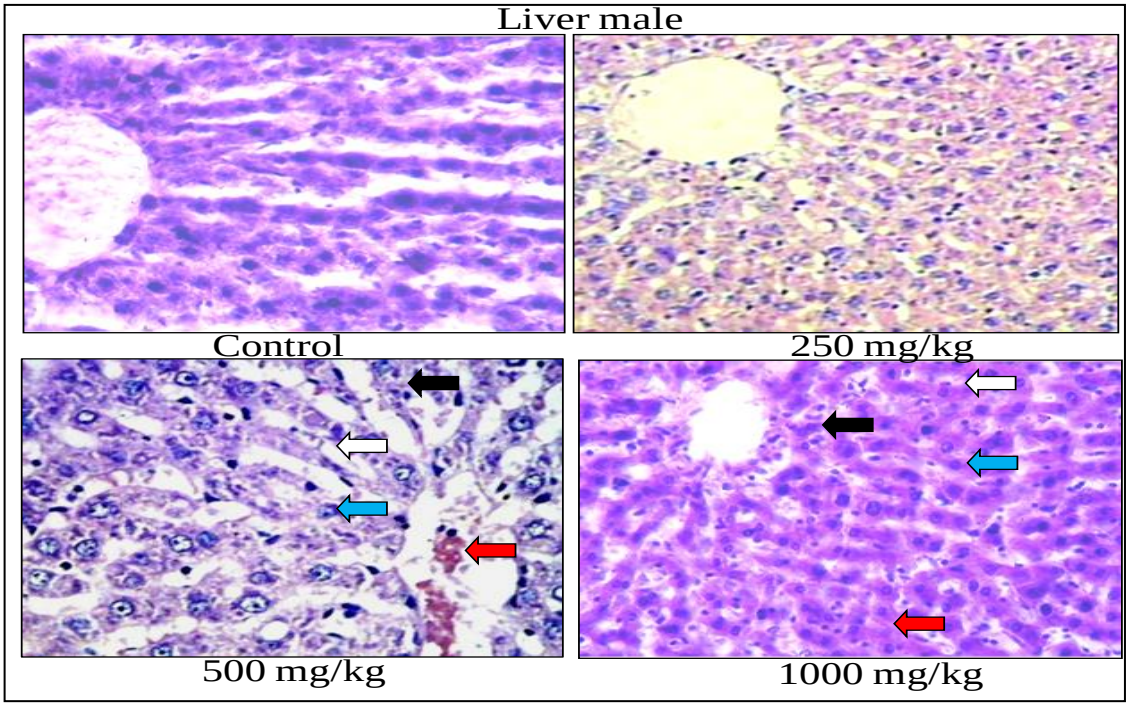
Effect of SA Extract on histopathology of Spleen in subacute oral toxicity study in male rats



***P**hotograph of H & E staining of spleen.*

➡	Red pulp congestion	⇨	Depopulation	➡	White pulp structure

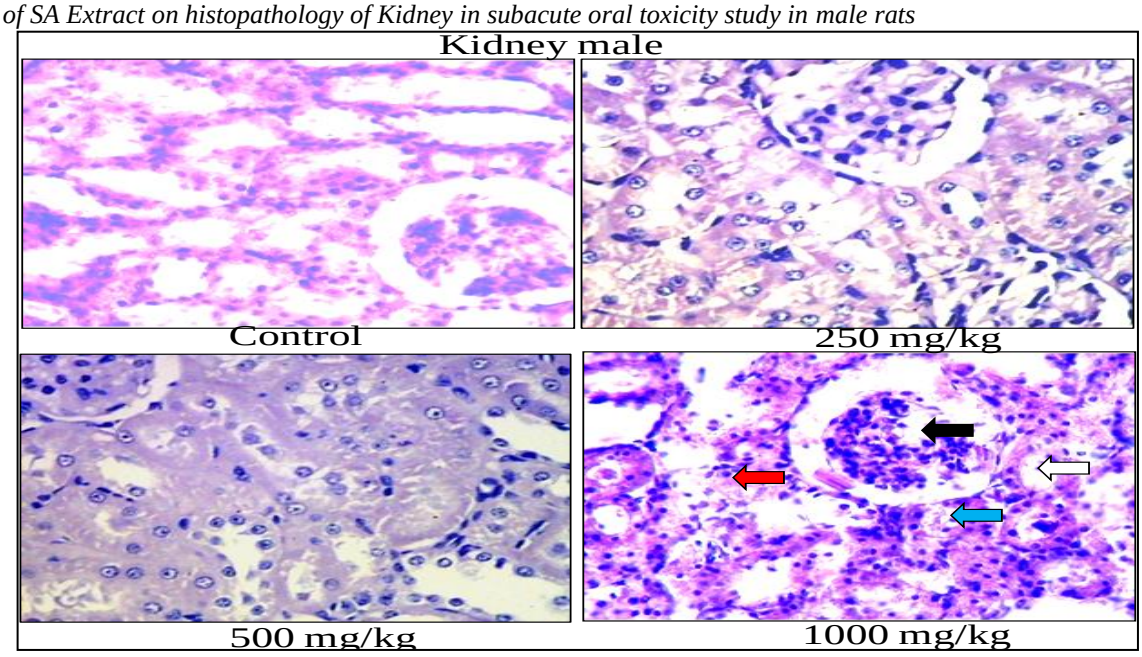
Effect of SA Extract on histopathology of Liver in subacute oral toxicity study in male rats



Photograph of H & E staining of liver

➡	Vascular changes	➡	Degeneration	➡	Leucocytic infiltration	⇨	Derangement of hepatic cords

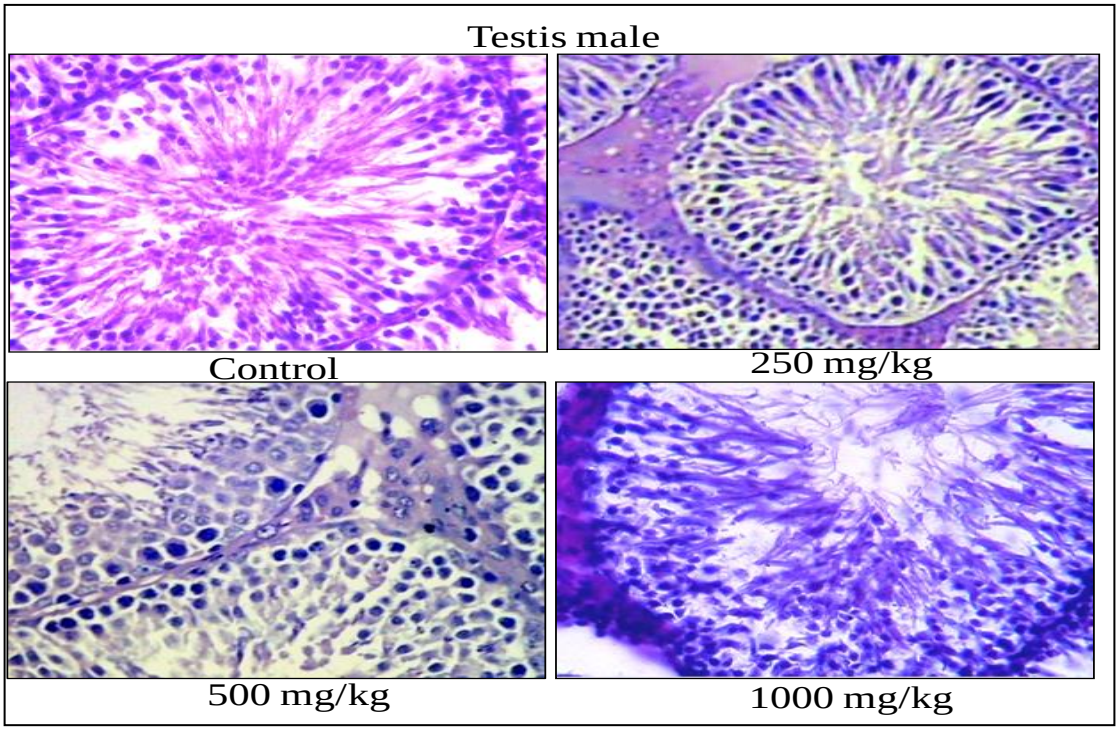
Effect of SA Extract on histopathology of Kidney in subacute oral toxicity study in male rats



Photograph of H & E staining of kidney

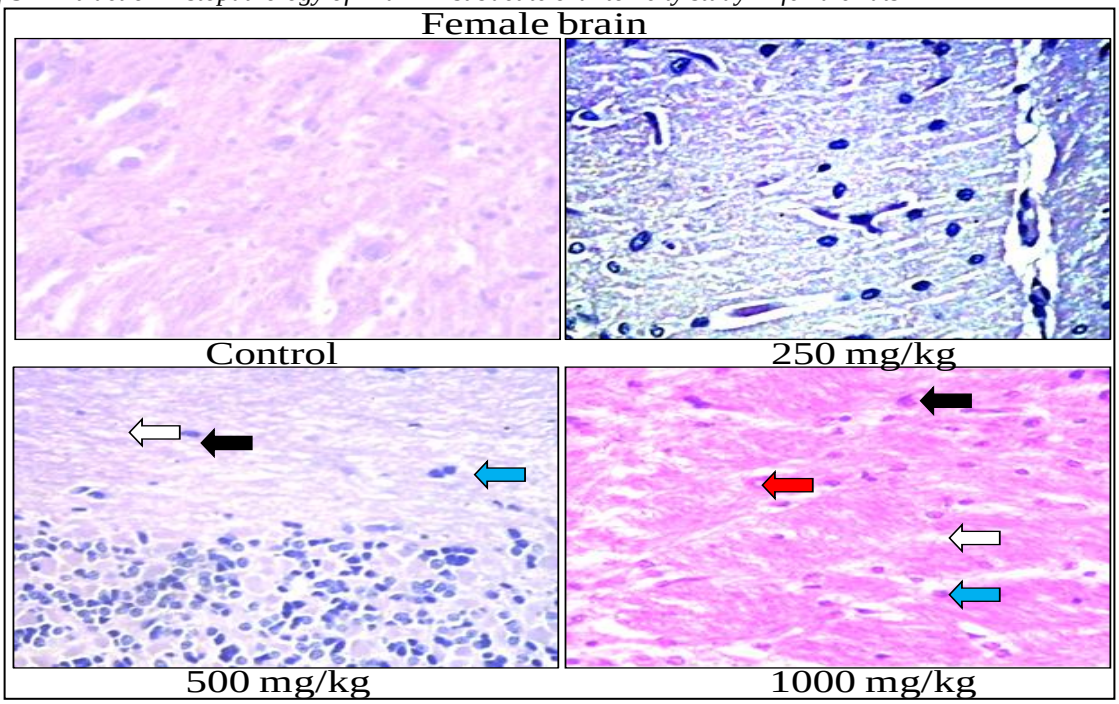
➡	Vascular changes	➡	Glomerulopathiae	➡	Leucocytic infiltration	⇨	Tubular degeneration

Effect of SA Extract on histopathology of Testis in subacute oral toxicity study in male rats



Photograph of H & E staining of testis

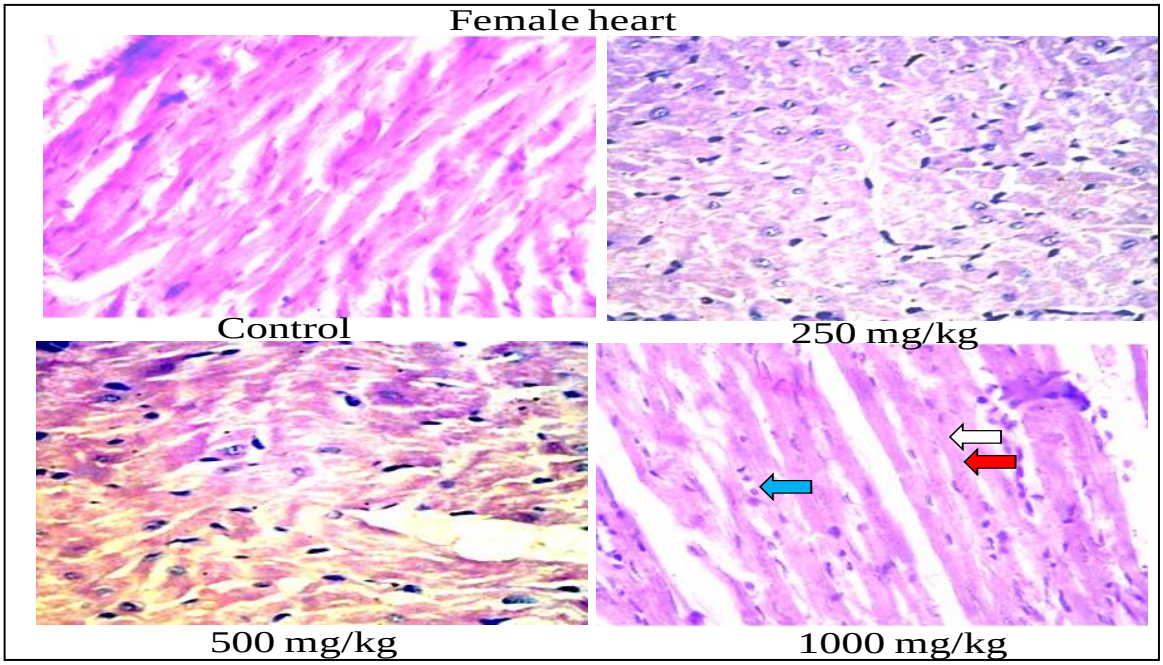
Effect of SA Extract on histopathology of Brain in subacute oral toxicity study in female rats



Photograph of H & E staining of brain

➡	Vascular changes	➡	Neuronal degeneration	➡	Microglial infiltration	⇨	Mild hypoxic changes

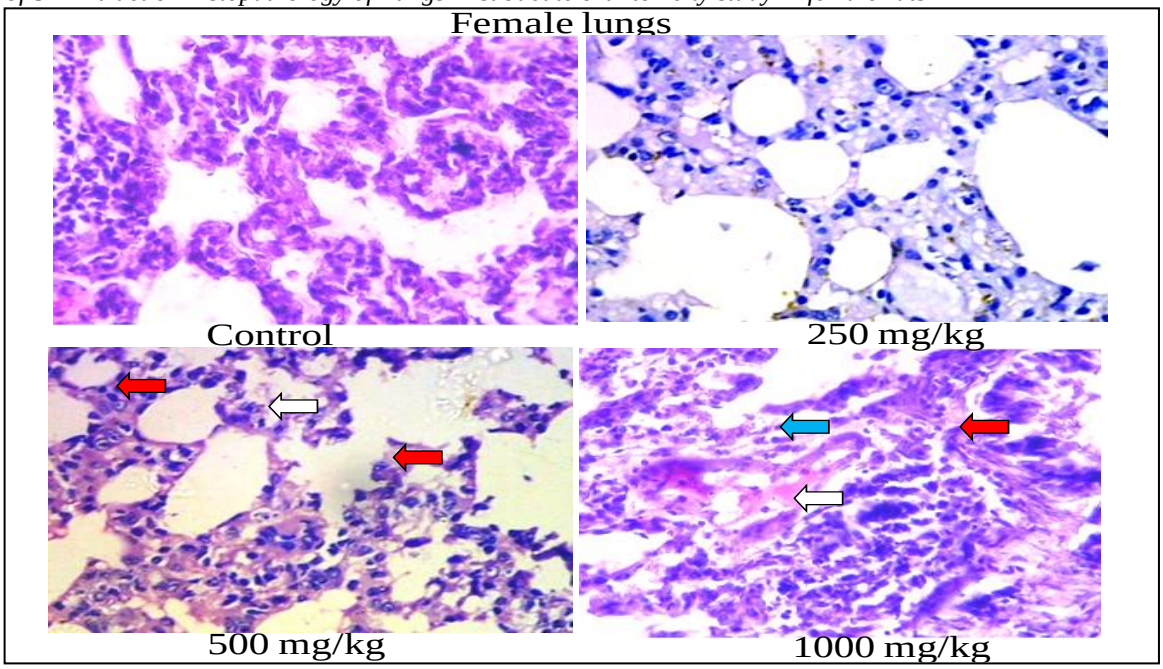
Effect of SA Extract on histopathology of Heart in subacute oral toxicity study in female rats



Photograph of H & E staining of heart.

➡	Vascular changes	⇨	Myocardial degeneration	➡	Leucocytic infiltration

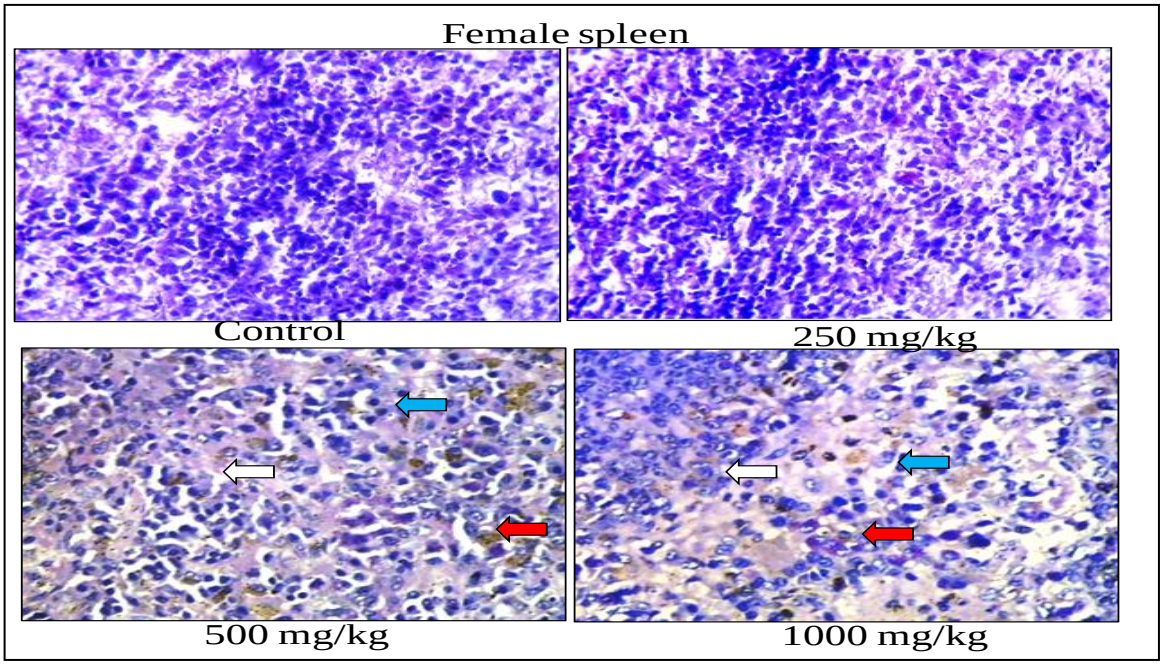
Effect of SA Extract on histopathology of Lungs in subacute oral toxicity study in female rats



Photograph of H & E staining of lung

➡	Vascular changes	⇨	Emphysema structure	➡	MNC infiltration

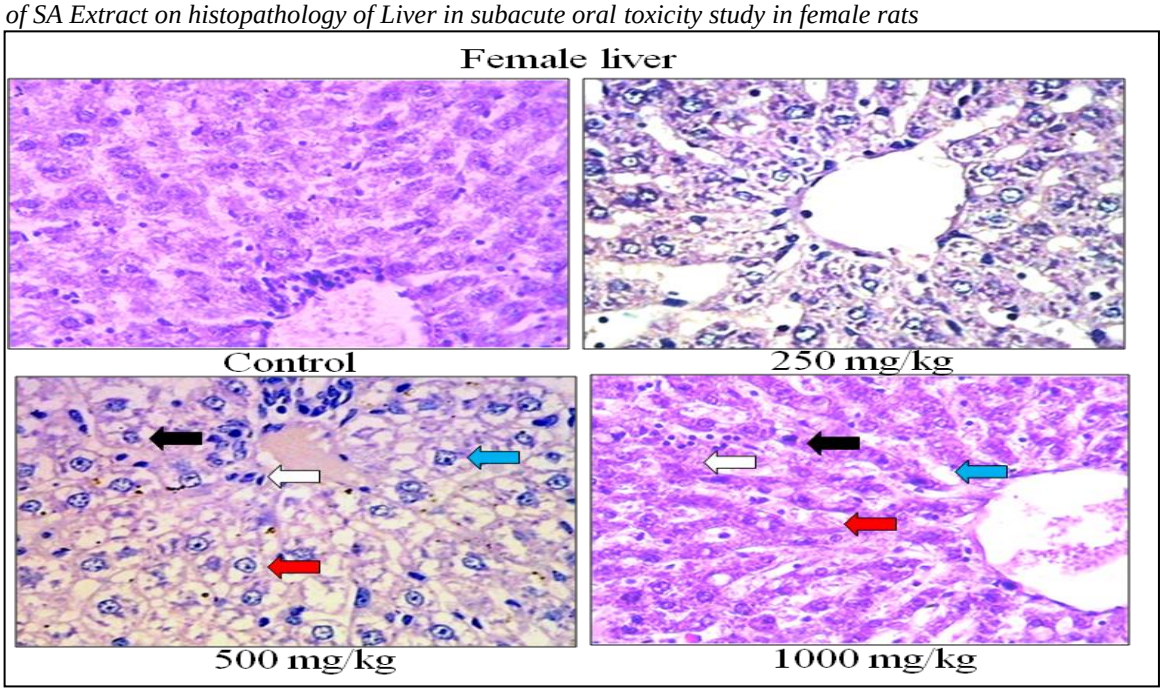
Effect of SA Extract on histopathology of Spleen in subacute oral toxicity study in female rats



Photograph of H & E staining of spleen

➡	Red pulp congestion	⇨	Depopulation	➡	White pulp structure

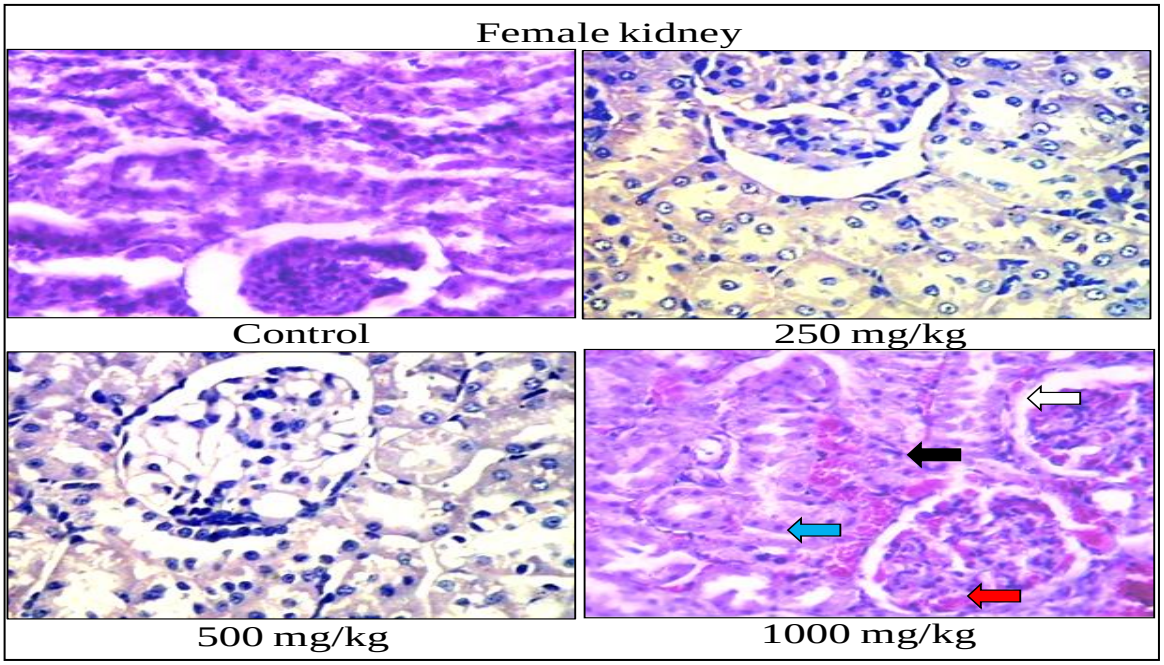
Effect of SA Extract on histopathology of Liver in subacute oral toxicity study in female rats



Photograph of H & E staining of liver

➡	Vascular changes	➡	Degeneration	➡	Leucocytic infiltration	⇨	Derangement of hepatic cords

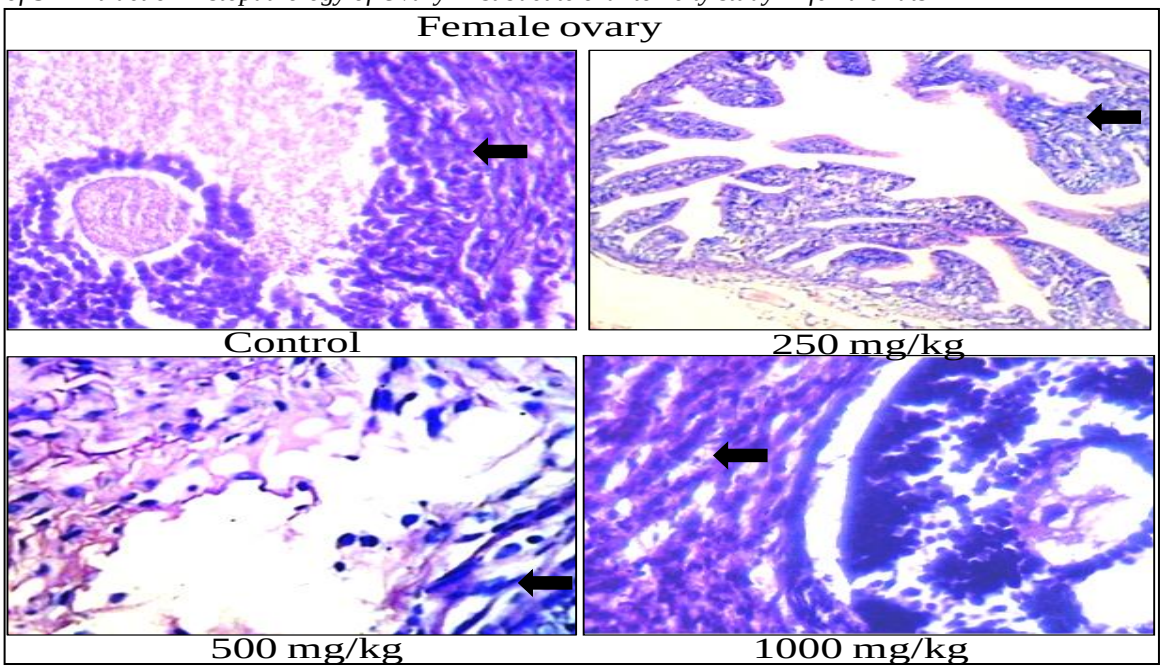
Effect of SA Extract on histopathology of Kidney in subacute oral toxicity study in female rats



Photograph of H & E staining of kidney

➡	Vascular changes	➡	Glomerulopathiae	➡	Leucocytic infiltration	⇨	Tubular degeneration

Effect of SA Extract on histopathology of Ovary in subacute oral toxicity study in female rats



Photograph of H & E staining of kidney.

➡	Different stages of follicular development

4. Discussion and conclusions:

The most widely used traditional medicine globally is the use of medicinal herbs. They have non-traditional indications, are extracted differently, and are administered at varying doses. In line with the growing interest in complementary and alternative medicine in many affluent nations, herbal medicines are becoming increasingly common in the primary

healthcare of individuals and communities. Herbs have always been seen as mild, non-toxic, and even innocuous due to their natural origins. In recent years, there has been a growing number of reports of adverse medication responses and poisonings linked to the use of herbal remedies (Deng et al., 1997).

There is an urgent need for additional information regarding herbal toxicity because many cases of herbal poisoning in daily practice have not been appropriately recognized or treated. Even though therapeutic plants are thought to be safe, over consumption of life-sustaining plants can have hazardous effects. Knowing how much of a drug dose can affect safety and efficacy is therefore crucial to understanding the toxicity of materials. Hence, the scientific approach through experimental and clinical validation of efficacy and documentation of useful herbs, herbal preparations and other formulations is necessary, as is done in modern medicine, animal toxicity studies are also required to establish the potential adverse effects (Reddy, 2010).

Many ancient medical systems have utilized the herb *Saraca asoca*, which has been shown to be pharmacologically active against a variety of clinical conditions. Traditionally, bark, flowers, and seeds have been used to treat a variety of illnesses. *Saraca asoca* plant is reported to contain glycosidic principles, non-phenolic, sapogenetic glycoside, sterols and aliphatic alcohols. *Saraca asoca* has extensive applications in homeopathy, ayurveda, and unani medicine in addition to its use in pharmacological activities such as antibacterial, anti-oxytoxic, anti-cancer, and antimenorrhagic. It is useful for a variety of conditions, including genito-urinary disorders, CNS disorders, and skin infections. *Saraca asoca* is available as various marketed formulations such as Ashokarishta, Ashokkwath, Seed powder, Flower powder, Ashokghrita, decoction, pill, paste, herbal wine, syrup, capsules etc containing bark extract (Hegdea et al., 2007). Acute oral toxicity was already performed on flower and leaves extract.

Acute toxicity tests (single dose) and subacute toxicity tests (28 days repeated dosage) are two types of toxicity studies that are conducted. Typically, acute dose-finding exercises are the first step in long-term research. Additionally, in the context of the manufacture, handling, and use of chemicals, the data on acute systemic toxicity produced by the test is utilized in hazard identification and risk management.

The animal's food and water intake and food utilization showed no significant changes, suggesting a normal metabolism. Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substance (Teo et al., 2002).

Comparing the experimental group to the control group, no significant variations were observed in the weight growth or internal organs (liver, lung, heart, spleen, kidney, and brain). Additionally, there was no anomaly found during gross morphological analyses of the internal organs.

There is absence of protein, glucose, ketone bodies, bile pigments and in microscopical examination such as pus cells, red blood cells, epithelial cells, crystals and cast in both male and female rats in control and treated groups. Also the aqueous extract of bark of *Saraca asoca* showed statistically nonsignificant effect on urine volume. This proves that aqueous extract of bark of *Saraca asoca* has no effect parameters of urine during both toxicity studies.

The haematological studies conducted over a 28-day period showed a significant decrease in the red blood cell count at 500 mg/kg and 1000 mg/kg, while the count was found to be normal at lower doses. This was reflected in an increase in hemoglobin and hematocrit, which measure the "percentage" of red blood cells, in the group of animals treated with *Saraca asoca* compared to the control. The altered red cell production may be attributed to the constituents of the plant such as steroids and potency of this plant as hematopoietic activity enhancer (Pawar et al., 2006). However parameters like MCV, MCH, and MCHC did not affect significantly as compared with that of control group at all dose. At 500 mg/kg and 1000 mg/kg dose neutrophils, eosinophils, and monocytes count was decreased but at the same time lymphocyte count increased as result of WBC count shows elevated level. Neutrophils represent an important part of total leucocyte cells. They are known to interact with microorganisms and other foreign compounds and to destroy them that is why they are responsible for congenital immune response (Ewuola and Egbunike, 2008). Investigations show a significant fall in the platelet count, which may result in hematological diseases. This suggests that the SA Extract may affect platelet formation or produce thrombocytopenia, a reduction in the amount of platelets in the blood. Thus, the hematological estimation results above, which show that the Saraca asoca extract does not harm circulating red blood cells, have disrupted the generation of red blood cells (Mukinda, 2007).

At low dose (250 mg/kg) no change in nearly biochemical parameter except in blood glucose level and creatinine phosphate kinase at subacute oral toxicity study. Blood glucose level shows significant change at all doses. Decreased in blood glucose indicate the hypoglycemic effect on animals. CPK level shows significant change at all doses in 28 day treatment. It indicates the increased risk of myocardial infarction. Total protein, albumin and globulin not show any significant change after 28 days dosing (But et al., 1994). When compared to normal, the blood levels of SGOT and SGPT in rats receiving a 1000 mg/kg dosage in a subacute toxicity investigation show no discernible alterations. There is significant decrease in the SGPT and SGOT levels in animals from all dose groups, which indicate normal functioning of heart as this enzyme shows increase level in myocardial infarction. Following 28 days of treatment at doses of 500 and 1000 mg/kg, creatinine levels are shown to have decreased. In a subacute toxicity trial, total and indirect bilirubin levels increased at 500 and 1000 mg/kg, respectively. This implies a possible impairment in liver function as well as hemolytic activity. After 28 days of therapy, blood urea nitrogen (BUN) and blood urea level (BUN) at 500 and 1000 mg/kg dosages decreased. Although a low BUN often doesn't mean much, it can be caused by liver issues, malnutrition (not getting

enough protein from food), or binge drinking. A low BUN can be caused by intravenous fluid over hydration. In subacute toxicity, triglycerides and cholesterol are decreased at the 1000 mg/kg dosage level. This behaviour suggests that the extract has a hypolipidemic effect (Carson and Relay, 1995).

Histologically, the control and 250 mg/kg dosage sections display normal neuroglial tissue. The observed modest hypoxic changes in brain matrix, microglial infiltration, vascular abnormalities, and neuronal degeneration in the 500 mg/kg and 1000 mg/kg groups could perhaps be attributed to a limited increase in blood volume within dilated capillaries within an organ or tissue. The phytoconstituents flavonoids, saponins, and tannin found in SA bark extract are responsible for neurodegeneration and neurotoxicity, which are characterized by early events of synaptic degeneration and neuronal loss. These processes are accompanied by an inflammatory response that includes activation of microglia, perivascular monocytes, and recruitment of leukocytes (Nath, 2002).

Heart section of control dose shows normal myocardial tissue histologically. In 250, 500 mg/kg and 1000 mg/kg myocardial degeneration, leucocytic infiltration and vascular changes observed. From biochemical parameters (level in CPK enzyme) and extract cause myocardial degeneration which indicates sign of myocardial infarction.

In lungs histopathology of animals receiving extract of bark of emphysema, congestion, mononuclear cell infiltration (MNC infiltration) was observed.

Hematopoietic organ spleen, kidney and liver show pathological alteration.

Spleen section of control dose shows normal tissue histologically. In 250, 500 mg/kg and 1000 mg/kg red pulp congestion, white pulp and depopulation observed. The pharmacological effect of herbal extract undoubtedly influenced the cellular responses observed. The marginal zone and the red pulp were crowded with cells, most of which were hemocytoblasts. These cells contain numerous polysomes the sites of protein synthesis and probably correspond to the large round, antibodyntining cells found at the same time. Splenic macrophages were numerous. They were packed with fragmented erythrocytes, remnants of cells, none of which were recognible as lymphocytes. It was possible to follow the transition of this cell to well-defined, but mature, plasmacytes. In addition to proplasmacytes, numerous activated lymphocytes and reticular cells were found immediately after the disappearance of hemocytoblasts, an indication that the latter may undergo a variety of morphologic transformations.

The liver sections of the control and 250 mg/kg dose exhibit normal tissue histology, with the exception of the 250 mg/kg dose's observed bile stasis. Degeneration, necrosis, leucocytic infiltration, and vascular alterations were noted in the 500 mg/kg and 1000 mg/kg hepatic cord derangements. Critical biomolecules can be harmed by reactive oxygen species, which are produced by a number of metabolic events in our body. The use of antioxidants in the management of degenerative disorders where oxidative stress has been linked has garnered a lot of attention in recent years (Satyanarayana et al., 2001).

Kidney section of control dose, 250 and 500 mg/kg shows normal tissue histologically. In 1000 tubular degeneration, vascular changes, glomerulopathiae and leucocytic infiltration observed. It may occur due to cytolysis same as in liver.

In testis of test male group and ovary of female rats found to be normal during the study.

In conclusion, the subacute oral administration of SA to wistar rats was linked to a number of noteworthy clinical and pathological alterations. The study's findings support the notion that there is a significant hemolytic risk.

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