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Acute and 28-Day Repeated Dose Subacute Toxicological Evaluation of Coroprotect Tablet in Rodents

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ABSTRACT

Introduction: Ayurvedic medications are extensively used across the world for illness prevention and treatment. The components found in the Coroprotect tablet are *Ashwagandha* ext., *Suddhashilajit* ext., *Giloy*ext., *Dadim* ext., *Haridra* ext., *Saragavo* ext., *Brahmi* ext., *Papaya* ext., *Methi*ext., *Punarnava* ext., *Kalmegh* ext.etc. all are found to be safe and used in Ayurvedic system since many years.

Objective: In Rodents, acute and subacute toxicity of coroprotect tablet was studied after a single and repeated 28-day oral dosage administration.

Method: In acute toxicity testing, 2000 mg/kg single dose of coroprotect tablet have been employed, whereas doses of 100, 200, 400mg/kg have been utilized in subacute toxicity testing over a 28-day period in rodents.

Result: With coroprotect tablet administration, no treatment-related fatalities or toxic signshave been identified in an acute toxicity investigation. There have been no significant variations in body weight changes, water/food intake, haematology, or clinical biochemistry content among the control and coroprotect tablet groups in the repeated dosage research. Between the control and coroprotect pill groups, no gross pathological abnormalities or differences in relative organ weights were found. With coroprotect tablet therapy, histopathological investigation indicated no abnormalities.

Conclusion: In a repeated dosage toxicity study in rodents, the coroprotect tablet was determined to be safe at all dose levels.

Key Words: Coroprotect tablet, Acute toxicity, 28-Day Repeated Dose toxicity, Subacute Toxicity, Wistar rat, Ayurvedic supplement

INTRODUCTION

Coroprotect Tablet is an Ayurvedic supplement that includes enhanced extracts of *Ashwagandha, Shilajit, Giloy, Dadim, Haridra, Moringa, Brahmi, Papaya, Methi, Punarnava* and *Kalmegh.* Coroprotect Tablet have been specially created using ancient Ayurvedic principles as a possible preventative and therapeutic intervention for coronavirus illness in 2019. (COVID-19).Coroprotect Tablet's ingredients have been used in therapeutic practise for millennia in India and other parts of the world. Two tablets twice a day has been authorised as the daily dosage of Coroprotect Tablet. Given Coroprotect Tablet's established *in-vitro* and *in vivo* antioxidant effectiveness, it's also critical to evaluate its non-clinical safety, with the goal of determining its preventative benefit and therapeutic potential in COVID-19-infected human individuals. As a consequence, we evaluated the possible health concerns of Coroprotect Tablet following repetitive oral dosing to rats at different dosages throughout 28 days in the current study. The reversibility, durability, and delayed development of toxic effects in a satellite group of animals given a high dose of Coroprotect Tablet for a 14-day treatment-free interval were investigated. The study's purpose was to learn more about Coroprotect Tablet's main toxic effects, target organs, and No Observed Adverse Effect Level (NOAEL) in rats. The study was carried out using OECD criteria 423[1] and 407[2] for acute and 28-day repeated-dose toxicity, respectively.

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MATERIALS AND METHODS

Ethical statement

The study carried out in line with the requirements of the Organization for Economic Cooperation and Development (OECD) for acute and 28-day repeated-dose toxicity. which were published on October 16, 2008, and February 8, 2002, respectively.

Animals

Wistar rats, both Male and female were used in the study. All toxicological testing was done in accordance with the Organization for Economic Cooperation and Development's guidelines (OECD).

Study Material

Coroprotect Tablet was used as a test drug. A suspension of Coroprotect Tablet was created using methylcellulose as the suspending agent for administration to the animals. The study's other reagents and chemicals were all of the highest commercial quality.

Study design

For acute toxicology investigation:

Step 1: Three female rats were given a single dosage of Coroprotect Tablet by oral gavage at a concentration of 2000 mg/kg body weight (BW) (suspended in distilled water) and were monitored for 24 hours for evidence of toxicity or mortality.

Step 2: An additional set of three female rats received Coroprotect Tablet as a single oral gavage dosage of 2000 mg/kg BW and were monitored for 24 hours for evidence of toxicity or mortality.

All six animals were observed individually after each dosage for a total of 14 days, at least once within the first 30 minutes, sometimes over the first 24 hours, with special attention provided within the first 4 hours, and daily afterwards to evaluate delayed toxicity.

For 28 days Repeated dose toxicity investigation:

Six rats each of both sexes had given oral gavage of coroprotect tablets daily at dosages of 100 mg/kg/day (test group 1), 200 mg/kg/day (test group 2), and 400 mg/kg/day (test group 3) body weight, and were slaughtered on day 28 to assess toxicity.

Dose preparation

Just before the dosage schedule, the test product Coroprotect Tablet was crushed, sieved and suspended in distilled water to achieve the appropriate concentration of mg/ml.

Administration of test article

Using a graduated syringe and a stainless-steel intubation needle, an animals have been dosed via oral gavage on the first day, if practicable. The dose volume given to each rat was modified based on its most recent body weight. For the acute toxicity research, rats have been observed for 14 days, and for the repeated dose toxicity research, they were monitored for 28 days.

Clinical pathology

Under carbon dioxide anesthesia, blood obtained from the retro-orbital plexus of all rodents in a test and control groups on the day the study finished. The specimens had been taken in test tube holding the anticoagulants Heparin (for clinical chemistry) and K-EDTA (for hematology). The animals' food was taken away overnight to be sampled for laboratory studies.

OBSERVATIONS

During the therapy period, the following observations were made. Animals in the satellite group were also monitored for 14 days after treatment to assess the permanence or reversibility of any toxic effects, as well as the incidence of delayed toxicity.

Mortality

Throughout the research, all animals were examined first thing in the morning and again in the afternoon to look for dead or moribund animals so that necropsy examinations could be performed throughout the day's working hours. A similar practice was followed on weekends and public holidays, with the exception that the final check was done about midday.

Clinical Signs

Individual animals had all indicators of illness documented, as well as any behavioural changes or treatment responses.

General Clinical Examinations

The animals were submitted to general cage side clinical exams on a daily basis, at the same time each day, and at appropriate intervals following medication, taking into account the peak period of predicted effects.

Detailed Clinical Examinations

Before starting the medication (to enable for within-subject comparisons), the animals were submitted to extensive clinical tests, which were repeated weekly during the treatment period. These observations were taken in a regular arena, ideally at the same time, outside the home cage. Changes in eyes, hair, skin, and mucous membranes, incidence of

fluids and excretions, and autonomic activity such as pupil size, piloerection, and peculiar breathing style being among the signs identified. Changes in stride, posture, and reaction to handling, and also the presence of clonic or tonic movements, stereotypes, or strange behaviour, were all reported.

Body Weights

A weights of a ratshave been recorded at a time of group assignment, which was one day previous to the start of therapy, weekly thereafter, and at necropsy. During the post-treatment period and at necropsy, the weights of Satellite Group Rats were recorded weekly.

Food Consumption

The amount of food eaten by the animals in each cage was recorded on the first day of treatment and then every week after that. The quantity of food supplied to and left in each cage was used to determine food consumption. During the post-treatment phase, the Satellite Group Rat's food intake was tracked weekly.

Clinical Pathology

All animals in the test and control groups had blood samples obtained from the retroorbital plexus on the day the trial ended, under carbon dioxide anesthesia. The samples were taken in tubes containing anticoagulants Heparin (for clinical chemistry) and K-EDTA (for hematology). Food was taken away from the animals overnight to be sampled for laboratory research. On glass slides, blood stains were also created. Clinical chemistry samples were centrifuged for 5 minutes at 3000 rpm.

Hematology

Estimates of hematology were made and recorded in observations, along with their units of measurement.

Clinical Chemistry

The parameters of serum chemistry, as well as their units of measurement, were examined and recorded in observations.

Urinalysis

All of the animals had their urine tested. A battery of specially built stainless steel urine collecting cages was used to collect urine samples. This was where each Mouse was kept. Over the course of three hours, urine samples were collected. During this time, no food or drink was provided. Color, Appearance, Specific gravity, pH of the urine was determined.

Necropsy Examination

All surviving animals were killed by exsanguinations under carbon dioxide anesthesia after 28 days of treatment and were submitted to a full necropsy. The necropsy performed at the end of the trial was staggered and completed at the conclusion of the research. All tissues were kept in 10% neutral buffered formalin, according per OECD 407 recommendations. In addition, samples of any macroscopically aberrant tissues, as well as samples of neighboring tissue, were regularly maintained.

Organ Weights

Organs were dissected devoid of fat and weighed moist as soon as feasible to prevent drying from all animals slaughtered at the planned sacrifices.

Ethical Consideration

The CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Fisheries, Animal Husbandry, and Dairying, Government of India, New Delhi, oversaw all animal experiments. The research has been authorized by the Institutional Animal Ethics Committee (IAEC) procedure DYPIPSR/IAEC/JULY/21-22/P001, and the animals were evaluated and given a week to adjust to their new surroundings before the tests began.

Statistical Analysis

Investigation of the data,the mean and standard deviation were computed using a data from all of the groups. The data on body weight, hematological, clinical chemistry, urinalysis (for specific gravity and urine pH), and relative organ weight were evaluated with the help of a one-way ANOVA and Dunnett's test.

RESULTS AND DISCUSSION

The Coroprotect Tablet had no detrimental effects on the growth, general health, behavioral, neurological, or gross of the tissues/organs of the rat treated at the dose level of 2000 mg/kg BW, based on the findings of the acute toxicity study. The NOAEL (no observed adverse effect level) of Coroprotect Tablet in rats after single oral dose was determined to be more than 2000 mg/kg body weight, according to the results of this investigation. Coroprotect Tablet was tested on rats for 28 days after daily oral treatment at three dosage levels: low, middle, and high dose.

In Wistar rats, an active component in Coroprotect tablets, Ashwagandha, had no toxicity or death.[3,4] Similarly in our research alsothe female test participants (at a dose of 2000 mg/kg BW) showed no rat-related mortality or adverse clinical signs. (table-1). As a result, according to the OECD 423 recommendations, the lethal dosage (LD50) had determined to be larger than 2000 mg/kg. As well as within male and females of the test article (low dose, Middle dose and high dose body weight) and control group (table-2). All of these symptoms were only seen during the therapy time. Because of the same occurrence of this discovery in both groups, it was assumed to be coincidental.

The body weight of a female rat given Coroprotect Tablet at a dose of 2000 mg/kg BW showed no significant alterations (table-3). In a subacute oral toxicity investigation, the body weights of treating mice did not change significantly from those of control animals after a 28-day treatment period. (table-4).

The data on food and water intake was judged to be normal, and weight increase was consistent throughout research, suggesting that a Coroprotect tablet had no harmful impact on the animals' development. The average daily food intake of female rats given Coroprotect tablets at a dosage of 2000 mg/kg BW did not change substantially over a 14-day period, and was equivalent or slightly higher for all six females. The average daily food intake of both the sexes of rats given Coroprotect tablets at low, middle, and high dosages was determined to be equivalent to the control group's. Over the course of 28 days, the average daily food consumption per rat was calculated. Coroprotect Tablet satellite groups at low, middle, and high doses were equivalent to control rats. Similarly, the test article-treated female rats ate about the same amount of food as the control rats on a daily basis. The intermediate dosage group Rat's daily food consumption increased after treatment ended, as well as throughout the recovery period.

Organ weight is a helpful measure in toxicity studies because it predicts toxicity, enzyme induction, physiologic abnormalities, and acute injury; it corresponds with any histopathological alterations; and it has a low inter-animal variability.[5] The mean absolute and relative weights of the testes/ovaries, epididymis/uterus, adrenals, kidneys, brain, liver, spleen, hearts, and thymus of both the sexes of rats treated at a low dosage, middle dose, and high dose were found to be approaching those of the control group at the conclusion of the research (table-5).

Furthermore, the weight of the organs in the control and treatment groups did not differ significantly in a study of subacute oral toxicity.

A hematopoietic system is a critical indication of pathological and physiological states in people and animals, and it is one of the most sensitive locations for toxicity. [6]Total erythrocyte count (RBC), hemoglobin (Hb), hematocrit (PCV), differential leucocyte counts (DLC), total leucocyte count (WBC), platelet count, and total leucocyte count (WBC) were the group mean values of hematological parameters[7]exposed to coroprotect tablet (at low dosage, middle dose, and high dose) in both the sexes of rats neither in the Therapeutic nor in the Control groups shown any treatment impact throughout the study's duration or at its conclusion. (table-6).

In toxicological investigations, serum biochemistry is crucial for evaluating liver function. [8,9] Male and female treatment The blood biochemistry parameters of rats treated with coroprotect tablet at low, middle, and high doses showed no significant therapy impact [10] (table-7).

After 28 days of medication, in both male and female rats in the low, medium, and high dosage body weight groups, there was no significant change in hematological and biochemical markers as compared to the control. The values of these parameters were all the same as those found in the present control groups.[11]

The treatment of both sexes of rats with Coroprotect tablets (at doses of 100, 200, and 400 mg/kg/day) had no effect on their urine parameters when compared to a control group. The qualitative properties of urine examined for all Rats in this investigation, as well as a microscopic inspection of their urine sediment, showed no significant treatment-related alterations.

Coroprotect Tablet did not induce any significant and therapy-related gross pathological abnormalities in any of the treated rat's organs or tissue at a dosage of 2000 mg/kg. (table-8). In the test group and satellite group of low, middle, and high dose body weight, Coroprotect Tablet did not induce any notable and therapy-related gross pathological abnormalities in any of the organs / tissues of treated rats.

At low, middle, and high dose, all microscopic alterations observed in this investigation seemed likely be coincidental, given that the frequency and severity of these events had been similar in the control and treated rats. Under a same experimental circumstances, Coroprotect Tablet at low dosage, middle dose, and high dose body weight had no histopathological effects in rats [12,13] (table-9).

Table 1: Clinical signs and mortality data of acute toxicity

Group	Days																		
	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	12 Hr	2	3	4	5	6	7	8	9	10	11	12	13	14
Coroprotect Tablet Limit test step 1 2000 Mg/Kg (female rat)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Table 1: ***(Continued)***

Group	Days																		
	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	12 Hr	2	3	4	5	6	7	8	9	10	11	12	13	14
Coroprotect Tablet	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Limit test step 2 2000 Mg/Kg (female rat)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N=No Clinical abnormality

Signs= Nostril discharge, Abdominal breathing, Gasping, Somnolence, Ataxia, Prostration, Tremors, Convulsion- Clonic, Convulsion- Tonic, Clonic/Tonic Convulsions, Excessive urination/ Polyurea, Ptosis, Excessive Lacrimation, Excessive Salivation, Piloerection, Diarrhea, Red ear, Hair loss.

Table 2: Clinical signs and mortality data of test group 1,2,3 and control group

Sex/Group	Days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Female - C/F001	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F002	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F003	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/F004	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M001	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M002	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M003	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M004	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Sex/Group	Days												
	16	17	18	19	20	21	22	23	24	25	26	27	28
Female - C/F001	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F002	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F003	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/F004	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F005	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - C/ F006	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M001	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M002	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M003	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M004	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M005	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - D/M006	N	N	N	N	N	N	N	N	N	N	N	N	N

N=No Clinical abnormality

Signs= Nostril discharge, Abdominal breathing, Gasping, Somnolence, Ataxia, Prostration, Tremors, Convulsion- Clonic, Convulsion- Tonic, Clonic/Tonic Convulsions, Excessive urination/ Polyurea, Ptosis, Excessive Lacrimation, Excessive Salivation, Piloerection, Diarrhea, Red ear, Hair loss.

Table 3: Body weight (g) data of acute toxicity

Dose (mg/kg)	Particulars	Day 0	Day 7	Day 14	Avg % Gain
Coroprotect Tablet Limit test step 1 2000 Mg/Kg	Female - CT/F001	285.5	286.4	286.8	
	Female - CT / F002	285.4	286.6	286.7	
	Female - CT / F003	285.6	286.5	286.6	0.42%
	Mean	285.5	286.5	286.7	
	SD	0.1	0.1	0.1	
Coroprotect Tablet Limit test step 2 2000 Mg/Kg	Female - CT /F004	177.7	177.9	179.6	
	Female - CT / F005	178.1	178.1	180.9	
	Female - CT / F006	178.6	178.6	180.3	1.24 %
	Mean	178.1	178.2	180.3	
	SD	0.451	0.361	0.651	

Table 4: Body weight (g) data of test 1,2,3 and control group

Group	Time period	Low dose	Middle dose	High dose	Control
Female	day 0	218.33±15.31	216.83±10.59	158.5±18.75	222.83±11.86
	day 14	230.67±16.42	229.5±10.63	171.5±18.39	233.83±11.29
	day 28	240.83±16.68	240.5±11.5	184.33±19.3	245.17±12.22
Male	day 0	410.5±43.96	304.33±31.81	266±50.04	316.83±21.33
	day 14	426.17±47.38	314.83±31.32	276±49.51	327.17±19.89
	day 28	436±51.28	327.17±32.11	291.33±49.89	340±19.3

The average ± standard deviation (n = 12) are shown by each value.

Table 5: Animal organ weights of test 1,2,3 and control group

Group	Organ	Low dose	Middle dose	High dose	Control
Female	Adrenals	0.065±0.001	0.066±0.002	0.065±0.001	0.064±0.002
	Ovary	0.078±0.001	0.078±0.001	0.078±0.001	0.076±0.002
	Brain	1.898±0.001	1.898±0.001	1.898±0.001	1.897±0.002
	Kidney	1.481±0.004	1.481±0.004	1.481±0.003	1.479±0.007
	Liver	8.132±0.009	8.127±0.004	8.130±0.005	8.130±0.005
	Heart	0.652±0.001	0.652±0.001	0.653±0.002	0.653±0.002
	Spleen	0.67±0.01	0.67±0.01	0.67±0.01	0.68±0.01
	Epididymis	NA	NA	NA	NA
	Thymus	0.230±0.002	0.230±0.002	0.230±0.001	0.230±0.002
	Uterus	0.71±0.01	0.71±0.01	0.71±0.01	0.70±0.01
Male	Adrenals	0.065±0.001	0.066±0.002	0.064±0.002	0.066±0.002
	Ovary	0.077±0.001	0.078±0.001	0.078±0.001	0.077±0.002
	Brain	1.898±0.001	1.898±0.001	1.897±0.001	1.898±0.001
	Kidney	1.481±0.003	1.481±0.003	1.481±0.003	1.481±0.003
	Liver	8.131±0.005	8.131±0.005	8.131±0.004	8.132±0.005
	Heart	0.652±0.001	0.652±0.001	0.652±0.001	0.652±0.002
	Spleen	0.68±0.01	0.68±0.01	0.68±0.01	0.69±0.01
	Epididymis	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.02
	Thymus	0.229±0.001	0.233±0.009	0.229±0.001	0.230±0.001
	Uterus	NA	NA	NA	NA

The standard ± standard deviation (n = 12) are shown by each value.

Table 6: Animal haematology parameters of test 1,2,3 and control group

Group	Parameters	Low dose	Middle dose	High dose	Control
Female	Total leukocyte count (x 10^3/µl)	11.83±0.14	21.66±0.77	9.20±0.36	9.70±0.46
	Total Rbc count(x 10^6/µl)	6.37±0.11	6.37±0.46	5.50±0.71	7.65±0.46
	Hemoglobin (hb) (g/dl)	11.2±0.14	10.87±0.22	10.32±0.32	13.95±1.83
	Platelet count (x 10^3/µl)	720.83± 11.58	801.83±41.09	416.83±24.29	722.33±15.46
	Neutrophils (%)	18.67±2.94	46.33±4.89	32.50±1.87	20.00±5.87
	Lymphocytes (%)	67.50±7.77	46.67±3.20	67.00±2.90	77.67±3.72
	Basophils (%)	0.20±0.14	0.00	0.10±0.13	0.10±0.15
	Eosinophils (%)	0.48±0.21	0.70±0.24	0.28±0.15	0.25±0.10
	Monocytes (%)	2.83±1.47	2.60±1.36	0.50±0.55	1.83±1.17
Male	Total leukocyte count (x 10^3/µl)	11.73±0.30	21.86±0.84	9.12±0.34	9.77±0.82
	Rbc count (x 10^6/µl)	6.28±0.21	6.27±0.46	5.58±0.82	7.93±0.50
	Hemoglobin (hb) (g/dl)	11.43±0.18	10.83±0.34	10.40±0.47	14.12±1.88
	Platelet count (x 10^3/µl)	721.33±12.53	805.33±28.32	416.00±25.88	724.67±23.36
	Neutrophils (%)	18.50±4.68	45.83±4.07	31.83±3.97	19.00±6.66
	Lymphocytes (%)	68.00±5.62	47.33±4.03	66.17±5.60	78.33±5.65
	Basophils (%)	0.12±0.12	0.00	0.12±0.12	0.15±0.21
	Eosinophils (%)	0.45±0.21	0.70±0.37	0.25±0.10	0.20±0.11
	Monocytes (%)	3.00±1.41	2.83±1.60	0.33±0.52	1.50±1.05

The average ± standard deviation (n = 12) are shown by each value at the p 0.05 level of significance from the control means, one-way ANOVA (Dunett's test) and Student's t test utilising graph pad prism.

Table 7: Animal biochemical parameters of test 1,2,3 and control group

Group	Parameters	Low dose	Middle dose	High dose	Control
Female	Total Bilirubin (mg/dl)	0.53±0.22	0.76±0.18	0.67±0.16	0.80±0.14
	Alkaline phosphatase (u/l)	393.00±17.39	720.00±16.76	150.67±17.14	252.83±34.63
	Uric acid(mg/dl)	1.50±0.53	1.55±0.54	1.40±0.50	2.52±0.57
	Urea (mg/dl)	31.67±8.59	32.17±2.93	36.67±6.19	31.00±2.76
	Creatinine (mg/dl)	0.28±0.11	0.62±0.12	0.61±0.27	0.37±0.22
	Total protein (g/dl)	6.69±1.87	7.55±1.12	5.75±0.98	7.80±0.97
	ALT (iu/l)	63.83±12.40	84.33±8.14	40.17±10.50	75.33±4.80
	AST (iu/l)	219.67±17.29	225.33±14.43	134.67±13.14	261.50±34.54
Male	Bilirubin total (mg/dl)	0.60±0.22	0.82±0.25	0.72±0.19	0.78±0.26
	Alkaline phosphatase (u/l)	399.17±17.97	723.83±18.40	148.83±19.91	255.50±31.61
	Uric acid(mg/dl)	1.48±0.66	1.62±0.37	1.42±0.37	2.57±0.73
	Urea (mg/dl)	33.33±13.19	33.50±6.41	38.00±7.62	31.83±6.31
	Creatinine (mg/dl)	0.27±0.12	0.68±0.18	0.60±0.28	0.32±0.21
	Total protein (g/dl)	7.07±1.95	7.73±0.60	5.55±1.27	7.67±0.88
	ALT (iu/l)	60.67±17.70	84.00±9.19	41.00±8.17	74.67±4.80
	AST (iu/l)	217.50±9.31	226.17±9.24	133.50±17.10	261.67±33.22

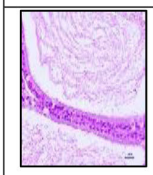
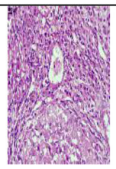
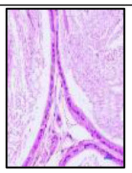
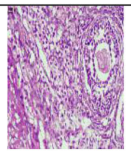
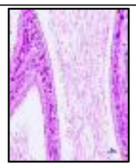
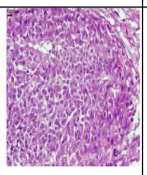
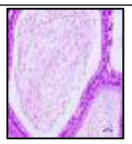
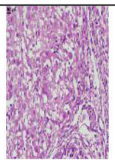
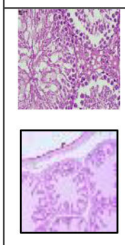
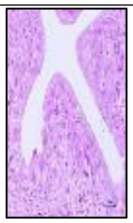
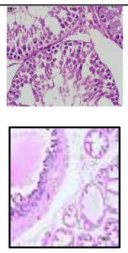
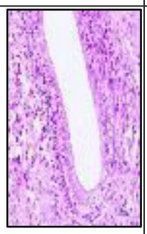
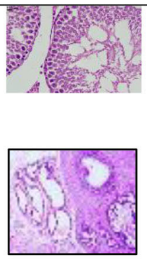
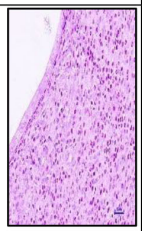
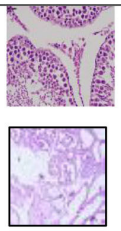
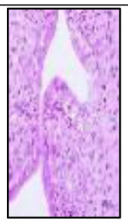
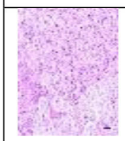
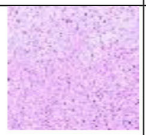
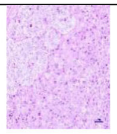
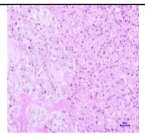
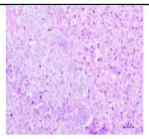
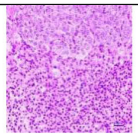
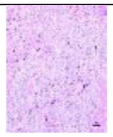
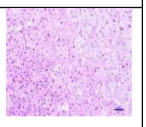
The average ± standard deviation (n = 12) are shown by each value at the p 0.05 level of significance from the control means, one-way ANOVA (Dunett's test) and Student's t test utilising graph pad prism.

Table 8: Gross pathological changes of acute toxicity

Dose (mg/kg)	Particulars	Gross pathological changes after necropsy
Coroprotect Tablet Limit test Step 1 2000 Mg/Kg	Female - CT/F001	N
	Female - CT / F002	N
	Female - CT / F003	N
Coroprotect Tablet Limit test Step 2 2000 Mg/Kg	Female - CT /F004	N
	Female - CT / F005	N
	Female - CT / F006	N

N=No gross pathological changes after necropsy

Table 9: Histopathology of test group 1,2,3 and control group

Test group 1		Test group 2		Test group 3		Control group	
Male	Female	Male	Female	Male	Female	Male	Female
							
Epididymis	Ovary	Epididymis	Ovary	Epididymis	Ovary	Epididymis	Ovary
							
Testes and prostate	Uterus	Testes and prostate	Uterus	Testes and prostate	Uterus	Testes and prostate	Uterus
							
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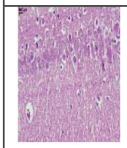
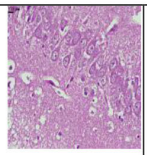
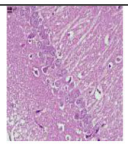
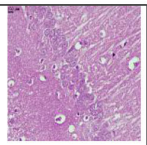
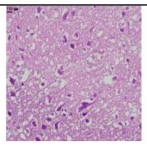
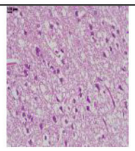
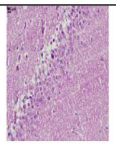
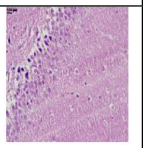
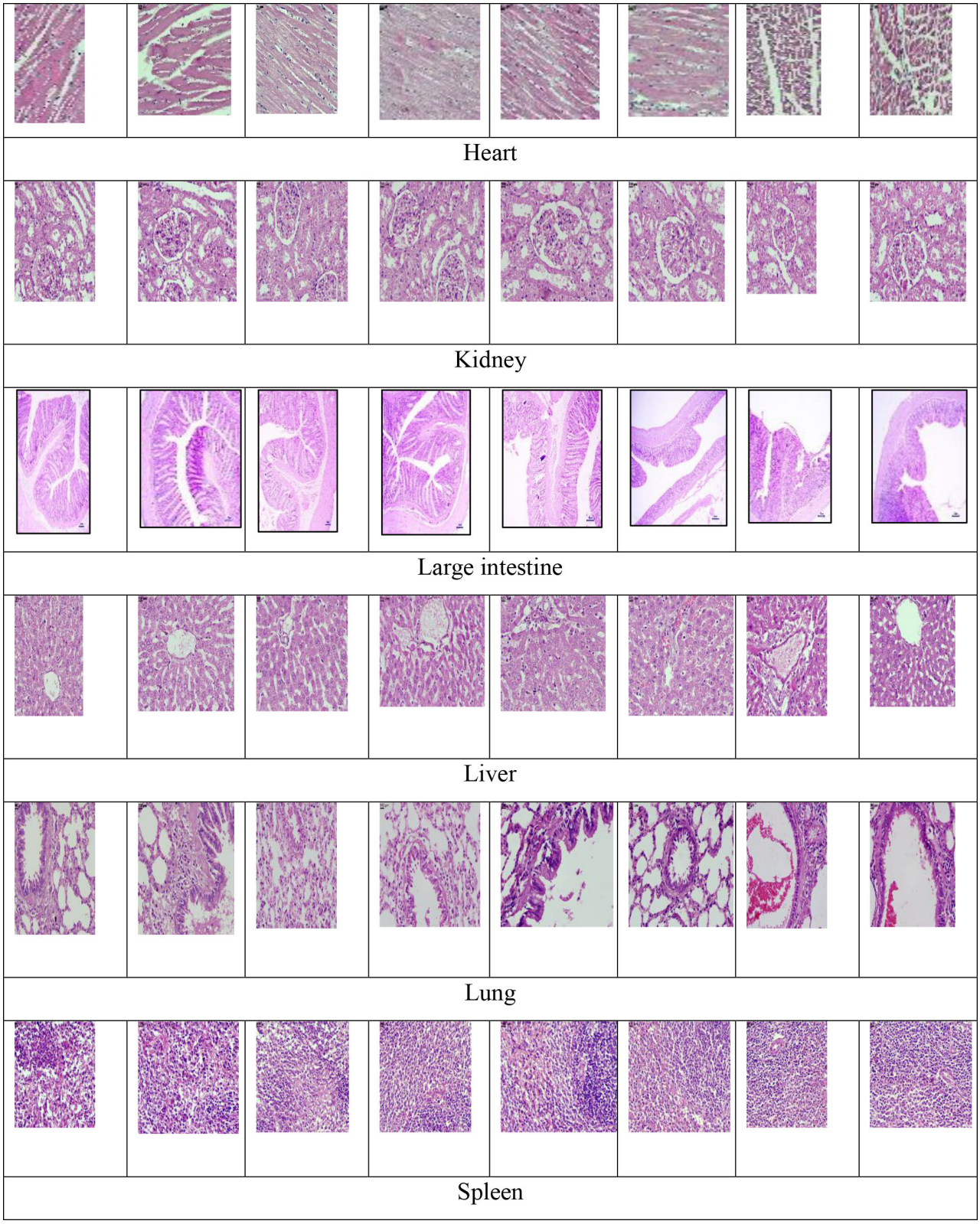
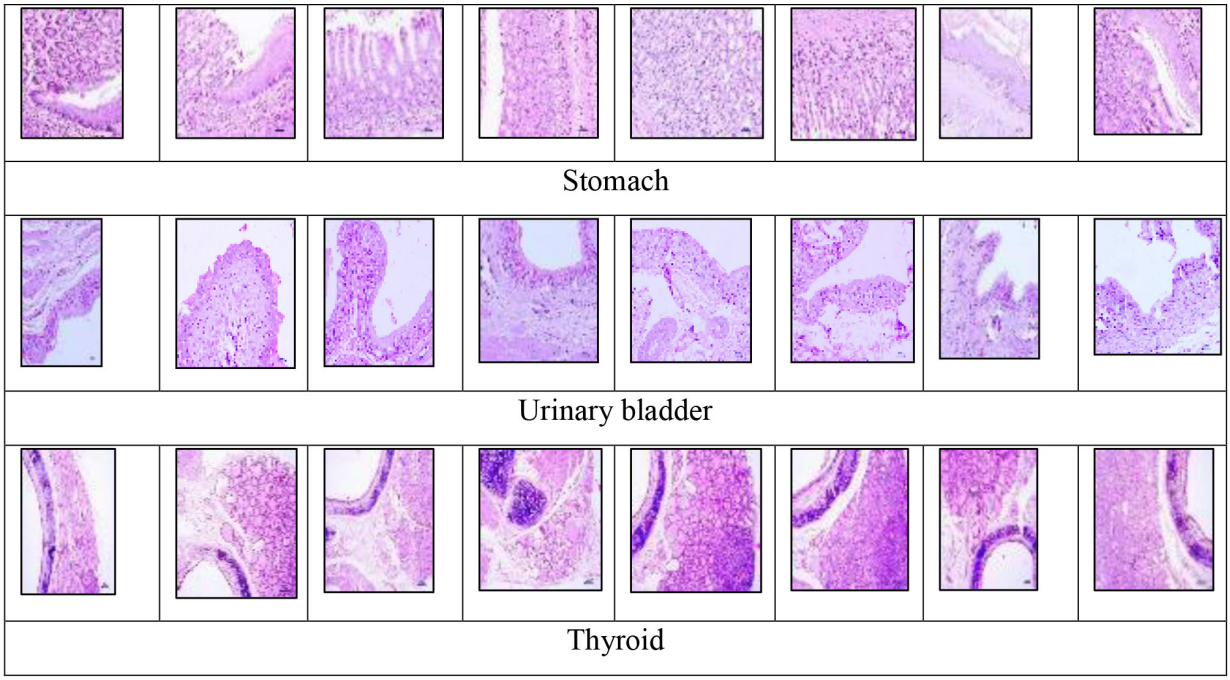
							
Brain							


Heart
Kidney
Large intestine
Liver
Lung
Spleen



CONCLUSION

A Coroprotect Tablet had no adverse impacts on the rat's development, general health, neurological, behavioral, hematological, urinalysis, clinical chemistry, gross tissue/organ weights, or organ weights. When administered at a dose of 2000 mg/kg body weight for acute research, as well as a low, middle, and high dose body weight for subacute research. Coroprotect Tablet has a NOAEL (no observable adverse effect level) of more than 2000 mg/kg body weight in rats following a single oral dosage. According to the findings of this study, at doses of 100, 200, and 400 mg/kg/day, Coroprotect Tablet did not produce any toxicity signs or symptoms in rats.

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DATA AVAILABILITY

The data used to support the findings of this study may be obtained from the corresponding author upon request.

CONFLICT OF INTEREST

There are no conflicting interests disclosed by the authors.

Authors' Contribution: All authors contributed equally towards the data collection, data analysis & compilations.

REFERENCES

1. OECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing Paris, https://doi.org/10.1787/9789264071001-en.
2. OECD (2008), Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070684-en.
3. Prabu PC, Panchapakesan S, Raj CD. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. Phytotherapy Research. 2013 Aug;27(8):1169-78.
4. Patel SB, Rao NJ, Hingorani LL. Safety assessment of *Withania somnifera* extract standardized for Withaferin A: Acute and subacute toxicity study. J-AIM. 2016 Mar 1;7(1):30-7.
5. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, etal., Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicologic pathology. 2007 Aug;35(5):742-50.
6. Wang L, Li Z, Li L, Li Y, Yu M, Zhou Y, etal., Acute and subchronic oral toxicity profiles of the aqueous extract of Cortex Dictamni in mice and rats. Journal of ethnopharmacology. 2014 Dec 2; 158:207-15.
7. Yu HY, Gao DM, Zhou W, Xia BB, He ZY, Wu B, etal., Acute and sub-chronic toxicity study of recombinant bovine interferon alpha in rodents. J. Vet. Res. 2021 Jun 1;65(2):183-92.

8. Khoo ZY, Teh CC, Rao NK, Chin JH. Evaluation of the toxic effect of star fruit on serum biochemical parameters in rats. Pharmacognosy magazine. 2010 Apr;6(22):120.
9. Oshaghi EA, Tavilani H, Khodadadi I, Goodarzi MT. Dill tablet: a potential antioxidant and anti-diabetic medicine. Asian Pac. J. Trop. Biomed. 2015 Sep 1;5(9):720-7.
10. Belemkar S, Shendge PN. Toxicity profiling of the ethanolic extract of *Citrullus lanatus* seed in rats: behavioral, biochemical and histopathological aspects. Bioscience reports. 2021 Jan 29;41(1).
11. Chandrama Singh S, Choudhary M, Mourya A, Khatri DK, Singh PK, Madan J, et al. Acute and Subacute Toxicity Assessment of Andrographolide-2-hydroxypropyl-β-cyclodextrin Complex via Oral and Inhalation Route of Administration in Sprague-Dawley Rats. The Scientific World Journal. 2022 Mar 28;2022.
12. Balkrishna A, Sinha S, Varshney A. 28-day repeated dose toxicological evaluation of Coronil in Sprague Dawley rats: Behavioral, hematological, biochemical and histopathological assessments under GLP compliance. Drug and Chemical Toxicology. 2022 Feb 9:1-4.
13. Lee SS, Tan NH, Fung SY, Pailoor J, Sim SM. Evaluation of the sub-acute toxicity of the sclerotium of *Lignosusrhinocerus* (Cooke), the Tiger Milk mushroom. J. Ethnopharmacol. 2011 Oct 31;138(1):192-200.