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

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ABSTRACT

Introduction: Ayurvedic medications are extensively used across the world for illness prevention and treatment. The active ingredients of Coroprotect dry syrup are *Jethimadh* ext, *Ajma* ext., *Tulsi* ext., *Pipper* ext., *Sunth* ext., *Kalamari* ext., *Vasa* ext., *Taj* ext., *Lavang* ext., *Nagarvel* ext., *Haridra* ext., *Kakamachi* ext., *Pushkarmool* ext., and *Phudina ful*, etc. Because there is no scientific proof that this formulation is safe, a comprehensive toxicity study in wistar rats was conducted.

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

Method: Six wistar female rats were given a single dose of coroprotect dry syrup at 2000 mg/kg by oral gavage, while doses of 100, 200 and 500 mg/kg/day were given over the course of 28 days in a repeated-dose subacute toxicity study.

Results: In an acute toxicity investigation involving coroprotect dry syrup administration, no therapies fatalities or toxic symptoms were found. In the repeated dosage study, there were no substantial differences in body weight fluctuations, food/water ingestion, haematology, or clinical biochemistry content among the control as well as coroprotect dry syrup groups. There were no gross pathological abnormalities or variations in relative organ weights between the control and coroprotect dry syrup groups. Histopathological investigation revealed no abnormalities after therapy with coroprotect dry syrup.

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

Key Words: Coroprotect dry syrup, Acute toxicity, Repeated dose 28-day oral toxicity study, Subacute toxicity, Wistar rat, Ayurvedic supplement.

INTRODUCTION

Modern medicine coexists in India alongside indigenous medical systems such as Ayurveda, Unani, and Siddha, which are widely used by large segments of the population.[1]

Our test preparation, Coroprotect dry syrup, is also an Ayurvedic supplement that contains enhanced extracts of *Jethimadh, Ajma, Tulsi, Pipper, Sunth, Kalamari, Vasa, Taj, Lavang, Nagarvel, Haridra, Kakamachi, Pushkarmool, and Phudina ful*, among others. Ancient ayurvedic concepts were used in the development of Coroprotect dry syrup, which is intended to be used as a prophylactic and therapeutic intervention for coronavirus disease in 2019 (COVID-19). Ingredients in Coroprotect dry syrup has been utilised in medicinal practise for centuries in India and other regions of the world. 10 ml thrice a day has been authorised as the daily dosage of Coroprotect dry syrup. The documented antioxidant efficacy of Coroprotect dry syrup *in vitro* and *in vivo* makes it imperative to assess its non-clinical safety with the objective of identifying whether it has a prophylactic benefit and therapeutic potential in human patients who were infected with COVID-19. As a result, we evaluated the potential health concerns connected with Coroprotect dry syrup after it was regularly orally delivered to rats at various doses over the course of 28 days. The reversibility, durability, and delayed development of toxic effects in a satellite group

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of animals given a high dose of Coroprotect dry syrup for a 14-day treatment-free interval were investigated. There was a need to understand more about Coroprotect dry syrup's principal toxic effects, target organs, and No Observed Adverse Effect Level (NOAEL) in rats, which is why this research was conducted. To assess the drug's toxicity, the investigators employed the OECD criteria 423 [2] and 407 [3] for acute and 28- day repeated dosage toxicity, respectively.

MATERIALS AND METHODS

Ethics statement

The research was done in accordance with the Organization for Economic Cooperation and Development's (OECD) criteria for acute and 28-day repeated-dose toxicity. On October 16, 2008, and February 8, 2002, respectively, they were published.

Animals

Male & female Wistar rats were employed in the experiment. All of the toxicity tests were carried out in accordance with the recommendations set forth by the Organization for Economic Cooperation and Development (OECD).

Study material

Coroprotect dry syrup was used as a test drug. A suspension of Coroprotect dry syrup was created using methylcellulose as the suspending agent for administration to the animals. The reagents and chemicals used in the investigation were all of the highest commercial grade.

Study design

For acute toxicology investigation:

Step 1: 3 female rats were administered a single dose of Coroprotect dry syrup by oral gavage at a dose of 2000 mg/kg body weight and were observed for 24 hours for signs of toxicity or death.

Step 2: Three female rats were given a single dose of 2000 mg/kg body weight of Coroprotect dry syrup by oral gavage and were followed for 24 hours for signs of toxicity or mortality.

All six rats were evaluated separately after the dose for a total of 14 days, at least once during the first 30 minutes, sometimes over the first 24 hours, with special attention devoted during the first 4 hours, and everyday thereafter to determine delayed toxicity.

For 28 days Repeated dose toxicity investigation:

Six male rats & six female rats were administered daily doses of Coroprotect dry syrup by oral gavage at doses of 100, 200 & 500 mg/kg/day bodyweight for 28 days before being slaughtered to determine toxicity.

Dose preparation

The test product Coroprotect dry syrup was suspended in distilled water just before the dosing schedule to achieve the required concentration of mg/ml.

Administration of test article

On the first day, the rats were dosed by oral gavage with a graduated syringe and, if possible, a stainless steel intubation needle for acute toxicity testing (18G).The medication volume given to each rat was changed based on its most recent body weight measurement. Rats were observed for clinical signs and symptoms for a sum of 14 days. For the subacute toxicity study, the animals were dosed by oral gavage at roughly the same time each day, using a graduated syringe with a stainless-steel intubation needle (18G). Each rat's drug dosage was adjusted based on its most recent body weight assessment. The control group of rats received the vehicle (saline) via oral gavage at the same dosage amount of 10ml/kg body weight. For a total of 28 days, the therapy was repeated in this approach.

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 were 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. The animals in the satellite group were also tracked for 14 days following treatment to see if any harmful effects were permanent or reversible, as well as the incidence of delayed toxicity.

Mortality

Throughout the study, all animals were checked 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 mid-day.

Clinical signs

Individual animals were all indicators of illness recorded, as well as any behavioral changes or treatment reactions.

General clinical examinations

The rats were submitted to general cage side clinical examinations on a daily basis, at the same time each day, and at appropriate intervals following dosage, taking into account the peak period of expected effects after dosing.

Detailed clinical examinations

Before starting the medication (to allow for within-subject comparisons), the rats were submitted to extensive clinical tests, which were repeated weekly throughout the treatment period. Outside the home cage, these observations were made in a regular arena, preferably at the same exact time. Physical indications included alterations in skin, fur, eyes, and mucous membranes, the frequency of fluids and excretions, and autonomic activity such as piloerection, pupil size, and unusual breathing patterns. Changes in stride, posture, as well as responsiveness to handling were also observed, & the occurrence of clonic or tonic movements, stereotypiesor odd behavior if present.

Body weights

The weights of rats were recorded when they were assigned to groups, which was one day previous to the start of therapy, weekly afterward, and at necropsy. The weights of the Satellite group rats were recorded weekly during the post-treatment period and at necropsy.

Food consumption

On the first day of therapy and every week following that, the amount of food ingested by each rat in each cage was recorded. The amount of food supplied to and left in each cage was used to calculate food consumption. During the post-treatment phase, the satellite group rat's food consumption was tracked weekly.

Clinical pathology

Under carbon dioxide anaesthesia, blood samples were obtained from the retroorbital plexus of all male as well as female rats in the test and control groups on the last day of the study. Heparin (for clinical chemistry) and K-EDTA (for anticoagulation) were used to collect the samples (for hematology). The rats' feed was taken away overnight to be sampled for lab experiments. On glass slides, bloodstains 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. The criteria that were assessed were total red blood cell (RBC), haemoglobin (Hb), total white blood cell (TWBC), neutrophils %, lymphocyte, eosinophils %, monocytes %, & platelet count.

Clinical chemistry

The parameters of serum chemistry, as well as their units of measurement, were examined and recorded in observations. Blood urea[4], creatinine[5], serum glutamic pyruvic transaminase (SGPT)[6], serum glutamic oxaloacetic transaminase (SGOT)[7], total protein[8], alkaline phosphatase (ALP)[7], total bilirubin[9] and uric acid[10]were the measures examined.

Urinalysis

All of the animals had their urine tested. Urine samples were collected using a battery of specially designed stainless steel urine collection cages. 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. The urine's color, appearance, specific gravity, and pH were all measured.

Necropsy examination

After 28 days of treatment, all survivor rats were exsanguinated under co2 anaesthesia and subjected to a thorough necropsy. The necropsy was done after day 28 and was dispersed towards the end of therapy. All tissues from animals were kept in 10% neutral buffered formalin, as per OECD 407 recommendations. In addition, samples of any macroscopically aberrant tissues, as well as samples of neighboring tissue, were routinely maintained.

Organ Weights

Organs from all animals slain during the scheduled sacrifices were dissected and weighed wet as quickly as possible to prevent drying.

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

The data was investigated using One-Way ANOVA (Dunnett's test) and Student's t-test utilising graph pad prism at $p<0.05$ at the level of significance from the control mean ranges.

RESULT AND DISCUSSION

Polyherbal formulations are frequently used in industrialized nations for the therapy of a number of illnesses when compared

to allopathic therapy.[13] Even though the side effects are modest, indigenous knowledge cannot be used to determine the exact safety of herbal compositions for approval at this time.[11]

There was no rat exposure mortality and adverse clinical sign among the female test subjects (at a dose of 2000 mg/kg body weight). [Table no. 1] as well as within Male & female rats subjected to the test item (low dose (100 mg/kg/ day), middle dose (200 mg/kg/ day), and high dose (500 mg/kg/ day) body weight) had no mortality and adverse clinical sign as well as a control group. [Table no. 2] All of these symptoms surfaced solely throughout the course of treatment. Because this discovery occurred in both of these groups at the same time, it was dismissed as purely accidental.

Female rats given Coroprotect dry syrup at a dose of 2000 mg/kg body weight had no noticeable or significant changes in body weight. [Table no. 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 no. 4]

The daily average food consumption of female rats given Coroprotect dry syrup at a dose of 2000 mg/kg body weight was shown to be consistent over a 14-day period, with intake remaining constant or slightly increasing for all six females. Coroprotect dry syrup at low doses (100), intermediate doses (200), & high doses (500 mg/kg/day) had found to be comparable to the control group in male and female rats. Over a 28-day period, the average daily food consumption per rat was measured. The low dosage (100 mg/kg/day), middle dose (200 mg/kg/day), and high dose (500 mg/kg/day) satellite groups of Coroprotect dry syrup were comparable to the control rat. Similarly, the average daily food consumption of female rats given the test article at higher dose levels was similar to that of control rats. The values of daily food intake by the intermediate dosage group rat were shown to be steadily increasing after treatment termination, as well as during the recovery period.

The kidneys, liver, adrenals, testes/ovaries, spleen, brain, epididymis/uterus, thymus, as well as hearts of male and female rats treated at low dose (100), middle dose (200), and high dose (500 mg/kg/ day) were found to be similar to the control group rat at the end of the treatment. [Table no. 5]

In humans and animals, the hematopoietic system is among the most susceptible sites for harmful substances and an important indicator of physiological and pathological status.[12] Male and female rats exposed to Coroprotect dry syrup (at low dose (100), middle dose (200), and high dose (500 mg/ kg/ day)) had hematological parameters such as hemoglobin (Hb), hematocrit (PCV), and total erythrocyte counts (RBC), total leucocyte count (WBC), differential leucocyte counts (DLC), and platelet count. Neither the treatment nor the control groups demonstrated any treatment effect over the course of the research or at the end. [Table no. 6]

On serum biochemistry parameters, treatment of male & female rats with Coroprotect dry syrup at low dose (100), middle dose (200), and high dose (500 mg/kg/ day) had no discernible effect. [Table no. 7]

There was no significant difference in hematological and biochemical parameters between the low dose (100), intermediate dose (200), and high dose (500 mg/kg/ day) body weight groups after 28 days of therapy in both male as well as female rats. All of the values discovered for these parameters were equivalent to those observed in the control groups.

The urine parameters of male as well as female rats treated with Coroprotect dry syrup (at low dose (100), middle dose (200), and high dose (500 mg/kg/ day) and the control group did not show any significant therapeutic effect. The urine qualitative indicators tested for all rats in this investigation, as well as a microscopic inspection of their urine sediment, revealed no significant treatment-related changes.

Coroprotect dry syrup did not induce any significant and therapies gross pathological abnormalities from any of the treated rat's organs or tissues at a dose of 2000 mg/kg. [Table no. 8] Coroprotect dry syrup did not induce any significant and therapies related gross pathological abnormalities in any of the organs / tissues of treated rats at low doses (100), middle doses (200), and high doses (500 mg/kg/day), as well as the satellite group (1000 mg/kg body weight).

All microscopic changes reported in this study appeared to be coincidental at low doses (100), medium doses (200), and high doses (500 mg/kg/day), as their frequency and intensity remained comparable in the control and treated animals. Coroprotect dry syrup at low doses (100), intermediate doses (200), and high doses (500 mg/kg/day) body weight exhibited no histological effects in rats under the identical experimental settings. [Table no. 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 dry syrup 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
Coroprotect dry syrup 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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

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

Sex/Group	Days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Female - A/F001	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F002	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F003	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - A/F004	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M001	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M002	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M003	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M004	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/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 - A/F001	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F002	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F003	N	N	N	N	N	N	N	N	N	N	N	N	N
Female - A/F004	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F005	N	N	N	N	N	N	N	N	N	N	N	N	N
Female -A/ F006	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M001	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M002	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M003	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M004	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/M005	N	N	N	N	N	N	N	N	N	N	N	N	N
Male - B/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 dry syrup Limit test step 1 2000 Mg/Kg	Female - CS/F001	247.1	251.8	252.7	2.48%
	Female - CS / F002	247.2	251.9	253.9	
	Female - CS/ F003	247	252.6	253.1	
	Mean	247.1	252.1	253.2	
	SD	0.1	0.4	0.6	
Coroprotect dry syrup Limit test step 2 2000 Mg/Kg	Female - CS /F004	178.3	177.8	180	1.18 %
	Female - CS / F005	177.8	178.5	180.4	
	Female - CS / F006	178.1	178.9	180.1	
	Mean	178.1	178.4	180.2	
	SD	0.3	0.6	0.2	

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	214.83±15.15	230.2±8.2	164.00±24.07	222.83±11.86
	day 14	226.83±16.29	242.5±8.3	176.00±22.31	233.83±11.29
	day 28	237.5±16.19	252.7±8.0	194.83±15.01	245.17±12.22
Male	day 0	207.5±16.31	242±36.3	156.33±27.93	316.83±21.33
	day 14	219.33±16.68	253.5±37.2	168.17±25.02	327.17±19.89
	day 28	228.83±16.48	265.3±37.3	192.17±12.09	340±19.3

Each value represents the mean ± standard deviation ($n = 12$).

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	0.065±0.001	0.064±0.002	0.064±0.002
	Ovary	0.077±0.000	0.077±0.000	0.073±0.003	0.076±0.002
	Brain	1.897±0.001	1.897±0.001	1.890±0.009	1.897±0.002
	Kidney	1.478±0.004	1.478±0.004	1.471±0.005	1.479±0.007
	Liver	8.126±0.002	8.126±0.002	8.126±0.002	8.130±0.005
	Heart	0.652±0.001	0.652±0.001	0.652±0.000	0.653±0.002
	Spleen	0.671±0.004	0.671±0.004	0.665±0.018	0.68±0.01
	Epididymis	NA	NA	NA	NA
	Thymus	0.230±0.001	0.230±0.001	0.220±0.006	0.230±0.002
	Uterus	0.70±0.010	0.70±0.010	0.68±0.04	0.70±0.01
Male	Adrenals	0.065±0.001	0.064±0.001	0.064±0.001	0.066±0.002
	Ovary	0.077±0.000	0.077±0.001	0.075±0.003	0.077±0.002
	Brain	1.897±0.001	1.897±0.002	1.890±0.010	1.898±0.001
	Kidney	1.478±0.004	1.478±0.005	1.464±0.015	1.481±0.003
	Liver	8.126±0.002	8.127±0.003	8.123±0.006	8.132±0.005
	Heart	0.652±0.001	0.652±0.001	0.652±0.001	0.652±0.002
	Spleen	0.671±0.004	0.665±0.008	0.656±0.024	0.69±0.01
	Epididymis	0.30±0.010	0.30±0.010	0.30±0.01	0.30±0.02
	Thymus	0.230±0.001	0.228±0.004	0.230±0.001	0.230±0.001
	Uterus	NA	NA	NA	NA

Table 6: Animal hematology parameters

Group	Parameters	Low dose	Middle dose	High dose	Control
Femle	Total leukocyte count (x 10^3/µl)	9.2±0.1	19.1±0.1	9.20±0.36	9.70±0.46
	Rbc count (x 10^6/µl)	6.6±0.04	7.8±0.1	5.50±0.71	7.65±0.46
	Hemoglobin (hb) (g/dl)	12.3±0.2	13.2±0.4	10.32±0.32	13.95±1.83
	Platelet count (x 10^3/µl)	438.8±100.8	626.2±21.3	416.83±24.29	722.33±15.46
	Neutrophils (%)	25.7±1.2	21.33±0.52	32.50±1.87	20.00±5.87
	Lymphocytes (%)	71.2±3.6	78.67±0.52	67.00±2.90	77.67 ±3.72
	Basophils (%)	0.2±0.4	0.12±0.12	0.10±0.13	0.10 ±0.15
	Eosinophils (%)	0.3±0.5	0.50±0.55	0.28±0.15	0.25±0.10
	Monocytes (%)	0.2±0.4	0.17±0.41	0.50±0.55	1.83±1.17
Male	Total leukocyte count (x 10^3/µl)	9.3±0.3	19.2±0.1	9.12±0.34	9.77±0.82
	Rbc count (x 10^6/µl)	6.6±0.1	7.8±0.1	5.58±0.82	7.93±0.50
	Hemoglobin (hb) (g/dl)	12±0.7	13.3±0.3	10.40±0.47	14.12±1.88
	Platelet count (x 10^3/µl)	479.7±71.7	591.5±70.2	416.00±25.88	724.67±23.36
	Neutrophils (%)	26.5±1.8	20.67±0.82	31.83±3.97	19.00±6.66
	Lymphocytes (%)	74.0±4.4	78.17±1.17	66.17±5.60	78.33±5.65
	Basophils (%)	0.3±0.5	0.15±0.16	0.12±0.12	0.15±0.21
	Eosinophils (%)	0.2±0.4	0	0.25±0.10	0.20±0.11
	Monocytes (%)	0.3±0.5	0.33±0.52	0.33±0.52	1.50±1.05

Each value represents the mean ± standard deviation ($n = 12$).One-Way ANOVA (Dunett's test) and Student's t test using graph pad prism at the level of significance from the control means at $p < 0.05$.

Table 7: Animal biochemical parameters

Group	Parameters	Low dose	Middle dose	High dose	Control
Female	Bilirubin total (mg/dl)	0.5±0.4	0.9±0.4	0.8±0.4	0.80±0.14
	Alkaline phosphatase (u/l)	224.2±16.3	352.5±17.4	195.8±19.2	252.83±34.63
	Uric acid(mg/dl)	2.7±0.5	2.3±0.5	2.5±0.6	2.52±0.57
	Urea (mg/dl)	37.5±0.7	26.1±0.1	37.59±0.73	31.00±2.76
	Creatinine (mg/dl)	0.5±0.0	0.4±0.0	0.52±0.02	0.37±0.22
	Total protein (g/dl)	5.6±0.5	5.8±0.5	5.6±0.5	7.80±0.97
	ALT (iu/l)	59.1±5.4	74.7±3.8	58.78±5.65	75.33±4.80
	AST (iu/l)	117.0±6.8	180.0±9.0	117.15±6.73	261.50±34.54
Male	Bilirubin total (mg/dl)	0.4±0.3	1.1±0.5	0.6±0.7	0.78±0.26
	Alkaline phosphatase (u/l)	223.0±25.3	365.2±26.6	185.8±19.4	255.50±31.61
	Uric acid(mg/dl)	2.2±0.7	2.5±0.3	2.7±0.2	2.57±0.73
	Urea (mg/dl)	36.6±0.8	25.9±0.4	36.45±0.80	31.83±6.31
	Creatinine (mg/dl)	0.5±0.0	0.4±0.1	0.51±0.02	0.32±0.21
	Total protein (g/dl)	5.8±0.5	5.8±0.6	5.76±0.57	7.67±0.88
	ALT (iu/l)	56.9±1.8	77.4±3.4	56.53±2.15	74.67±4.80
	AST (iu/l)	108.8±7.5	144.3±31.7	108.59±7.84	261.67±33.22

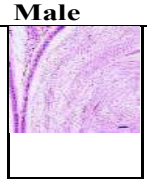
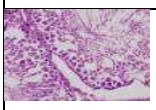
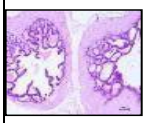
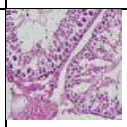
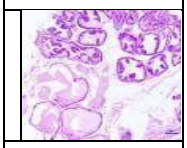
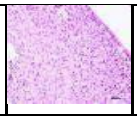
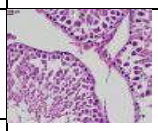
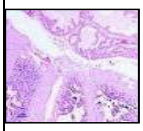
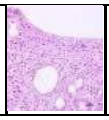
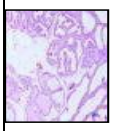
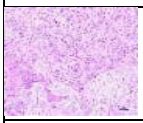
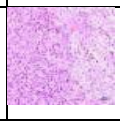
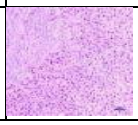
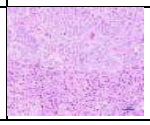
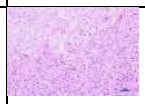
Each value represents the mean ± standard deviation ($n = 12$).One-Way ANOVA (Dunett's test) and Student's t test using graph pad prism at the level of significance from the control means at $p < 0.05$.

Table 8: Gross pathological changes of acute toxicity

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

N=No gross pathological changes after necropsy

Table 9: Histopathology

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 & prostate	Uterus	Testes & prostate	Uterus	Testes & prostate	Uterus	Testes & prostate	Uterus
							
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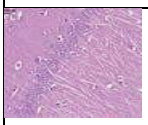	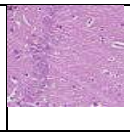	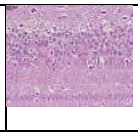	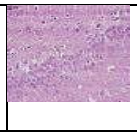	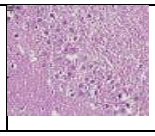	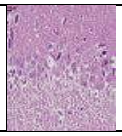		
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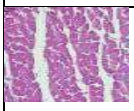	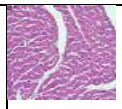	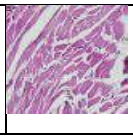	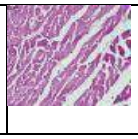				
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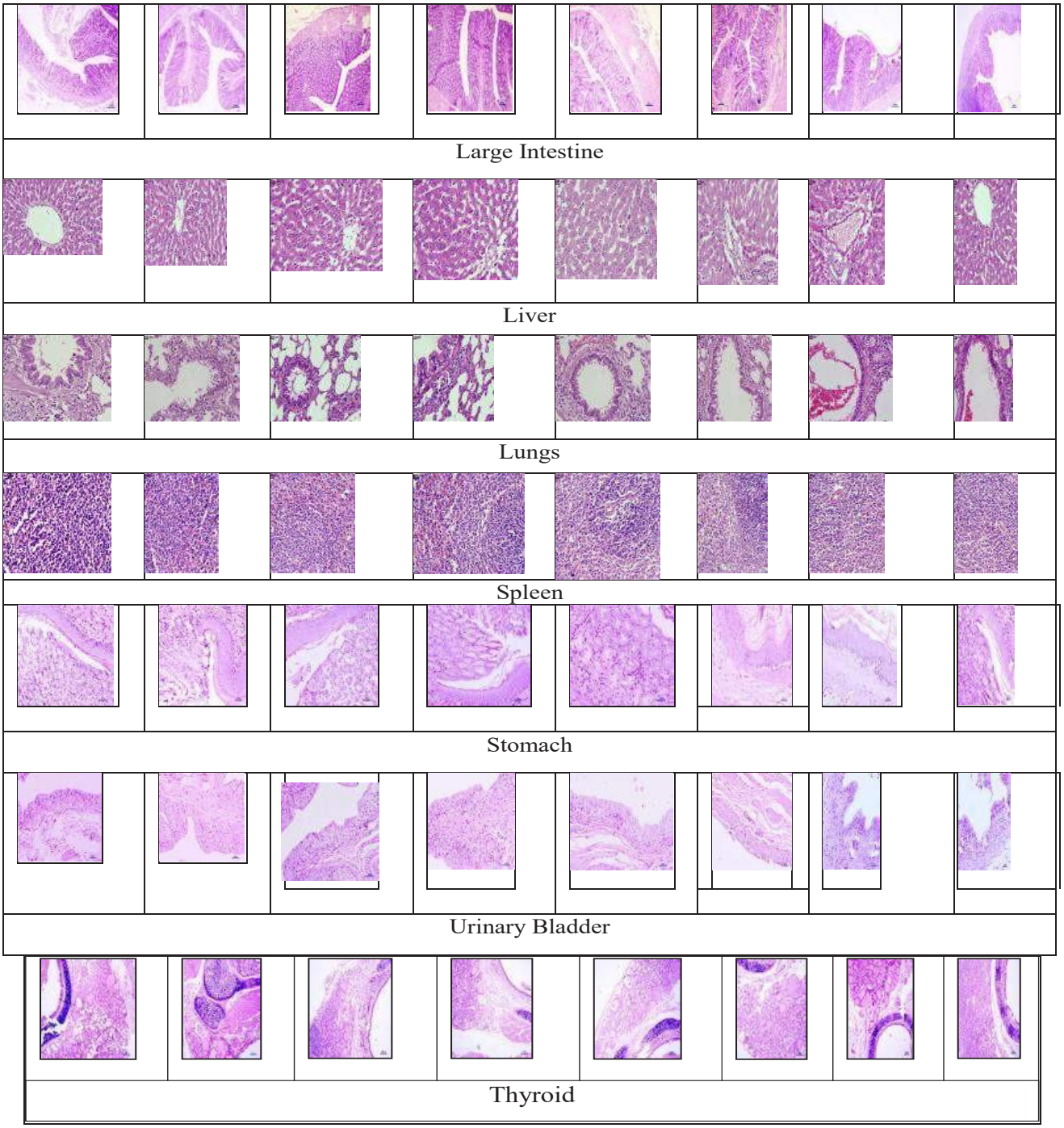
Kidney							



CONCLUSION

Following a single dose oral administration of 2000 mg/kg to groups of six female rats for 14 days, and repeated daily oral administration of low dose (100 mg/kg/ day), middle dose (200 mg/kg/ day), and high dose (500 mg/kg/ day) to groups of six rat per sex for 28 days, researchers concluded that there was no treatment-related mortality in female rats, no incidence of any remarkable abnormal clinical signs, and no adiposity. The study found that Coroprotect dry syrup had no negative effects on general health, growth, behavior, neurological function, or the gross appearance of tissues and organs. The no observed adverse effect level (NOAEL) of Coroprotect dry syrup in rats following a single oral dose was determined to be more than 2000 mg/kg body weight, according to the findings of this investigation.

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DATA AVAILABILITY

On request, the data used to support the findings of this study can be obtained from the corresponding author.

CONFLICTS OF INTEREST

There are no conflicting interests disclosed by the authors

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

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