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Safety Assessment of Tuberous Rhizome of *Kaempferia rotunda* L. By Acute and 28-days Repeated Dose Toxicity Studies

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Abstract: The acute and 28-days repeated dose toxicity studies of the ethanolic extract of rhizome, tuber, rhizome fractions and the essential oil of *Kaempferia rotunda* L. were performed on Wistar albino rats to substantiate the tribal claims of its use as a safe wound healing and anti-ulcerogenic drug. The extracts, the essential oil and the rhizome fractions (petroleum ether, chloroform, methanol, aqueous and the acetone-chloroform) of *K.rotunda* were found to be non toxic or non corrosive in the acute dermal toxicity studies when tested topically on the skin of the animal and were safe for use up to 20 % (w/w) /day. The individual acute oral toxicity of the extracts and fractions were carried out at a single test dose of 2000 mg/kg p.o. and there was no treatment related mortalities or toxic signs observed in any of the treated animals. Further, the 28-days repeated dose study (250, 500 and 1000 mg/kg p.o. / day) with a post trial 14-day no treatment observation period at high dose level (1000 mg/kg p.o. / day) showed a mixed trend of both increase and decrease in haematological, enzymological and biochemical parameters. But all these changes were observed to be within the normal range and were further supported by histopathological evaluation of the organs. The results of the study reveals that the extracts and fractions of *K. rotunda* is not acutely toxic up to 2000 mg/kg of body weight/day p.o and the no-observed-adverse-effect level (NOAEL) of the extract and fractions for both male and female rats is considered to be greater than 1000 mg/kg of body weight/day, p.o. for 28 days.

Key words: K. rotunda tuberous rhizome · NOAEL · Wound healing · 28-days repeated dose toxicity · Acute dermal toxicity

INTRODUCTION

Kaempferia rotunda L. (Zingiberaceae) is a handsome aromatic herb with very fragrant subglobose yellow-white tuberous rhizome, commonly known as 'Chengazhineerkkizhangu' in traditional medicine of Kerala. The plant is distributed throughout India [1, 2] from eastern Himalaya to Sri Lanka and the Malay Peninsula to Malay Island [3]. The rhizomes and root tubers of the plant have a bitter, pungent and camphoraceous taste and has been widely used as a vegetable and a food flavouring spice in India and in Southeast Asia including Malaysia, Indonesia and Thailand [4]. The fragrant rhizomes, roots and tubers of the plant are traditionally used for abdominal pain, dysentery, diarrhoea, cold, obesity and preparation of

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cosmetics and it is locally applied to tumours, swellings, bruises, wounds and ulcers. They are also considered anti-inflammatory, stomachic and given in gastric complaints [2, 3, 5,6]. In 'Ayurveda', the rhizome of the plant has been used for the preparation of a drug 'Hallakam', which is stomachic, anti inflammatory to wounds and bruises, improves complexion, cures burning sensation, mental disorders and insomnia [6-9].

The previous phytochemical analysis conducted in the rhizome of *K.rotunda* revealed the presence of many active phytoconstituents that included, cyclohexane diepoxides, crotepoxide, (-)-zeylenol [10-12], six polyoxygenated cyclohexane derivatives and a triacylated derivative of salicin[13], further the GC and GC/MS analysis of the volatile constituents of the rhizomes oil of *K.rotunda* reported the presence of four main constituents which included pentadecane, bornyl acetate, benzyl benzoate and camphor [14, 15].

As per the ethno botanical information collected from the 'Kurichiya' tribe of Wayanad District, Kerala, India, the rhizome and tubers of the plant *K.rotunda* is used for diverse medicinal and therapeutic purposes including wound healing, anti-ulcer and antipyretic activities which has not been scientifically validated. Unpublished *in vivo* studies conducted in our laboratory revealed the wound healing, anti-inflammatory, anti-nociceptive and anti-ulcerogenic activity of the rhizome, tuber, rhizome fractions and the essential oil isolated from the rhizome and tuber of *K.rotunda*. Despite its widespread use, no toxicological data is available regarding the safety assessment of the tuberous rhizome of *K.rotunda.* Hence the present study was undertaken to assess the safety of rhizome and tuber extract of *K.rotunda* after single and 28-day repeated dose administration.

MATERIAL AND METHODS

Plant Material: The fresh rhizomes and tubers of *K.rotunda L.* (Zingiberaceae) were collected from Malayinkeezhu, Thiruvananthapurm, Kerala, during September, 2007. They were authenticated by Dr. Mathew Dan, plant taxonomist of the Institute. A voucher specimen, TBGT 57031, dtd 11/09/2007 has been deposited at the herbarium of the Institute, JNTBGRI.

Preparation of Plant Extracts: The fresh rhizomes and tubers were collected, washed, shade dried and powdered individually. The powder (100 g) was successively extracted with 1000 ml of ethanol overnight with constant stirring. The extracts were filtered and the filtrates were then concentrated under reduced pressure in a rotary evaporator. The yield of ethanol extract of rhizome was found to be 9.24 % (w/v) and this crude extract was referred to as KRE. The yield of the ethanol extract of tuber was found to be 4.10 % (w/v) and was referred to as KRT.

Preparation of Rhizome Fraction: Fresh rhizomes of *K.rotunda* (100 g) were collected, washed, chopped finely and macerated for 24 hrs with 1000 ml ethanol under constant stirring. The extract was filtered and the filtrate was then concentrated to dryness under reduced pressure in a rotary evaporator and the crude extract was referred to as KRE1. The yield of the extract was found to be 8.86 % (w/v). The extract was partitioned with a range of different solvents with increasing polarities, including petroleum ether (KRE1P), chloroform (KRE1C) and methanol (KRE1M). Each fraction was then concentrated to dryness under reduced pressure in a rotary evaporator and the yield was observed to be, 0.57% (w/w), 5.88 % (w/w) and 2.41 % (w/w) respectively. In addition to this, an aqueous and acetone extract of the fresh rhizomes of *K. rotunda* were prepared individually by macerating the finely chopped fresh rhizome (100 g) for 24 hrs with 1000 ml Milli-Q water and acetone respectively under constant stirring. The extract was filtered, the filtrate was concentrated and the yield of the extract was found to be 9.25 % (w/v) and 6.10 % (w/v) for crude aqueous (KRAq) and acetone (KRAc) extract respectively. The crude acetone extract was successively partitioned with the solvent petroleum ether (KRAcP), chloroform (KRAcC) and butanol (KRAcB). From this the choloroform (KRAcC) extract was further concentrated and the yield of the extract was found to be 5.56 % (w/w). Compared to the acetone-chloroform fraction, the yield of petroleum ether and butanol fraction was less and therefore only the acetone-chloroform fraction was further studied.

Extraction of Essential Oil by Hydrodistillation: The fresh rhizomes and tubers of *K.rotunda*, were harvested and approximately 300 gm of fresh unpeeled rhizomes and tubers were washed and chopped into small pieces and hydrodistilled individually using a Clevenger distillation apparatus for 5h and the essential oil from the fresh rhizome (KRRO) and tuber (KRTO) were collected individually and their yield was observed to be 0.6 % (w/w) and 0.04 % (w/w) respectively. The isolated oil, KRRO and KRTO was dried over anhydrous sodium sulphate and stored at 4-6°C.

Route of Drug Administration: In acute dermal toxicity, the drug was tested topically on the skin surface of the animals, whereas in acute oral and in 28-day repeated dose toxicity, the drug was administered orally to the animals.

Topical Treatment: For topical application, the crude rhizome and tuber extract (KRE and KRT) and rhizome fractions (KRE1P, KRE1C, KRE1M, KRAq, KRAc and KRAcC) were reconstituted in simple ointment base material (white petroleum jelly) as vehicle and formulated in to ointment containing crude rhizome and tuber extract (KRED and KRTD respectively) and isolated fractions of rhizome (KRE1PD, KRE1CD, KRE1MD, KRAqD, KRAcD and KRAcCD) respectively with the highest concentration of 20 % (w/w) for topical application. In addition to this, a topical formulation of essential oil was prepared by dispersing the essential oil extracted from the fresh rhizomes and tubers (KRRO and KRTO) of *K.rotunda* in corn oil as vehicle and formulated into a pharmaceutical oil formulation of rhizome and tuber, KRROD and KRTOD respectively at the highest concentration of 20 % (w/w) for topical application.

Oral Administration: For oral administration, the crude rhizome, tuber extracts (KRE and KRT respectively) and the rhizome fractions (KRE1P, KRE1C, KRE1M, KRAq, KRAc and KRAcC) were re-suspended in 1 % Tween-80 as vehicle and administered in required concentrations per orally.

Experimental Animals: Male and female Wistar rats (150-200 g) obtained from the Institute's animal house were used for the present study. They were maintained under standard conditions and fed commercial diet (Lipton India Ltd, Mumbai, India) and boiled water, *ad libitum,* during the experiment. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee.

In Vivo Toxicity Studies

Acute Dermal Toxicity of Rhizome, Tuber and Rhizome Fractions of K. rotunda: The acute dermal toxicity with the single highest test dose was conducted as per the OECD guideline 402 [16] with slight modification. Wistar albino rats (150-200 g) were selected and acclimatised to laboratory conditions for at least five days prior to the experiment. The fur was removed from the dorsal area of the trunk of all the animals by clipping or shaving and care was taken to avoid abrading the skin and only animals with intact skin were used for the present study. At least 10 % of the body surface area of each animal was cleared and the control and test substances were applied uniformly over this area. The animals were then divided in to ten groups (six each) and each animal was caged individually and left undisturbed for 24 hrs before the study. Group I, the control animals were treated with physiological saline. Group II, the vehicle control animals were treated with simple ointment base and the animals of Group III- Group X were topically applied with a highest concentration of 20 % ointment prepared from rhizome, tuber (KRED, KRTD) and the rhizome fractions (KRE1PD, KRE1CD, KRE1MD, KRAqD, KRAcD and KRAcCD) respectively on the shaven area of the animals. They were held in contact with the skin using porous gauze dressing and non-irritating tape for a period of 24 hrs, whereas for the control and vehicle control groups, gauze moistened with physiological saline and Tween-80 respectively was applied and held in contact with the skin using porous gauze dressing and non-irritating tape as described in treated groups. After 24 hrs of exposure the gauze and tape were carefully removed so as not to damage the skin and the test site was rinsed with distilled water and the animals were examined at grading intervals of 30 min, 4 hrs, 24 hrs, 48 hrs and kept under observation for 72 hrs. They were observed for the changes in skin, eyes and mucous membranes, behavioural patterns, diarrhoea, salivation and tremors. Mortality was recorded during the course of study.

Acute Dermal Toxicity of Essential Oil: The acute dermal toxicity of the essential oil extracted from the rhizome and tubers (KRRO and KRTO) were evaluated with the single highest test dose mentioned in OECD guideline 402 [16]. For the experiment, the animals were grouped in to four groups of six animals each. Group 1, the control animals were treated with physiological saline. Group II, the vehicle control animals were treated topically with corn oil as vehicle.Groups III and IV animals were topically applied with an oil formulation containing essential oil isolated from the rhizome (KRROD) and tuber (KRTOD) respectively with a highest concentration of 20 % on the shaven area of the animal. After the commencement of drug application the same procedure was followed as that done in the previous experiment.

Acute Oral Toxicity Studies of Rhizome, Tuber and Rhizome Fractions: Acute oral toxicity studies were performed as per OECD-423 guidelines with a slight

modification[17], where the limit test dose of 2000 mg/kg body weight p.o. was used as the test dose. The animals were fasted prior to the experiment for 18 hrs with free access to water and they were divided into ten groups of six animals each. Group 1, the control animals were orally administered with 1ml saline. Group II, the vehicle control animals were administered with of 1 ml of 1 % Tween-80 p.o., as vehicle. Groups III and IV animals were orally administered with ethanolic extract of *K.rotunda* rhizome and tuber respectively with a single limit test dose of 2000 mg/kg p.o., Groups V-X animals were administered orally with a single limit test dose of 2000 mg/kg rhizome fractions including KRE1P, KRE1C, KRE1M, KRAq, KRAc and KRAcC respectively. All the animals were then allowed free access to food and water and observations of toxic symptoms were made and recorded systematically for the first 4 hrs after administration. Further these animals were observed for 24 hrs and thereafter once a day for the next 14 days. Mortality was recorded during the course of study, if any. The toxicological effect was assessed on the basis of mortality and expressed as LD_{50}.

Repeated Dose 28-day Oral Toxicity Study: A 28-day repeated oral toxicity study was performed according to the OECD guideline, TG 407[18] with minor modifications. Rats of both sexes were randomly assigned into twenty three groups: a control, vehicle control and twenty one treatment groups (n = 12; 6 males and 6 females). The animals of the normal control and vehicle control groups were administered with 1ml of saline and 1 % Tween-80 p.o. and the animals of the treatment groups were administered with ethanolic extract of the rhizome, tuber (KRE and KRT) and *K.rotunda* rhizome fractions (KRE1P, KRE1C, KRE1M, KRAq, KRAc and KRAcC) with a graded dose of 250, 500 and 1000 mg/kg p.o. respectively. All the experimental animals were orally administered with a single dose/ day for a period of 28 days. In order to determine the reversibility or recovery from toxic effects, if any, an additional recovery groups for the animals in the normal, vehicle control and high dose treated groups (ten animals each) were preset, for follow-up observations for the next 14 days without control and/or vehicle or test drug administration, but food and water was provided to determine any delayed effects of the treatment. All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment. Body weights of the animals were recorded once a week. At the end of experiment (28 days), the overnight fasted (water allowed) animals were anaesthetized under CO_2 inhalation and blood samples were collected via carotid artery into heparinised and non-heparinised tubes for haematological and biochemical analysis.

Haematological Parameters: The heparinised blood was used for the analysis of haematological parameters such as, haemoglobin (HGB) and the differential cell count including red blood cell (RBC), white blood cell (WBC) and platelet (PLT) count were made by using an automated haematological analyzer (Sysmex K4500, Japan).

Serum Biochemical Analysis: The serum was separated from non-heparinized blood and the serum biochemical parameters including, glucose (GLU), total cholesterol (TC), triglycerides (TG), total protein (TP), total bilirubin (TBIL), creatinine (CRE) and tissue damage enzyme parameters such as serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT) and lactate dehydrogenase (LDH) were analysed using standard diagnostic kits (Crest Bio Systems, Coral Clinical Systems, Goa, India).

Histopathological Analysis: After blood collection, the animals were euthanized by carbon dioxide exposure followed by exsanguination and were necropsied in a randomized order. The external surface of the body and all organs/tissues in the thoracic and abdominal cavities were examined. The organs including liver, spleen, lung and kidneys were collected and preserved in 10% buffered formalin and a detailed histopathological examination of control and treated animals was performed.

Statistical Analysis: Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 20. Data are expressed as the mean ± S.D. and were analyzed using one-way analysis of variance (ANOVA). Significant differences between the control and treatment groups were determined by post- hoc Least Significant Difference (LSD) method for multiple comparisons and the values of $P < 0.05$ was considered to be statistically significant.

RESULTS

The values obtained from the non-solvent control and solvent control (petroleum ether, chloroform, acetone, methanol and ethanol) did not show any marked difference. Therefore the data obtained from solvent control was not compared with the experimental values.

Acute Dermal Toxicity of Ethanolic Extract of Rhizome, Tuber and the Rhizome Fractions: The dermal application of ethanolic extract of *K. rotunda* rhizome and tuber extracts formulated in white petroleum jelly (KRED and KRTD) with a highest concentration of 20% produced no toxic symptoms in male and female rats. No signs of erythema and oedema were observed in the skin of treated animals and the cage side observations also did not show any changes in the fur, eyes and skin in treated and control animals. Based on these findings, the acute dermal LD_{50} of KRED and KRTD in rats were estimated to be greater than a concentration of 20 % (w/w). In addition, the dermal applications of *K.rotunda* rhizome fractions formulated in white petroleum jelly (KRE1PD, KRE1CD, KRE1MD, KRAqD, KRAcD and KRAcCD) did not result in mortality or any clinical symptoms at 30 min, 4 hrs, 24 hrs, 48 and 72 hrs post treatment. Therefore the acute dermal LD_{50} of *K.rotunda* rhizome fractions was observed to be greater than a concentration of 20 % (w/w).

Acute Dermal Toxicity of Essential Oil Isolated from Rhizome and Tuber: Topical treatment with the oil formulation, KRROD and KRTOD at a concentration of 20 % (w/w), did not produce any burning sensation or rashes on the skin of experimental animals. Further no mortality was seen throughout the observation period either in the control or in any of the treated groups. Therefore the essential oil isolated from the rhizome and tuber of *K.rotunda* were observed to be safe up to a concentration of 20 % (w/w) topically and their acute dermal LD_{50} was found to be greater than 20 % (w/w).

Acute Oral Toxicity Studies of Rhizome, Tuber Extracts and Rhizome Fractions: No mortality or adverse symptoms were observed with the ethanolic extract of *K. rotunda* rhizome, tuber and vehicle treatment of animals in the 14-day study period, with a limit test dose of 2000 mg/kg p.o. The control and treated animals showed a normal behavioural pattern and no mortality was reported. Thus the LD_{50} values of the extracts, KRE and KRT were found to be greater than 2000 mg/kg per orally.

Further, no morbidity or mortality was recorded in the *K.rotunda* rhizome fractions (KRE1P, KRE1C, KRE1M, KRAq, KRAc and KRAcC) treated animals during the experimental period. The animals did not show any changes in general behaviour. Thus the LD_{50} of the rhizome fraction treated animals were found to be greater than 2000 mg/kg body weight p.o. In addition no statistically significant differences in body weight, food and water consumption were noted in any of the treated groups in comparison with the control animals (data not shown).

Repeated 28 - Day Oral Toxicity Study: There were no treatment-related toxicity signs or mortality observed in both sexes of rats treated with KRE, KRT and rhizome fractions at a dose of 250, 500 and 1000 mg/kg orally for a period of 28 days. Daily cage side observations also did not reveal any changes in eye, fur, skin, respiratory system and behavioural patterns and no toxicologically significant changes in body weight was observed among male and female rats (data not shown).

The hematological parameters, which included total red blood cells (RBC), total white blood cells (WBC), platelet count (PLT) and hemoglobin concentration (HGB) in rats of both sexes treated with KRE, KRT and rhizome fractions of *K.rotunda* (250, 500 and 1000 mg/kg) did not differ significantly (P □ 0.05) from those of control treated rats (Tables 1 and 2). All these parameters were found to be within normal limits throughout the experimental period. In addition, the changes observed in the haematological parameters of both the sexes were considered to be of no toxicological significance, instead these changes observed were considered to be sex related changes (P□0.05).

In the biochemical analysis, a statistically significant decrease in glucose concentration was observed at a dose of 1000 mg/kg in KRE treated female rats for 28 days (P< 0.05). The other parameters (TC, TG, TP, TBIL, CRE, AST, ALT, GGT and LDH) showed no significant changes during the treatment period (Tables 3-6).

The necropsy performed on 29th day of the experiment did not reveal any gross pathological abnormalities in the organs of the treated animals in comparison to the control groups. The histopathological analysis of the KRE, KRT and rhizome fraction-treated liver showed occasional mild fatty changes and no hepatic lesions were observed. Enhanced hematopoietic cells with occasional depletion of red pulp were observed in the treated and control spleen of animals. In addition, mild bronchial hyperplasia and peri-bronchial lymphoid accumulation were frequent in the lung tissue of the treated and control animals. Moreover, type-II pnumocyte hyperplasia, interstitial thickening and emphysema observed in the treated group were also common in the kidney of the treated and control animals. However, these changes observed in the treated group were common as that observed in the control samples. Therefore the changes observed can be deemed as background lesions (data not shown).

Table 1: Clinical hematological parameters after a 28-day repeated oral administration of *K. rotunda* L. rhizome, tuber and rhizome fractions in male rats.

Treatment	RBC (10^6/µl)	WBC (10^3/µl)	PLT (10^3/µl)	HGB (g/dl)
Control	7.66±0.51	13.83±1.47	839.16±64.37	14.83±1.83
Vehicle control	7.50±0.54	13.16±1.32	840.83±54.90	14.00±1.67
KRE (250 mg/kg)	7.50±0.54	12.66±1.03	799.16±52.38	13.16±1.32
KRE (500 mg/kg)	8.33±0.81	15.33±1.03	804.16±37.20	16.33±0.81
KRE (1000mg/kg)	7.83±0.75	14.16±1.16	866.66±50.56	15.66±1.36
KRT (250 mg/kg)	7.33±0.51	13.00±1.09	790.00±33.46	13.16±1.32
KRT (500 mg/kg)	8.00±0.63	14.50±0.83	805.83±24.98	15.16±1.16
KRT (1000 mg/kg)	7.83±0.75	14.16±0.75	820.83±48.00	14.83±0.98
KRE1P (250 mg/kg)	7.50±0.54	12.83±0.98	783.33±37.23	13.33±1.03
KRE1P (500 mg/kg)	7.83±0.75	14.00±1.7	800.83±29.73	14.66±1.03
KRE1P(1000 mg/kg)	7.66±0.81	13.66±0.81	810.00±42.54	13.66±1.63
KRE1C(250 mg/kg)	7.50±0.54	13.33±0.81	784.16±22.00	13.66±1.03
KRE1C(500 mg/kg)	8.16±0.75	15.50±0.83	798.33±25.62	16.16±0.98
KRE1C(1000 mg/kg)	7.83±0.75	15.16±0.98	832.50±47.93	15.66±1.36
KRE1M(250 mg/kg)	7.83±0.75	12.66±1.03	794.16±42.47	13.86±0.99
KRE1M(500 mg/kg)	8.16±0.75	15.16±0.98	805.00±16.43	16.50±0.54
KRE1M(1000mg/kg)	8.00±0.89	14.50±0.83	848.33±40.08	15.83±1.16
KRAq (250 mg/kg)	7.50±0.54	12.50±1.22	800.83±38.00	14.00±1.78
KRAq (500 mg/kg)	8.00±0.63	14.33±0.81	819.16±42.71	15.16±0.98
KRAq(1000 mg/kg)	7.50±0.54	13.16±0.98	850.00±48.68	13.33±1.21
KRAcC(250 mg/kg)	7.50±0.83	13.00±1.09	795.83±36.66	14.83±1.60
KRAcC(500 mg/kg)	8.33±0.51	15.50±0.83	826.66±47.92	16.33±0.81
KRAcC(1000 mg/kg)	7.83±0.75	14.83±0.98	854.16±48.00	15.50±1.04

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P□0.05).

Table 2: Clinical hematological parameters after a 28-day repeated oral administration of *K.rotunda* L. rhizome, tuber and rhizome fractions in female rats.

Treatment	RBC (10^6/µl)	WBC (10^3/µl)	PLT (10^3/µl)	HGB (g/dl)
Control	6.93±0.72	10.66±0.81	812.50±54.74	13.83±1.47
Vehicle control	6.94±0.83	9.66±1.03	778.33±54.09	13.00±0.89
KRE (250 mg/kg)	7.11±0.69	9.83±0.75	780.00±42.42	12.83±1.32
KRE (500mg/kg)	7.66±0.81	11.50±1.37	811.66±43.08	14.66±0.51
KRE (1000mg/kg)	7.53±1.07	10.50±1.04	827.50±52.22	13.83±0.98
KRT (250 mg/kg)	6.96±0.87	9.66±1.03	781.66±41.67	12.50±0.83
KRT (500 mg/kg)	7.31±0.79	10.33±1.36	801.66±41.19	14.16±1.16
KRT (1000 mg/kg)	7.06±0.49	9.83±0.75	825.00±49.69	13.83±1.47
KRE1P (250 mg/kg)	6.69±0.48	9.00±0.89	771.66±24.83	12.83±0.98
KRE1P (500 mg/kg)	7.33±0.51	11.66±1.03	799.16±19.08	14.00±1.09
KRE1P(1000 mg/kg)	6.92±0.65	9.83±1.47	810.00±33.46	13.16±0.98
KRE1C(250 mg/kg)	7.25±0.46	10.16±1.47	785.00±13.41	12.50±1.22
KRE1C(500 mg/kg)	7.72±0.44	12.16±1.16	798.33±40.20	14.66±0.51
KRE1C(1000 mg/kg)	7.50±0.54	11.16±0.75	846.66±34.88	14.33±0.51
KRE1M(250 mg/kg)	7.07±0.81	9.16±1.16	765.00±32.55	12.66±1.21
KRE1M(500 mg/kg)	7.34±0.63	11.66±1.21	810.00±28.10	14.83±0.40
KRE1M(1000 mg/kg)	7.14±0.96	9.83±2.04	850.00±41.10	13.66±1.03
KRAq (250 mg/kg)	6.85±0.55	9.16±1.32	770.83±18.28	12.83±1.32
KRAq (500 mg/kg)	7.47±0.40	10.50±1.76	806.66±32.19	14.16±0.75
KRAq(1000 mg/kg)	7.08±0.66	9.66±1.03	830.00±35.21	14.00±0.63
KRAcC(250 mg/kg)	7.08±0.37	9.50±0.83	799.16±27.46	12.92±1.44
KRAcC(500 mg/kg)	7.71±0.67	11.33±1.21	826.66±35.87	14.75±0.61
KRAcC(1000 mg/kg)	7.65±0.68	11.00±0.89	846.66±45.12	14.02±1.12

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P□0.05).

Table 3: Clinical biochemistry parameters after a 28-day repeated oral administration of *K.rotunda* L. rhizome, tuber and rhizome fractions in male rats.

Treatment	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	γ-GT (IU/l)	LDH (IU/l)
Control	104.16±11.14	31.33±3.50	143.33±12.90	5.17±0.98	178.00±10.95
Vehicle control	104.83±13.15	30.33±2.65	147.50±18.90	5.17±0.75	185.00±5.47
KRE (250 mg/kg)	104.00±8.36	31.33±1.63	133.83±2.31	5.50±0.54	180.00±8.36
KRE (500mg/kg)	112.33±6.59	32.50±2.81	145.00±11.40	5.83±0.40	183.33±7.52
KRE (1000mg/kg)	100.16±7.62	28.33±1.96	129.16±6.64	4.67±0.51	170.00±6.32
KRT (250 mg/kg)	103.33±6.83	30.33±1.96	142.66±9.62	5.83±0.40	183.33±7.52
KRT (500 mg/kg)	111.66±6.83	33.00±2.75	150.83±13.91	5.67±0.51	186.66±4.08
KRT(1000 mg/kg)	106.33±5.88	28.00±2.19	141.66±11.69	5.33±0.51	180.83±7.35
KRE1P(250 mg/kg)	106.00±6.78	32.00±1.78	150.83±12.00	5.67±0.51	185.00±5.51
KRE1P(500 mg/kg)	112.50±2.73	34.33±1.50	155.83±15.62	5.83±0.40	187.50±2.81
KRE1P(1000 mg/kg)	106.00±5.09	30.50±2.94	140.83±8.01	5.00±0.63	182.50±6.25
KRE1C(250 mg/kg)	106.00±7.48	29.33±2.73	156.66±8.75	5.67±0.51	180.00±9.57
KRE1C(500 mg/kg)	111.50±6.34	31.50±1.64	140.00±8.36	5.83±0.40	184.16±6.76
KRE1C(1000 mg/kg)	94.16±9.17	28.66±2.42	150.83±13.57	5.00±0.89	175.00±7.07
KRE1M(250 mg/kg)	106.83±6.17	31.16±1.83	145.16±7.35	5.50±0.54	182.16±10.10
KRE1M(500 mg/kg)	107.50±10.36	34.50±3.61	150.83±10.68	5.83±0.40	186.66±6.18
KRE1M(1000 mg/kg)	102.66±7.78	30.33±2.33	139.16±8.01	5.00±0.89	180.66±7.25
KRAq (250 mg/kg)	105.16±6.49	31.33±2.42	139.16±9.17	5.50±0.54	183.00±6.32
KRAq (500 mg/kg)	108.33±6.83	34.00±2.19	149.16±12.81	5.83±0.40	185.00±6.32
KRAq(1000 mg/kg)	102.50±7.58	30.33±2.94	140.33±8.52	4.83±0.75	179.16±7.35
KRAcC(250 mg/kg)	103.16±9.60	30.00±2.19	144.16±9.70	5.67±0.51	180.00±11.40
KRAcC(500 mg/kg)	108.33±6.83	33.33±2.33	141.66±10.32	5.67±0.51	183.50±12.70
KRAcC(1000 mg/kg)	96.66±9.83	29.66±1.50	138.33±6.83	4.50±0.54	173.33±9.30

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P□ 0.05).

Table 4: Clinical biochemistry parameters after a 28-day repeated oral administration of *K. rotunda* L. rhizome, tuber and rhizome fractions in female rats.

Treatment	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	γ-GT (IU/l)	LDH (IU/l)
Control	140.83±9.70	37.00±2.36	85.66±14.40	4.86±0.73	159.16±17.15
Vehicle control	133.33±6.05	37.00±3.52	81.66±8.77	4.83±0.40	158.33±12.11
KRE (250 mg/kg)	134.16±7.35	36.33±2.33	80.16±9.06	4.97±0.58	153.33±14.02
KRE (500mg/kg)	145.00±7.74	38.00±3.09	90.66±6.12	5.10±0.20	159.16±8.01
KRE (1000mg/kg)	131.66±6.05	34.66±3.01	76.66±9.30	4.25±0.41	141.66±11.69
KRT (250 mg/kg)	135.83±8.01	36.00±2.82	81.66±6.83	5.11±0.68	147.50±12.94
KRT (500 mg/kg)	146.66±4.08	37.50±2.81	89.66±5.00	5.26±0.75	157.50±10.83
KRT(1000 mg/kg)	135.83±8.01	35.66±1.36	79.16±8.61	4.81±0.40	152.83±20.98
KRE1P(250 mg/kg)	140.00±7.07	34.83±3.92	86.66±7.52	5.05±0.14	169.00±10.58
KRE1P(500 mg/kg)	145.83±4.91	37.33±2.42	90.50±2.34	5.02±0.17	165.66±13.06
KRE1P(1000 mg/kg)	134.16±8.61	34.66±2.06	82.50±8.80	4.96±0.57	155.83±14.28
KRE1C(250 mg/kg)	135.83±7.35	36.66±2.73	79.16±8.01	4.61±0.65	149.16±13.57
KRE1C(500 mg/kg)	142.50±8.21	38.00±2.52	88.33±5.16	5.11±0.58	152.50±11.72
KRE1C(1000 mg/kg)	131.66±5.16	34.66±1.63	76.50±10.55	4.23±0.57	147.50±18.90
KRE1M(250 mg/kg)	136.66±11.25	37.00±2.09	86.50±7.63	4.80±0.69	146.16±17.02
KRE1M(500 mg/kg)	147.50±6.89	38.33±1.96	91.00±5.09	5.10±0.20	157.66±10.61
KRE1M(1000 mg/kg)	134.16±9.70	34.16±1.32	81.66±7.55	4.40±0.49	146.66±9.83
KRAq (250 mg/kg)	139.16±7.35	34.83±0.75	83.50±5.78	4.83±0.68	150.00±17.02
KRAq (500 mg/kg)	147.50±5.24	37.00±3.52	85.66±7.11	5.10±0.20	157.50±13.32
KRAq(1000 mg/kg)	136.66±6.05	34.33±1.96	81.50±7.89	4.90±0.46	147.50±14.40
KRAcC(250 mg/kg)	135.83±4.91	35.66±2.33	77.50±8.47	4.91±0.47	149.16±6.64
KRAcC(500 mg/kg)	146.66±4.08	36.33±2.25	84.83±6.08	4.75±0.44	154.16±6.64
KRAcC(1000 mg/kg)	132.50±6.12	34.33±0.81	75.66±4.96	4.25±0.38	146.66±14.02

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P ? 0.05).

Table 5: Clinical biochemistry parameters after a 28-day repeated oral administration of *K. rotunda* L. rhizome, tuber and rhizome fractions in male rats.

Treatment	GLU (mg/dl)	TC (mg/dl)	TG (mg/dl)	TP (g/dl)	BIL (mg/dl)	CRE (mg/dl)
Control	113.66±16.69	65.00±6.32	54.50±7.17	5.76±0.75	0.64±0.11	0.59±0.06
Vehicle control	108.33±17.18	62.50±4.18	56.83±6.55	5.78±0.63	0.67±0.09	0.57±0.01
KRE (250 mg/kg)	103.33±6.05	64.16±2.04	51.83±6.52	6.25±0.75	0.62±0.11	0.57±0.03
KRE (500mg/kg)	120.00±4.47	66.66±2.58	56.16±5.30	6.20±0.67	0.72±0.08	0.62±0.07
KRE (1000mg/kg)	100.83±2.04	61.16±2.04	48.50±1.97	6.05±0.76	0.56±0.08	0.55±0.03
KRT (250 mg/kg)	105.83±10.68	66.66±2.58	50.00±5.47	6.16±0.98	0.64±0.13	0.57±0.02
KRT (500 mg/kg)	121.66±12.11	68.33±2.58	58.33±6.83	6.45±0.38	0.74±0.08	0.58±0.04
KRT(1000 mg/kg)	104.16±7.35	65.00±3.16	53.33±4.08	6.11±0.24	0.59±0.11	0.54±0.03
KRE1P(250 mg/kg)	106.66±9.30	65.83±3.76	54.16±7.35	5.96±0.15	0.66±0.12	0.58±0.05
KRE1P (500 mg/kg)	122.50±10.36	66.66±2.58	51.83±4.91	6.36±0.49	0.71±0.09	0.60±0.04
KRE1P(1000 mg/kg)	102.50±8.21	63.33±4.08	60.16±8.49	5.88±0.58	0.68±0.11	0.56±0.03
KRE1C (250 mg/kg)	105.83±8.61	65.00±3.16	50.33±3.26	6.11±0.58	0.60±0.08	0.54±0.03
KRE1C (500 mg/kg)	117.50±13.69	66.66±2.58	55.33±7.36	6.43±0.30	0.70±0.08	0.60±0.04
KRE1C(1000 mg/kg)	101.66±5.16	61.66±2.58	48.83±2.04	5.91±0.80	0.57±0.10	0.54±0.03
KRE1M (250 mg/kg)	106.66±6.05	65.83±3.76	49.16±5.84	6.13±0.52	0.59±0.07	0.55±0.03
KRE1M(500 mg/kg)	119.16±11.58	68.33±2.58	57.50±6.20	6.35±0.57	0.72±0.09	0.56±0.03
KRE1M(1000 mg/kg)	102.50±5.24	61.66±2.58	51.66±6.05	5.79±0.45	0.56±0.08	0.54±0.02
KRAq (250 mg/kg)	108.83±8.01	67.50±2.73	55.33±4.96	5.91±0.79	0.64±0.10	0.55±0.04
KRAq (500 mg/kg)	123.33±7.52	68.33±2.58	59.16±7.35	6.58±0.47	0.74±0.10	0.56±0.05
KRAq(1000 mg/kg)	108.33±8.16	67.50±2.73	51.66±4.08	5.85±0.69	0.60±0.06	0.54±0.03
KRAcC(250 mg/kg)	110.83±7.35	65.83±3.76	49.00±4.00	6.20±0.40	0.59±0.12	0.55±0.02
KRAcC(500 mg/kg)	125.83±7.35	67.50±2.73	53.16±4.02	6.46±0.44	0.65±0.10	0.58±0.03
KRAcC(1000 mg/kg)	104.16±4.91	60.83±2.04	48.00±2.44	6.00±0.89	0.54±0.06	0.54±0.04

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P□0.05).

Table 6: Clinical biochemistry parameters after a 28-day repeated oral administration of *K.rotunda* L. rhizome, tuber and rhizome fractions in female rats.

Treatment	GLU (mg/dl)	TC (mg/dl)	TG (mg/dl)	TP (g/dl)	BIL (mg/dl)	CRE (mg/dl)
Control	131.66±22.28	95.83±17.15	43.50±4.18	7.24±0.88	0.91±0.09	0.68±0.06
Vehicle control	128.33±24.83	99.16±8.01	40.33±7.65	7.11±0.80	0.90±0.08	0.64±0.08
KRE(250 mg/kg)	116.66±17.22	90.00±7.07	39.16±3.76	7.13±0.83	0.86±0.14	0.61±0.10
KRE (500mg/kg)	133.33±8.16	95.83±6.64	41.66±6.05	7.56±0.47	0.90±0.10	0.64±0.07
KRE(1000mg/kg)	101.33±7.73*	85.83±4.91	38.83±4.91	6.60±0.60	0.79±0.06	0.60±0.09
KRT(250 mg/kg)	121.66±12.11	100.83±9.17	43.33±6.94	7.01±0.82	0.87±0.11	0.63±0.09
KRT(500 mg/kg)	145.00±7.74	105.00±4.47	46.33±3.82	7.44±0.70	0.88±0.09	0.70±0.03
KRT(1000 mg/kg)	119.16±15.94	94.16±4.91	40.08±4.36	6.89±0.68	0.86±0.12	0.64±0.05
KRE1P(250 mg/kg)	121.66±17.51	92.50±6.12	39.33±2.06	7.00±0.70	0.88±0.10	0.65±0.07
KRE1P (500 mg/kg)	139.16±10.68	99.16±4.91	46.16±5.49	7.55±0.64	0.93±0.08	0.68±0.06
KRE1P(1000 mg/kg)	114.16±8.61	91.66±9.83	41.00±7.37	6.76±0.63	0.85±0.10	0.63±0.10
KRE1C(250 mg/kg)	127.50±16.35	90.83±6.64	40.00±5.47	7.08±0.24	0.85±0.09	0.61±0.09
KRE1C(500 mg/kg)	140.00±8.94	99.16±6.64	39.83±8.63	7.33±0.38	0.89±0.10	0.62±0.08
KRE1C(1000 mg/kg)	112.50±12.94	88.50±6.74	39.66±3.26	6.87±0.67	0.82±0.14	0.59±0.08
KRE1M(250 mg/kg)	120.83±12.41	98.33±8.16	38.33±6.05	7.16±0.54	0.90±0.08	0.63±0.07
KRE1M(500 mg/kg)	141.66±9.30	100.83±9.17	42.50±4.18	7.49±0.61	0.93±0.08	0.64±0.07
KRE1M(1000 mg/kg)	115.00±14.83	87.50±7.58	38.33±4.08	7.16±0.51	0.85±0.09	0.59±0.05
KRAq (250 mg/kg)	125.00±17.88	90.00±8.36	42.16±4.70	7.48±0.76	0.90±0.09	0.65±0.09
KRAq (500 mg/kg)	146.66±6.05	102.50±4.18	45.33±5.27	7.59±0.45	0.94±0.06	0.65±0.08
KRAq(1000 mg/kg)	112.50±9.35	88.33±9.30	41.66±4.08	7.06±0.77	0.85±0.10	0.61±0.10
KRAcC (250 mg/kg)	123.33±14.02	90.00±12.64	38.33±5.16	7.25±0.75	0.88±0.11	0.59±0.09
KRAcC (500 mg/kg)	138.33±10.32	93.33±11.69	43.33±6.05	7.67±0.38	0.91±0.07	0.60±0.10
KRAcC(1000 mg/kg)	119.16±16.85	85.00±6.32	37.50±6.12	6.80±0.40	0.82±0.10	0.59±0.10

The values of different parameters in each group were expressed as mean ± standard deviation; n=12(6/sex). The results of treated groups were compared with those of the normal control group. Differences were considered as significant by performing one way ANOVA; LSD post hoc multiple comparison test at a 95% confidence level. '*' indicates significantly different from the control (P □ 0.05).

The data obtained from the post trial 14-days no treatment observation period (recovery study) reveals that, the control and the treated animals of both sexes at 1000 mg/ kg of the recovery group did not show any toxicologically significant changes with respect to biochemical, hematological and histopathological parameters (data not shown). The data obtained from the recovery study also suggest a complete recovery after stoppage of the test drug administration.

DISCUSSION

The aim of the present study was to assess and compare the individual adverse effects of rhizome, tuber and rhizome fractions of *K.rotunda* in Wistar albino rats by different toxicity models. No *in vivo* toxicity studies on the tuberous rhizome of *K. rotunda* have been reported so far, therefore it is necessary to carry out the toxicity and biokinetics of the tuberous rhizome of *K.rotunda*.

The toxic changes in the skin are of significant importance to organisms because the skin is one of the largest organs of the body and possess functions vital to survival. Cutaneous exposure of humans or animals to xenobiotic substances may result in a toxicological reaction or dermatosis. This necessitates the evaluation of initial acute toxicity screening in animals followed by OECD guideline 402 [16] with limit test dose method for the safety dictated by the potential for and conditions of human exposure to the toxin.

The results obtained in the acute dermal toxicity studies indicate that single application of *K.rotunda* rhizome (KRED) and tuber ointment (KRTD) did not induce any skin responses indicative of dermal irritation in Wistar albino rats. Likewise, the single dermal application of formulated essential oil, KRRO and KRTO isolated from *K. rotunda* rhizome and tuber respectively did not produce any corrosive or ulcerative effects on the skin after the scheduled time period. Similar results have been observed in treatment with the *K.rotunda* rhizome fractions formulated ointment, KRE1PD, KRE1CD, KRE1MD, KRAcD, KRAcCD and KRAqD. This suggests the safe application of the extracts and essential oil isolated from the rhizome and tuber and the rhizome fractions of *K.rotunda* in the treatment of skin ailments in both humans and laboratory animals.

The results obtained in the acute oral toxicity studies reveals that the oral administration of the ethanolic extract of the rhizome, tuber and rhizome fractions of *K. rotunda* did not cause any behavioural and toxicological effects during the 14-days study period with a limited test dose of 2000 mg/kg. As no mortality is reported in any of the treated groups, it is safe to state that the oral LD_{50} value of the extracts KRE, KRT and the rhizome fractions of *K.rotunda* exceeds 2000 mg/kg.

Although an animal may survive the acute toxicity action of a xenobiotic or a chemical, some irreversible damage to normal homeostatic mechanisms may have occurred. Such non-lethal adverse effects can be studied via repeated dose 28-days experiment. Thus, unlike acute toxicity studies, the major end-point in a repeated-dose study is not mortality but some non-lethal parameter which can be defined by functional, biochemical and pathological effects [19].

Since haematological and biochemical profiles of blood can provide important information about the internal environment of the organism, the evaluation of haematological and biochemical characteristics in treated animals has become an important means of understanding normal, pathological and toxicological impacts[20]. Moreover, the haematopoietic system is known to be one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals [21]. The hematological evaluations conducted in the present study demonstrates that neither those of male nor female treated rats appeared to be toxicologically significant, being slightly higher or lower than those of the control group, indicating that the extract was neither toxic to circulating erythrocytes, leukocytes and platelets nor interfered with their production. Hence no significant alterations in the haematological parameters of the treated rats were observed.

As the liver and kidneys are frequently the target organs in toxicity studies, many serum enzymes have been used as markers of cellular and organ damage [22]. Generally, the enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been tested as markers of hepatotoxicity. The enzymes such as AST and LDH have a wide tissue distribution, hence they are not only specific for liver injury but also they are used as marker enzymes for cardiac and skeletal muscle injury. In addition to LDH, the amino transferases, AST and ALT are also used as serum marker enzymes for detecting myocardial damage and congestive cardiac failure. The enzymes, alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) are widely used as a marker for liver injury, particularly for cholestasis [23]. Total serum bilirubin concentration is a useful measure of liver dysfunction and the total serum bilirubin rises significantly with the increased exposure of hepatotoxins

and may results in extra hepatic obstruction and haemolytic jaundice [24-26]. Furthermore, an altered serum protein level has been reported to be an indicator of hepatocellular damage, with reduced hepatic synthesis of albumin, fibrinogen, á$_1$-antitrypsin, hepatoglobin, transferrin, caeruloplasmin and other proteins [22].

In the present study, there were no statistically significant differences in the serum amino transferases (AST and ALT) level observed between the control and treated animals at any of the treated doses. Hence, it can be stated that either the extracts (KRE and KRT respectively) or the rhizome fraction of *K.rotunda* does not cause hepatic toxicity. This was further confirmed by histopathological examination of the liver of treated and control rats, showing normal lesions. In addition, the serum marker enzyme levels of ALP, γ-GT and LDH of the treatment groups did not differ significantly in comparison with the control animals and the changes that appeared in both sexes were toxicologically irrelevant and comparable to normal control values.

As the kidney is a major site of organ damage caused by drug toxicity [27], it is necessary to prevent the use of such new nephrotoxic drugs, to best manage nephrotoxicity. When injury occurs in the kidney, the serum creatinine level exceeds the normal range and the elevated blood creatinine is a reliable indicator of a negative impact on kidney function or impaired glomerular flitration [28]. In the present study the serum creatinine level of the treatment groups did not differ in comparison with the control animals, showing the no-toxic effect of the extracts (KRE and KRT) and rhizome fraction of *K.rotunda* on kidneys. This was further confirmed by histopathological examination of the kidneys from treated and control groups, showing normal architecture.

The biochemical parameters like TC, TG, TP and TBIL of the treated animals did not show any difference when compared with the control groups. However, a significant decrease in the serum glucose level was observed in the female rats treated with 1000 mg/kg of KRE extracts than in the control animals. These changes were regarded as toxicologically irrelevant because these adverse results did not appear in both sexes. Moreover, the biochemical data of all the treated groups were consistent with histological evaluation of the liver, spleen and kidneys which did not reveal any significant changes due to administration of the extracts (KRE and KRT) and rhizome fraction of *K. rotunda* for a period of 28-days.

As reported previously, a lectin (KRL), purified from the tuberous rhizome of *K.rotunda* was found to possess an *in vivo* antiproliferative activity against Ehrlich ascites carcinoma (EAC) cells in mice and the study also demonstrated the antibacterial effect of the isolated lectin [29]. In addition to this an *in vivo* antimutagenic activity of the methanolic extract and the three known flavanones, namely 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone and 5, 7-dihydroxyflavanone isolated from the rhizome of *K. rotunda* were reported against cyclophosphamide induced mutagenesis by bone marrow micronucleus test in micronucleated polychromatic cell erythrocytes (MNPCE) from male Balb-c mice [30]. The study also demonstrates the ability of *K.rotunda* and isolated flavanone compounds to protect DNA and thereby prevent the chromosome fragmentation *in vivo*. These findings suggest the anticarcinogenic and antigenotoxic effect of the tuberous rhizome of *K.rounda*. As reported by Jantan [31], the crude methanolic extract and a compound isolated from the chloroform fraction of the rhizome of *K.rotunda* prevent increase in number and aggregation of platelets in human blood *in vitro* and thereby suggest its effect in protection against stroke and myocardial infarction. An experimental study has also shown that the methanolic extract of the rhizome of *K.rotunda* possess anti-hyperglycemic activity via *in vivo* glucose tolerance test in mice[32]. All these studies conducted in the animals support the non-toxic effect of the extracts (KRE, KRT), rhizome fraction and bioactive compounds of *K.rotunda*.

The present study thus reveals that the treatment with the extracts, KRE, KRT and rhizome fractions of *K.rotunda* in the animals up to 1000 mg/kg/day p.o. does not bring any toxic effects with respect to biochemical, hematological and histopathological parameters tested during the 28-days repeated dose administration and post trial 14- days no treatment observation period Based on these overall findings, the present study suggests that the no-observed-adverse-effect level (NOAEL) of the ethanolic extract of the rhizome, tuber and the rhizome fraction of *K.rotunda* exceeds 1000 mg/kg b.wt./day, p.o for both male and female rats.

CONCLUSION

The present study confirms the relatively safe use of the extracts of rhizome, tuber and rhizome fraction of *K.rotunda* when tested in rats through various routes of drug administration. The acute dermal toxicity study conducted with the ethanolic extract of the rhizome tuber, rhizome fractions and the essential oil isolated from the rhizome and tuber of *K. rotunda* did not show any dermal toxic effect when tested on the surface of the skin

topically. In addition, treatment with single oral dose of 2000mg/kg did not result in any observable adverse effect or mortality in the acute toxicity studies conducted in the animals. The daily oral administration of the extracts and rhizome fraction at doses, 250, 500 and 1000 mg/kg for a period of 28-days did not cause mortality, changes in body weight and body weight gain. Further no significant haematological, biochemical and histopathological alterations were observed at the end of the treatment period. Hence, the no-observed adverse-effect level of the extracts and rhizome fraction of *K.rotunda* in male and female rats were found to exceed 1000 mg/ kg/ day p.o. Based on the overall findings of the repeated dose toxicity evaluation, it can be concluded that the ethanolic extracts of the rhizome, tuber and rhizome fractions of *K.rotunda* was well tolerated in daily dose at 1000 mg/kg of body weight for a period of 28 days. Further investigations including larger doses and longer periods of treatment should be conducted before it is prescribed as a drug.

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