

Sub-Chronic Oral Toxicity Study of Kushta Qalai (A Unique Herbo-Mineral Unani Formulation) in Wistar Rats

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Abstract

Objective: Kushta Qalai, a Herbo-mineral effective formulation of Unani medicine used for the treatment of male sex disorders has as yet not been evaluated for pre-clinical toxicity study. The objective of the study was to investigate the Sub-Chronic oral toxicity study of Kushta Qalai Drug as well as Crude material (the un-detoxified form) in young, healthy Albino rats (Wistar Strain) of both the sexes in order to know their adverse effects, if any, and also to check the scientific validity of detoxification method.

Methods: The study was conducted as per OECD-408 Guideline, during which the test substance was administered orally to the rats at the limit dose of 1000 mg/kg, and its sub fractions 500 and 250 mg/kg body wt., daily for 90 days. Overnight fasted rats were sacrificed on the 91st day and blood sample for Haematological and Biochemical parameters were collected. At the end, the tissues were collected and subjected to histopathological examination by a veterinary pathologist.

Results: The results showed that, there were no statistically significant changes in the general behaviour, body weight gain, feed & water consumption, haematological parameters and biochemical parameters between test substance treated rats and control groups. Also there were no gross histopathological changes in vital organs of rats except the test substance treated rats showed degeneration of liver and kidney tissues, which were confirmed by the histopathological examination.

Conclusion: The drug Kushta Qalai showed impairment at 1000 mg/kg body weight while as the Crude material

showed at all the three dose levels, indicating that the method of detoxification used in preparing the Kushta reduces the elements of toxicity.

Keywords: Sub-chronic toxicity; Qalai; Biochemical parameters; Haematological parameters; Histopathology

Introduction

The word Kushta owes its origin to a Persian word “Kushtan” which means to kill or conquered [1]. Kushta is the finest powder form of the Unani medicinal preparations obtained by the calcinations of metal, mineral and animal drugs.

These drugs, by special process are calcined in closed crucibles and in pits of different sizes, having varying number of cowdung cakes and with different intensity of heat. According to the traditional healers these calcination techniques used in Kushta preparation are specialized processes wherein herbal juices are incorporated during preparation of ash [2].

It is claimed by the Traditional healers that these techniques increases the efficacy of drug and also either completely remove the elements of toxicity at all or downgrade them to the level where the drug can be safely used [3-5]. Kushta (Calcined product) is easily absorbed in the human body which accounts for its high efficacy [6].

The preparation of Kushta results in higher efficacy of the medicine and after its entry into the body, it discharges its curative role promptly and effectively. The composition of Kushta Qalai as mentioned in the National Formulary of Unani Medicine, 2008 is shown in Table 1.

Table 1: Constituents of Crude Kushta Qalai.

Ingredients	English / Scientific name	Quantity
Qalai	Stannum (TIN)	250 g
Phitkari	Aloe / Aloe vera	125 g
Aab-e-Gheekwar	Alum/ potassium alum	50 ml

Special Method of Preparation

Before making Kushta Qalai, Qalai and Phitkari are cleaned and purified. After this the drugs are ground in a pestle and mortar (Kharal) with Aab-e-Gheekwar for a specified period of time. Thereafter, small cakes of varying sizes and thickness are made. These cakes are well dried in the shade and are put in earthen discs and the process of Gil-e-Hikamat is followed and the whole apparatus is dried. After this a pit is dug in an open space. Half the pit is filled with cowdung cakes. The apparatus (sealed earthen discs) is now placed in the pit and the remaining space is filled with more cowdung cakes which are then ignited. After the calcinations is over, the pit is allowed to cool completely, the apparatus is removed and the contents, thus obtained, are again powdered with Aab-e-Gheekwar as many times till the proper fineness and the quality of Kushta Qalai is obtained [6]. In Unani system of medicine Kushta Qalai is used for the treatment of diseases like spermatorrhoea, excessive nocturnal emission, premature ejaculation, impotency, increases sex vigour and density of semen [7-9]. As per Unani medicine classical books the drug Kushta Qalai is very much efficacious, used for the treatment of male sexual disorders [10-12]. The ingredients of Kushta Qalai are Qalai, Aloevera and Phatkari. The Qalai (Tin) is one of the most popular metal known for its use since Vedic period [13]. Qalai is silver like metal, which is soft than the gold but harder than the lead, malleable and sparingly ductile with little elasticity [14]. It is used internally in the form of Kushta. Qalai is known by diverse vernacular names like rasas in Arabic, urziz in Persian, rang in Hindi, vanga in Sanskrit and Qalai in Gujarati. There are two varieties of QalaiKhuraka and mishraka only the Khuraka being acceptable for therapeutic applications [15]. The Qalai tin was grinded with heavy duty wiley mill to make the fine powder which was used. Aloevera (Aloe bardadensis) is an important component of Vrihani Gutika which is considered as the drug of highest potency useful in the treatment of sexual dysfunction [16]. Phatkari (Alum) is an important mineral origin drug of Unani medicine [17]. Since there is limited scientific data available about the difference between the calcinated material and Crude material to determine the effectiveness of detoxification method adopted while preparation of Kushta's. In this study, an effort has been made to scientifically validate these methods and also try to intensify the adverse effects of the Crude materials used in preparation of Kushta's.

Material and Methods

Test item (Drug and Crude material)

The drugs and Crude ingredients of Kushta Qalai were procured from the registered Unani drug dealer M/s. Ellahi

Dawakhana, Unani Ayurvedic Medicines, Habak, Naseem Bagh. The manufacturer of the drug of Kushta Qalai is Hamdard Laboratory India (P Certified) Mg. Lic No U-212/78. All the Crude material was identified by Dr. M.A.Wajid (Unani Pharmacologist), Ex. Deputy Director, RRIUM, Srinagar. The drug and crude material powder was mixed with RO water to make the suspension for oral administration.

Experimental Animals and Maintenance

Young adult Albino Wistar rats of either sex were procured from IIM, Jammu. These rats were monitored closely during the quarantine and acclimatization period [18]. A detailed veterinary examination was conducted on the rats prior to and at the end of the acclimatization and quarantine period of 14 days. The five rats of same sex were housed in a polypropylene cage under standard environmental conditions i.e. temperature of 20 – 250 C with 12 hour light/dark cycle and had a free access to pelleted feed procured from Pronow Agro Industries, New Delhi and RO water ad libitum. The use of animals in the study was approved by the Institutional Animal Ethics Committee (IAEC) of Regional Research Institute of Unani Medicine, Srinagar which is registered with the Committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA, India) with registration No. 927/GO/C/06/C PCSEA.

Experimental Design

The doses were selected according to Organization of Economic Co-operation and Development guideline (OECD guideline- 408) [19]. One highest dose of 1000 mg/kg body weight was selected with the aim of inducing some toxic effects but not death or severe suffering and also two sub fractions i.e. 500 mg and 250 mg were selected with the aim to check any dose related response at the lowest dose level. The rats were randomly divided into 14 groups as shown in table 2 (7 male and 7 female groups) each group consisted of 5 rats. All the animals were closely observed for the first 1 to 4 hours after dosing to examine any adverse toxic signs, behavioural changes etc. The body weight of the rats was evaluated weekly. Feed and water consumption/rat/24 hours were recorded before dosing and then weekly upto 13 weeks. On the 91st day, after overnight fasting, all the animals were sacrificed by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under ISOFLURANE anaesthesia. 2 ml of blood was collected in EDTA vacutainer for haematological studies and 3 ml in red top vacutainer for serum biochemistry. All the animals were dissected to check macroscopic examination of the body organs. The vital organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/testes and heart were collected to determine the relative organ weight followed by grossing for the collection of tissues to evaluate Histopathological studies.

Dosage of Kushta Qalai in Humans: 125 mg a day orally with water [6]. This amounts to about 2 mg/kg/day in human subjects. Table 3 shows the corresponding dose level in rats. Curry et al., 2011 has previously calculated interspecies dose

conversion from rats weighing 100 gm body weight to humans with 60 kg body weight. The equivalent surface area dosage conversion factor from rat to humans was 1/720.

Table 2: Description of rat groups.

Group No.	Male/Female	Description	Dose for 90 days (Daily in the morning)
Group I	Male	Control	RO water (Vehicle only)
Group II		Drug Treated	1000 mg/kg body wt.
Group III		Drug Treated	500 mg/kg body wt.
Group IV		Drug Treated	250 mg/kg body wt.
Group V		Crude Treated	1000 mg/kg body wt.
Group VI		Crude Treated	500 mg/kg body wt.
Group VII		Crude Treated	250 mg/kg body wt.
Group VIII	Female	Control	RO water (Vehicle only)
Group IX		Drug Treated	1000 mg/kg body wt.
Group X		Drug Treated	500 mg/kg body wt.
Group XI		Drug Treated	250 mg/kg body wt.
Group XII		Crude Treated	1000 mg/kg body wt.
Group XIII		Crude Treated	500 mg/kg body wt.
Group XIV		Crude Treated	250 mg/kg body wt.

Table 3: Corresponding dose level in rats.

Particulars of Study	Sub-Chronic toxicity study
Dose level	1000, 500 and 250 mg/kg body weight (Corresponding to 70X, 35X and 17.5X the extrapolated dose in rats)
Dosing Schedule	Repeated daily dosing by oral route
Period of Observation	90 Days
Observation Parameters	See Text

Biochemistry parameters

Biochemical parameters were studied in serum obtained after centrifugation of blood at 2000 RPM for 15 minutes on the same day of the rat sacrifice. Biochemical parameters were determined on fully automatic biochemistry analyzer (XL- 640 TRANSASIA) using ERBA kits.

Liver function tests- aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase ALP, Total bilirubin, total protein, and albumin, and kidney function tests- blood urea, serum uric acid and serum Creatinine were done. In addition to this other Metabolic function tests such as blood glucose, serum Cholesterol and serum Triglyceride were estimated.

Haematological parameters

Haematological parameters were analyzed in freshly collected blood in blue top vacutainer containing EDTA anticoagulant. The

blood was gently mixed with the EDTA anticoagulant coated on the tube walls. Haematological parameters were determined on fully automatic haematological analyzer (Sysmex XT- 2000iV Sysmex Corporation Japan) having animal version software.

Haematological parameters such as Haemoglobin Conc. WBC count, RBC count, haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin, Platelet Count, differential leukocyte count – Neutrophil %, Lymphocyte %, Monocyte %, Eosinophil % and basophil %, and Reticulocyte count were studied.

Histopathology

Tissue samples were collected from the organs of control as well as treated male/female rats. The tissue collected from the organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/testes and heart were numbered for identification and then transferred to tissue cassettes (SS) to

enable fixation in 10 % formalin for 3 to 5 days followed by the tissue processing which was carried on Automatic tissue processor Model No. 1020 (LIECA make Germany).

The tissue processing included dehydration in graded isopropyl alcohol, clearing in xylene I & II, impregnation in paraffin wax and finally tissue blocks was prepared on paraffin block maker Model No. 1150 H+C (LIECA make Germany).

Section cutting of tissue blocks was done using microtome (YARCO) to the thickness of 3 – 5 microns. The tissue sections were fixed on the slide by heat technique following by the staining, Haematoxylin and Eosin stain were used.

The staining was done with Automatic slide stainer (THERMO MAKE Germany). After staining the tissue section were covered with cover slip using DPX mountant to prevent any damaging of tissue section which was done on Auto cover slipper Model No. CV5030 (LIECA make Germany).

The stained tissue sections were examined under microscope 10x and 40x objective to check the adverse effects of drug on cell morphology as well as on the cell organelles.

Statistical analysis

All results are expressed as mean $\pm$ standard deviation. Comparison of all results on body weight, feed & water consumption, haematological value, biochemical values, were performed using one way analysis of variance (ANOVA) method using statistical software Graph Pad Prism version 6.07. Probability of 0.05 or small ($p \leq 0.05$) was used as the criterion of significance.

Results

Average mean body weight

The rats treated with Kushta Qalai drug and crude material at the dose of 1000, 500 and 250 mg/kg of body weight were found to grow and gain body weight normally and there was no any adverse effect found in the body weight gains. Figure 1a, 1b, 1c and 1d shows the body weight of rats in sub-chronic oral toxicity study.

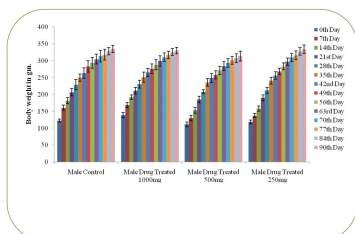


Figure 1a : Average body weight gain of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. n=5 in each group. * $p < 0.05$ as compared with the controls at the same time (one-way ANOVA).

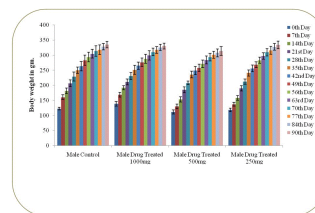


Figure 1b: Average body weight gain of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. n=5 in each group. * $p < 0.05$ as compared with the controls at the same time (one-way ANOVA).

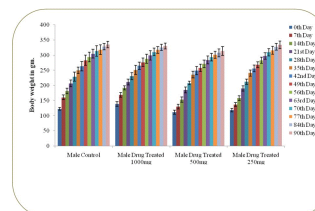


Figure 1c : Average body weight gain of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. n=5 in each group. * $p < 0.05$ as compared with the controls at the same time (one-way ANOVA).

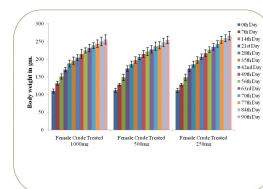


Figure 1d: Average body weight gain of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. n=5 in each group. * $p < 0.05$ as compared with the controls at the same time (one-way ANOVA).

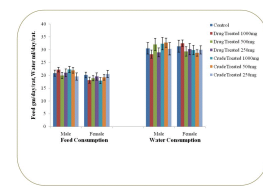


Figure 2: Average feed and water consumption of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. n=5 in each group. * $p < 0.05$ as compared with the controls at the same time (one-way ANOVA).

Mean feed and water consumption

Relative the average feed and water consumption of both treated groups were found to be unaffected by the Kushta Qalai drug and crude material as there were no statistically significant changes in the average feed and water consumption when compared with the respective controls as shown in Figure 2.

Relative organ weight of male and female rats in sub-chronic oral toxicity study (Gram/100 gram of body weight)

The relative organ weight of male and female rats in sub-chronic oral toxicity study were found unaltered by the Kushta Qalai drug and Crude material when comparing treated rats with the respective controls as shown in table 4a and 4b.

Table 4a: Relative organ weight of male rats in sub-chronic oral toxicity study of Kushta Qalai and its Crude material.

Organ	Male							
	Control		Drug 1000mg Treated	Drug 500mg Treated	Drug 250mg Treated	Crude 1000mg Treated	Crude 500mg Treated	Crude 250mg Treated
Brain (gm)	0.602 ± 0.12		0.584 ± 0.11	0.633 ± 0.15	0.608 ± 0.10	0.623 ± 0.12	0.613 ± 0.13	0.600 ± 0.14
Spleen (gm)	0.295 ± 0.12		0.297 ± 0.14	0.318 ± 0.10	0.282 ± 0.12	0.277 ± 0.08	0.277 ± 0.09	0.312 ± 0.11
Right adrenal (gm)	0.012 ± 0.008		0.011 ± 0.006	0.013 ± 0.007	0.012 ± 0.009	0.014 ± 0.008	0.010 ± 0.008	0.012 ± 0.009
Left adrenal (gm)	0.011 ± 0.007		0.012 ± 0.008	0.013 ± 0.007	0.012 ± 0.006	0.013 ± 0.09	0.010 ± 0.008	0.011 ± 0.007
Heart (gm)	0.282 ± 0.09		0.303 ± 0.07	0.262 ± 0.08	0.338 ± 0.06	0.312 ± 0.08	0.312 ± 0.09	0.299 ± 0.07
Lung (gm)	0.539 ± 0.11		0.554 ± 0.14	0.535 ± 0.15	0.494 ± 0.16	0.503 ± 0.13	0.499 ± 0.12	0.600 ± 0.13
Right kidney (gm)	0.293 ± 0.11		0.319 ± 0.13	0.289 ± 0.14	0.318 ± 0.12	0.321 ± 0.16	0.321 ± 0.11	0.314 ± 0.10
Left kidney (gm)	0.268 ± 0.11		0.307 ± 0.12	0.278 ± 0.13	0.303 ± 0.10	0.312 ± 0.12	0.312 ± 0.13	0.302 ± 0.14
Right testis (gm)	0.426 ± 0.15		0.481 ± 0.12	0.397 ± 0.12	0.395 ± 0.11	0.397 ± 0.11	0.394 ± 0.10	0.433 ± 0.13
Left testis (gm)	0.411 ± 0.13		0.454 ± 0.12	0.376 ± 0.10	0.385 ± 0.15	0.383 ± 0.14	0.393 ± 0.10	0.400 ± 0.12
Liver (gm)	2.804 ± 0.55		3.309 ± 0.54	2.761 ± 0.43	2.809 ± 0.59	2.824 ± 0.61	2.744 ± 0.38	2.833 ± 0.40
Pancreas (gm)	0.188 ± 0.05		0.171 ± 0.07	0.151 ± 0.07	0.177 ± 0.04	0.192 ± 0.05	0.192 ± 0.05	0.180 ± 0.08
The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).								

Table 4b: Relative organ weight of female rats in sub-chronic oral toxicity study of Kushta Qalai and its Crude material.

Organ	Female							
	Control		Drug 1000mg Treated	Drug 500mg Treated	Drug 250mg Treated	Crude 1000mg Treated	Crude 500mg Treated	Crude 250mg Treated
Brain (gm)	0.600 ± 0.11		0.624 ± 0.10	0.610 ± 0.14	0.575 ± 0.13	0.659 ± 0.12	0.661 ± 0.10	0.580 ± 0.14
Spleen (gm)	0.333 ± 0.10		0.300 ± 0.11	0.321 ± 0.09	0.321 ± 0.14	0.301 ± 0.11	0.341 ± 0.10	0.320 ± 0.12
Right adrenal (gm)	0.014 ± 0.009		0.016 ± 0.008	0.012 ± 0.007	0.011 ± 0.007	0.013 ± 0.009	0.014 ± 0.006	0.012 ± 0.008
Left adrenal (gm)	0.012 ± 0.008		0.014 ± 0.009	0.010 ± 0.007	0.009 ± 0.008	0.011 ± 0.009	0.012 ± 0.008	0.010 ± 0.008

Heart (gm)	0.288 ± 0.10	0.311 ± 0.11	0.300 ± 0.12	0.300 ± 0.12	0.265 ± 0.08	0.310 ± 0.10	0.280 ± 0.09
Lung (gm)	0.620 ± 0.13	0.640 ± 0.16	0.624 ± 0.15	0.592 ± 0.11	0.620 ± 0.15	0.568 ± 0.16	0.588 ± 0.13
Right kidney (gm)	0.298 ± 0.12	0.279 ± 0.13	0.302 ± 0.12	0.325 ± 0.13	0.325 ± 0.10	0.286 ± 0.11	0.305 ± 0.10
Left kidney (gm)	0.274 ± 0.11	0.270 ± 0.12	0.289 ± 0.13	0.311 ± 0.13	0.309 ± 0.10	0.267 ± 0.11	0.289 ± 0.13
Right Ovary (gm)	0.025 ± 0.009	0.024 ± 0.008	0.025 ± 0.009	0.022 ± 0.007	0.023 ± 0.008	0.018 ± 0.008	0.021 ± 0.009
Left Ovary (gm)	0.021 ± 0.008	0.020 ± 0.007	0.020 ± 0.007	0.018 ± 0.006	0.021 ± 0.008	0.017 ± 0.006	0.019 ± 0.007
Liver (gm)	3.019 ± 0.55	2.894 ± 0.39	3.347 ± 0.57	2.727 ± 0.44	3.458 ± 0.58	2.812 ± 0.47	2.976 ± 0.42
Pancreas (gm)	0.173 ± 0.07	0.169 ± 0.06	0.192 ± 0.04	0.209 ± 0.05	0.209 ± 0.06	0.201 ± 0.07	0.200 ± 0.09

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA)

Biochemistry Parameters.

The results of Biochemical parameters of both the treated rat groups did not show any statistically significant change when compared with the controls groups as shown in Figure 3a, 3b and 3c.

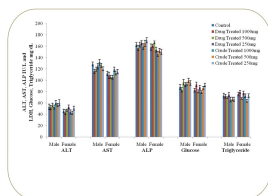


Figure 3a: Biochemistry parameters ALT, AST, ALP, Glucose and Triglyceride of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

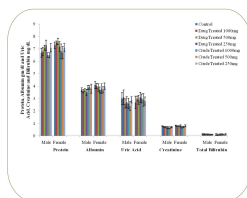


Figure 3b: Biochemistry parameters Total Protein, Albumin, Uric Acid, Creatinine and Total Bilirubin of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

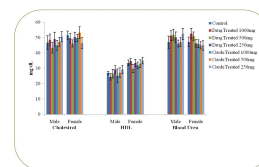


Figure 3c: Biochemistry parameters Serum Cholesterol, HDL and Blood Urea of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

Haematology Parameters

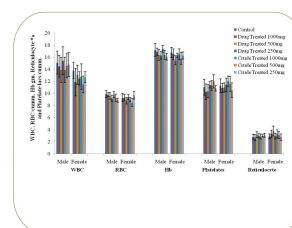


Figure 4a: Haematological parameters WBC Count, RBC Count, Haemoglobin, Platelet and Reticulocyte count of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

The results of Haematological parameters of the drug and Crude material treated males and females rats did not show statistically significant change in the values when compared with the respective controls. Figure 4a, 4b & 4c shows the haematological parameters of rats.

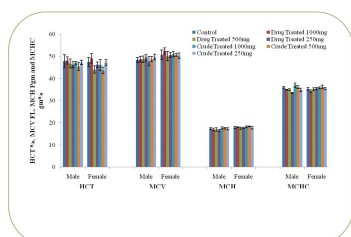


Figure 4b: Haematological parameters HCT, MCV, MCH and MCHC of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. $n=5$ in each group. $*p<0.05$ as compared with the controls at the same time (one-way ANOVA).

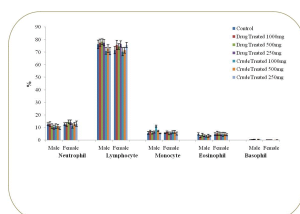


Figure 4c: Haematological parameter DLC – Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil percentage of male and female rats in sub chronic oral toxicity study of Kushta Qalai and its Crude material. The values are expressed as mean $\pm$ SD. $n=5$ in each group. $*p<0.05$ as compared with the controls at the same time (one-way ANOVA).

Histopathology

The Histopathological examination of vital organs such as Liver, Kidney, Lung, Pancreas, Spleen, Adrenal Gland, Testes/Ovaries, Heart and Brain shows normal architecture and did not reveal any pathology after the oral administration of Kushta Qalai and its crude material for 90 days as shown in figure 5 – 14, except there were mild degeneration in liver and kidney tissues of crude material treated rats as shown in figure 5 and 6. Effect of drug on the histology of tissues/organs in male and female rats in sub-chronic oral toxicity study of Kushta Qalai.

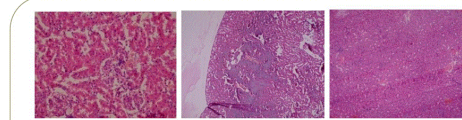
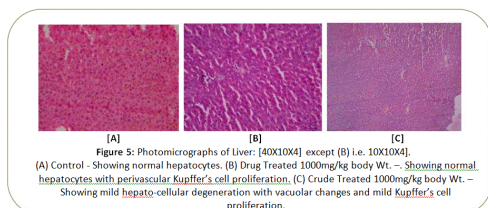


Figure 6: Photomicrographs of Kidney: [40X10X4] except (B & C) i.e. 10X10X4
(A) Control - Showing normal tubules and glomeruli. (B) Drug Treated 1000mg/kg body Wt. - Showing normal epithelium with glomerular congestion. (C) Crude Treated 1000mg/kg body Wt. - Showing glomerular congestion, swelling and degeneration.

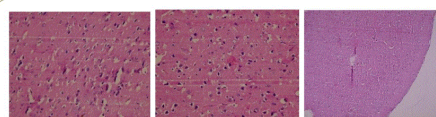


Figure 7: Photomicrographs of Brain: [40X10X4] except (C) i.e. 10X10X4
(A) Control - Showing normal neurons. (B) Drug Treated 1000mg/kg body Wt. - Showing normal neurons. (C) Crude Treated 1000mg/kg body Wt. - Showing normal neurons with meningeal congestion.

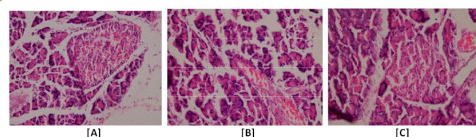


Figure 8: Photomicrographs of Pancreas: [40X10X4]
(A) Control - Showing exocrine gland and islet of langerhans. (B) Drug Treated 1000mg/kg body Wt. - Showing normal exocrine gland and islet's. (C) Crude Treated 1000mg/kg body Wt. - Showing normal exocrine gland and islet's.

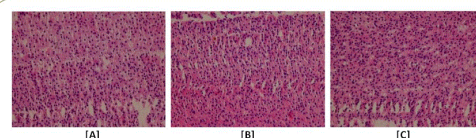


Figure 9: Photomicrographs of Adrenal gland: [40X10X4]
(A) Control - Showing adrenal cortex. (B) Drug Treated 1000mg/kg body Wt. - Showing normal adrenal cells. (C) Crude Treated 1000mg/kg body Wt. - Showing normal adrenal cortex.

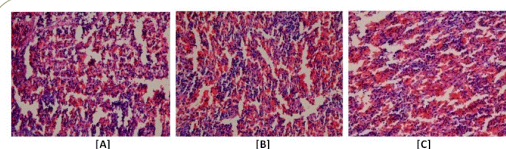


Figure 10: Photomicrographs of Spleen: [40X10X4]
(A) Control - Showing normal splenic corpuscles. (B) Drug Treated 1000mg/kg body Wt. - Showing normal morphology of spleen cells. (C) Crude Treated 1000mg/kg body Wt. - Showing normal splenic corpuscles.

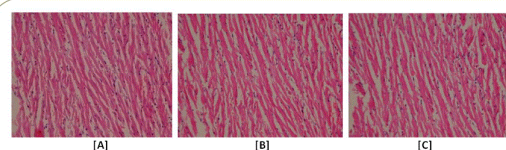


Figure 11: Photomicrographs of Heart: [40X10X4]
(A) Control - Showing normal myocardium. (B) Drug Treated 1000mg/kg body Wt. - Showing normal cardio-myocytes. (C) Crude Treated 1000mg/kg body Wt. - Showing normal morphology of cardiac muscle.

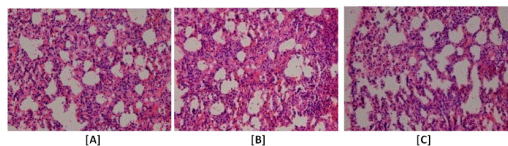


Figure 12: Photomicrographs of Lung: [40X10X4].
(A) Control - Showing normal lung alveoli. (B) Drug Treated 1000mg/kg body Wt. - Showing normal alveoli. (C) Crude Treated 1000mg/kg body Wt. - Showing normal alveoli with micro vascular congestion.

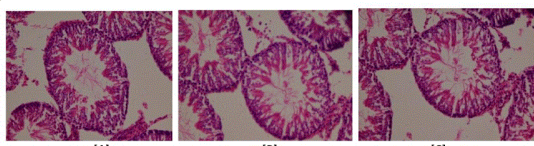


Figure 13: Photomicrographs of Testes: [40X10X4].
(A) Control - Showing normal seminiferous tubules with spermatozoa. (B) Drug Treated 1000mg/kg body Wt. - Showing normal spermatozoa in seminiferous tubules. (C) Crude Treated 1000mg/kg body Wt. - Showing normal spermatozoa and seminiferous tubules.

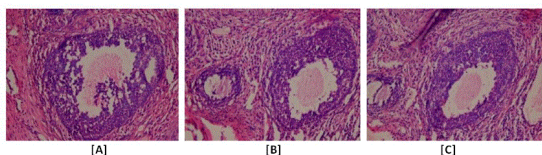


Figure 14: Photomicrographs of Ovary: [40X10X4].
(A) Control - Showing normal ovarian tissue, follicle and ovum. (B) Drug Treated 1000mg/kg body Wt. - Showing normal ovarian follicles. (C) Crude Treated 1000mg/kg body Wt. - Showing follicles normal with mild focal haemorrhages.

Discussion

World Health Organization survey indicates that about 70-80% of the world's population rely on non-conventional medicine mainly of herbal source in their primary healthcare [21]. The growing number of herbal drug users around the globe and lack of scientific data on the safety profile of herbal products make it necessary to conduct toxicity study of herbal products [22]. Therefore, the safety of these products has become an importance issue [23]. An increase in the number of cases due to poisoning by herbal drugs makes their pharmacovigilance and toxicity studies all the more imperative to ensure their safe use and to protect public health [24]. In view of the above facts the principle goal of sub-chronic oral toxicity study of Kushta Qalai and its Crude material, are to identify and characterise the specific organ/parameter that may be affected, after repeated oral administration for 90 days and also to check the validity of detoxification method adopted while preparation of Kushta.

In this study, the body/organ weight of Treated rats did not show any significant changes after oral administration of Kushta Qalai drug and crude material at the dose level of 1000, 500 and 250mg/kg body weight for 90 days when compared with the Control rats. The change in body / organ weight is simple and sensitive indicator of toxicological studies [25-27]. The Haematological parameters reveal anomalies in body metabolic

process. The haematological parameters furnish important information of the body response to injury, deprivation and stress. The haematological parameters remained unaltered between test substances treated rats and Control rats. The biochemical parameters were performed to check the liver, kidney, lipid and glycemic profile of the Kushta Qalai drug and crude material treated rats. In this study, the LFT (AST, ALT, ALP) of Treated rats did not show any significant change when compared with the respective Control rats. The KFT (Blood Urea, Serum Creatinine and uric acid) of Treated rats did not show any significant change when compared with the respective Control rats and also the other metabolic parameters of the Treated rats were also at par with the Control rats. The Histopathological examination of the vital organ did not reveal any morphological changes after oral administration of Kushta Qalai drug and crude material for 90 days at the dose level of 1000, 500 and 250 mg/kg body weight, except in liver and kidney mild degenerative changes were seen in case of Kushta Qalai drug Treated rats at the dose level of 1000mg/kg body weight and at all the three dose levels of Crude material. The glomeruli congestion and swelling was also seen in the kidney tissue due to overburden / overload of excretion/reabsorption as shown in figure 6. It may be noted that the Kushta Qalai drug is normally orally administered to the human subjects at the dose level of 125 mg/kg body weight per day for a period of 30 days. The aim of these animal toxicity studies was to unmask the damaging potential of the drug by very high and several doses were used to establish dose relatedness of the adverse effects.

Conclusion

The result therefore suggests that the Kushta Qalai drug was potentially safe for oral consumption at the therapeutic dose level however it was found to cause impairment in the liver and kidney tissues at the dose level of 1000 mg/kg body weight (70X, X is the extrapolated therapeutic dose in rats) while as the Crude material was found to be potentially cause degenerative changes at all the three dose levels due to the increase hepatic overburden in detoxification/removal process of Crude material. This study indicates that the method of detoxification adopted by the Unani System of Medicine while preparation of Kushta is scientifically affective in removing/decreasing the elements of toxicity.

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Conflict of interest statement

We declare that we have no conflict of interest.

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