TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 6, Issue 1, Winter 2021

Original Research

90-Days Repeated Dose Oral Toxicity Study of Sharbat-e-Deenar (A Hepatoprotective Unani Herbal Formulation)

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Received: 9 Jan 2021 **Revised:** 24 Feb 2021 **Accepted:** 27 Feb 2021

Abstract

Sharbat-e-Deenar (SDR) is a compound Unani pharmacopoeial formulation recommended for the treatment of Waram-e-Kabid (hepatitis), Waram-e-Rahem (uterine inflammation/ Pelvic Inflammatory Diseases), Yarqan-e-Suddi (obstructive jaundice), and Istisqa (ascites). The current study was carried out to investigate repeated dose oral toxicity study of SDR for 90 days in Sprague dawley (SD) rats. SDR was orally administered (gavage) at the doses of 4, 10 and 20 mL/kg bw/day. A periodic observation was performed for mortality, morbidity and any clinical sign of toxicity. Changes in body weight and feed consumption were observed weekly throughout study duration. After the treatment duration of three months, animals were anaesthetized and blood samples were subjected to haematological investigation and serum was subjected to different biochemical estimation. Gross necropsy was performed and internal organs/ tissues were processed for histopathological investigation. Treatment with SDR showed no incidence of mortality and no clinical sign of systemic toxicity. Body weight showed pattern of weight gain except significance decrease at mid and high dose at 13th week of study duration. Feed consumption exhibited a significant decrease as compare to control. Haematology and biochemistry profile found normal except certain isolated changes which was considered toxicologically not significant as the values lies in the normal physiological range. There were no changes observed in the gross necropsy and relative organ weight data of control and SDR treated rats. It is reported that few of the animals showed changes in liver at mid (2.5 times of therapeutic equivalent dose) and high dose (5 times of therapeutic equivalent dose) in SDR treated animals that may be attributed to SDR treatment, however, associated liver function parameters like ALT, AST and ALP did not show any alteration of liver function. Based on the results of this study, it may be indicated that liver may be the target organ for toxicity if SDR is used above recommended therapeutic dose for longer duration

Keywords: Hepatoprotection; Polyherbal formulation; Oral toxicity

Citation: Husain GM, Ahmad T, Fatima SH, Javed G, Kazmi MH, Urooj M. **90-Days Repeated Dose Oral Toxicity Study of Sharbat-e-Deenar (A Hepatoprotective Unani Herbal Formulation)**. Trad Integr Med 2021; 6(1): 5-18.

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Introduction

Sharbat-e-Deenar (SDR), a compound Unani pharmacopoeial formulation, is recommended for hepatitis, enlargement of liver and various inflammatory conditions such as uterine inflammation, pelvic inflammatory diseases, and pleurisy [1]. SDR is one among the potential formulation for the treatment of liver disorders used since decades. Hepatoprotective activity of SDR is confirmed in experimental animals using carbon tetrachloride and acetaminophen-induced hepatotoxicity model [2,3]. SDR was prepared using different medicinal plants as mentioned in Table 1. It has been reported in literature that some of the medicinal plants of this formulation like *Cichorium intybus* L.*, Rosa damascena* Herrm. and *Rheum emodi* Wall. possess hepatoprotective and antioxidant activity. SDR is presently used by Unani physician as alone or in combination for the treatment of liver disorders. The clinical dose of SDR as mentioned in classical literature is 20-40 ml per day [4,5]. It is well known that traditional herbal medicine (like Ayurveda and Unani) become popular in recent times especially in India. The distinguishable feature of Unani medicine is application of holistic approach towards individual treatment. Unani medicine focuses on strengthening the healing ability and defence system of body to cure the disease. Inspite of popularity, it has been reported that herbal products have undesirable effects. Sub standard quality, contamination and adulteration are the major causes leading to toxicities of such products. There is a lack of systematic toxicity data for this valuable formulation in order to support the long-term use of this polyherbal Unani formulation without causing any serious adverse effect. There is no scientific rationale to claim plant or their parts or derived products are intrinsically safe or beneficial [6,7]. Therefore, the present study was designed to evaluate the safety of traditional polyherbal Unani formulation SDR by performing 90 days repeated dose oral toxicity study in SD rats.

Table 1: Composition of SDR

Common Name	Traditional Name	Scientific Name	Parts Used
Chicory root	Post-e-Bekh-e-Kasni	*Cichorium intybus*	Root Bark
Dodder Seeds	Tukhm-e-Kasoos	*Cuscuta reflexa*	Seed
Chicory Seeds	Tukhm-e-Kasni	*Cichorium intybus*	Seed
Damask rose/ rose of Castile	Ghunacha-e-Gul-e-Surkh	*Rosa damascena*	Flower Bud
Himalayan rhubarb/Indian rhubarb	Rewand Chini	*Rheum emodi*	Root
Water Lilly	Gul-e-Nilofar	*Nymphaea alba*	Flower
Starflower	Gaozaban	*Borago officinalis*	Leaves
Water	Aab	-	-
Sugar	Qand Safaid	-	Product from cane sugar

Methods

Experimental animals

Sprague dawley (SD) rats (100 ± 20 g, 5-6 weeks old) were purchased from the National Institute of Nutrition, Hyderabad, India. Nulliparous and non-pregnant females were chosen for the study. Animals were kept in satndard cages in the air conditioned room. Protocol of the study was approved by the Institutional Animals Ethics Committee vide Protocol No. CRIUM/IAEC/2016/01/P03.

Animals were provided with standard feed pellets (National Institute of Nutrition, Hyderabad) and purified water *ad libitum*, as well as a 12:12 h light/dark illumination cycle. The temperature and relative humidity was maintained at 22°C ± 3°C and 30-70%, respectively as mentioned in CPCSEA guideline. Animals were habituated to the laboratory conditions for seven days prior to conduct experiment [8].

Dose Selection

Dose Calculation for Repeated Dose Toxicity Study

Therapeutic Dose of SDR: 20-40 mL per day [1] As a conservative approach, 40 mL human dose was used for dose calculation. Three dose groups were included for investigation i.e., Therapeutic Equivalent Dose (TED in rats is 4 mL/kg bw per day), 2.5X and 5X of TED (i.e., 10 mL/kg bw and 20 mL/kg bw per day, respectively) [9].

Table 2: Dose conversion for rats based on Body Surface Area (BSA)

Total Human Dose (mL/day)	Human Dose (mL/kg bw) (Human bw= 60kg)	Equivalent Rat Dose 'X' (mL/kg bw)	2.5X (mL/kg bw)	5X (mL/kg bw)
40	0.67	4	10	20

X= Human Equivalent Dose (HED), which is calculated on the basis of surface area as follows [3]:
HED (mg/kg) = Animal Dose (mg/kg) multiplied by (Animal Km/Human Km**)**

Drug / Formulation and Administration

The study drug SDR was prepared in pharmacy department at National research institute of Unani medicine for skin disorders, Hyderabad. The crude drugs were procured from local supplier Devkripa Herbals, Hyderabad. The procedure for the preparation of SDR begins with the washing of the plant parts as mentioned in table 1. All the ingredients were thoroughly washed and soaked overnight in water (six times the weight of all ingredients). The next morning the soaked content was boiled and allowed to cool. Then the plant parts were rubbed with hands to extract the maximum possible constituents in water. The contents were filtered through a piece of fine clothes and liquid was kept undisturbed for some time so that the heavier matter settles down at the bottom. Thereafter, the supernatant liquid part was transferred into another vessel. A required quantity of sugar was added to this liquid and boiled on a low fire to the required consistency. It was then filtered again through

a piece of fine cloth to obtain the Sharbat [10]. An aqueous suspension of SDR in purified water (< 2mL/100 g body weight (bw)) was freshly prepared every day. Control animals were administered with vehicle (purified water) only. Test drug was administered at three dose levels of 4, 10 and 20 mL/kg bw once daily for 90 consecutive days at same time each day to minimize variations.

Vehicle

Purified water (purified using Aqua guard purifier) was used as vehicle for oral administration of SDR.

Experimental design

The 90-day repeated dose oral toxicity study was performed according to the OECD test guideline-408 [9]. Male and female SD rats were divided into four groups with 20 animals (10 males + 10 females) in each group as follows:

• Vehicle control (purified water)
• SDR Low Dose (X = 4 mL/kg bw)
• SDR Mid Dose (2.5X = 10 mL/kg bw)
• SDR High Dose (5X = 20 mL/kg bw)

All animals were observed two times daily for mortality and morbidity during the study. Detailed observations, including functional observation parameters were performed to detect any possible sign of toxicity, everyday 1h after administration of the treatments. Animals body weight was weekly recorded. Additionally, feed intake for each sex was measured once a week by weighing the amounts of feed given to a cage group and leftovers on the next day. By the end of 90th day, the overnight fasted (water provided ad libitum) animals were anaesthetized with isofluorane inhalation (EZ Anaesthesia-1339), blood samples were collected by retro-orbital puncture in the EDTA vacutainers (for haematological) and serum vacutainers (for biochemical and electrolyte analysis).

Hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), haematocrit (HCT) and platelet (PLT) were analyzed using fully automated haematology analyzer (Swelab Autocounter-920EO+). Serum biochemical parameters such as glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), total cholesterol (TC), triglycerides (TG), total protein (TP) and albumin were analyzed using fully automatic analyzer (Erba-EM200). Serum sodium, potassium and chloride were measured with fully automated electrolyte analyzer (Allcare-AC9801). Finally, all euthanized animals were subjected to gross necropsy. Organs and tissues were examined macroscopically and internal organs/ tissues were isolated, trimmed and weighed. Organs/ tissues were preserved in the neutral buffer formalin and histologically examined. The tissues were processed for routine paraffin embedding and approximately 3-5 µ sections were stained with Mayer's Hematoxylin and Eosin stains.

Statistical analyses

Data were expressed as mean ± Standard Error of Mean (SEM) for ten animals. The mean difference between the control and treatment

groups was analysed by analysis of variance using GraphPad prism (version 5) GraphPad Software, Inc., CA, USA. p value ≤ 0.05 was considered statistically significant.

Results

Survival & Clinical Examination

Oral administration of SDR for 90 consecutive days did not cause any mortality in male or female rats at any tested dose level. Daily general examination and detailed observations conducted at various time points did not reveal any abnormal signs of toxicity in SDR treated or control animals at any tested dose.

Body Weight

Oral administration of SDR did not induce any significant effect on body weight gain in female rats compared to control except a decrease in low dose group only at 13th week ($p < 0.01$) (Figure 1). There was a significant decrease in body weight of male rats in mid dose ($p < 0.05$) and high dose group ($p < 0.01$) only at 13th week compared to control (Figure 2). Apart from these isolated changes, animals showed similar weight gain as that of control animals.

Feed Consumption

Administration of SDR in rats showed a pattern of reduced daily food consumption compared to control throughout the study duration. There was a significant reduction in feed consumption in females treated with low dose ($p < 0.05$), mid dose ($p < 0.001$) and high dose ($p < 0.01$) compared to control animals (Figure 3). Similarly, significant reduction in feed consumption was observed in male rats treated with low dose, mid dose and high dose SDR ($p < 0.001$) compared to control animals (Figure 4).

Haematology

Oral administration of SDR for 90 consecutive days at three dose levels did not alter the haematological profile. There is no statistically significant difference in Hemoglobin, RBC, WBC, HCT or differential leukocytes levels in SDR treated rats compared to control group in any sex. There was significant increase in platelet count in low and mid dose males ($p < 0.05$) Figure 5, 6). Peripheral blood smear showed normocytic and normochromic cells in all animals.

Biochemistry

In the present study, the serum level of ALT, AST, ALP, Bilirubin, and Albumin of SDR treated groups were found comparable to control group in both sexes (Table 3). The level of blood glucose was significantly decreased in females of low dose ($p < 0.05$), mid dose ($p < 0.01$) and high dose groups ($p < 0.001$) compared to control; whereas only mid dose males showed a significant decrease ($p < 0.05$) in glucose level compared to control. The level of globulin was significantly decreased in females of low dose ($p < 0.05$), mid ($p < 0.001$) and high dose groups ($p < 0.01$) compared to control. Similarly, males of SDR treated groups showed a significant decrease ($p < 0.001$) in globulin compared to control. Consequently, total protein level in SDR treated female and male rats showed a significant decrease ($p < 0.001$) compared to respective control groups.

There was no significant difference in the BUN level in any sex compared to control except a minor decrease ($p < 0.05$) in low dose males. The level of serum creatinine was significantly increased in females of low dose ($p < 0.001$), mid dose ($p < 0.001$) and high dose groups ($p < 0.05$) compared to control. Male rats of SDR treated groups showed a significant increase in serum creatinine at low and high dose ($p < 0.05$) compared to control. A trend of increase in the serum level of total cholesterol, triglycerides and HDL was observed in SDR treated male and female rats compared to control (Table 3).

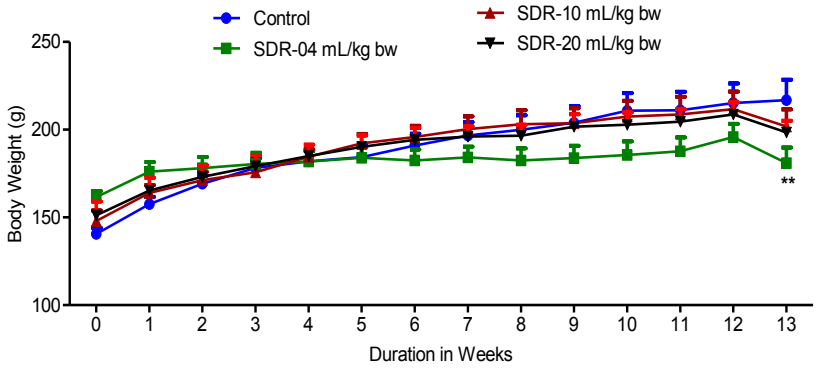

Figure1: Average body weight of control and SDR treated Female rats; **p < 0.01 vs. control

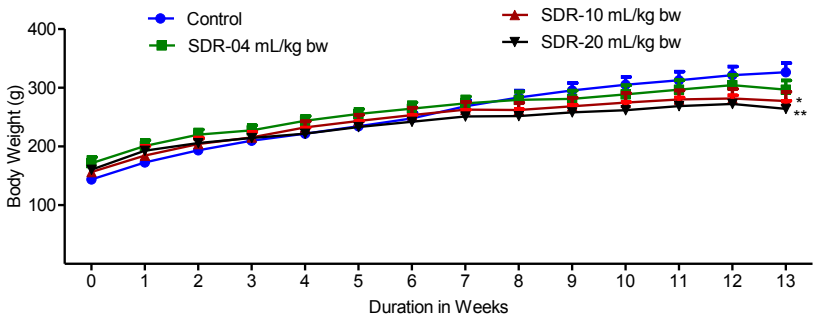

Figure 2: Average body weight of control and SDR treated Male rats; * p< 0.05; **p < 0.01 vs. control

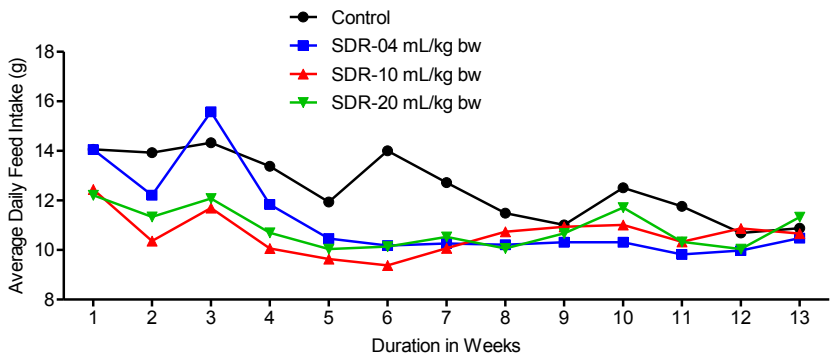

Figure 3: Average feed intake of control and SDR treated Female rats

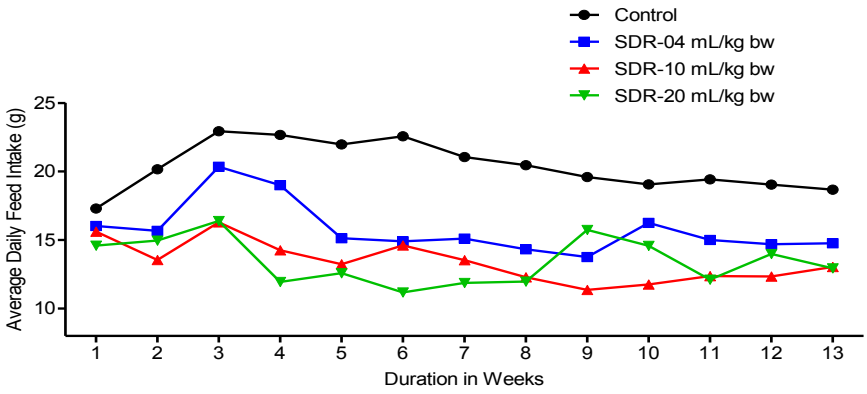

Figure 4: Average feed intake of control and SDR treated Male rats

Organ Weights

The oral administration of SDR at three dose levels i.e., 4, 10 and 20 mL/kg bw did not result any alterations in relative organ weight of Brain, Thymus, Heart, Lungs, Liver, Spleen, Adrenals, Kidney, Testis, Epididymis, Uterus and Ovaries. All the observed values of SDR treated animals were comparable to control group (Figure 7, 8).

Necropsy

Rats of control and SDR treated groups were subjected to necropsy after completion of dosing period of 90-days. No abnormal changes were observed either in control or treated animals. No gross lesions were observed in any organ/ tissue during necropsy in any group.

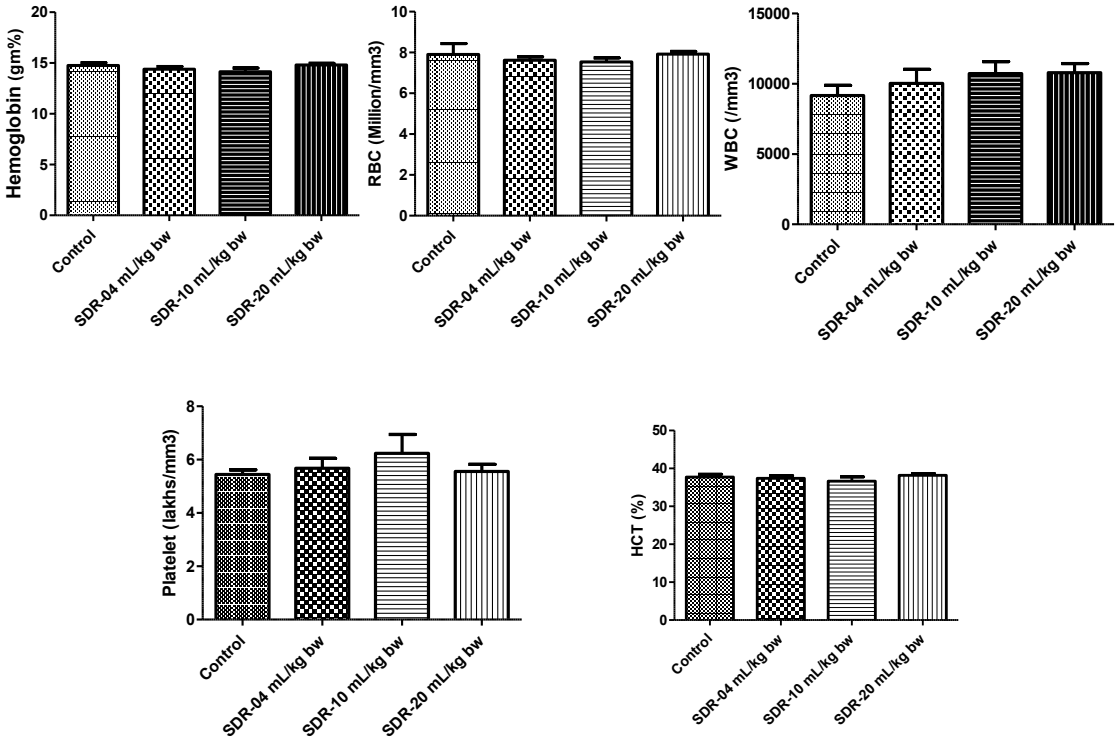

Figure 5: Effect of SDR on haematology in female rats

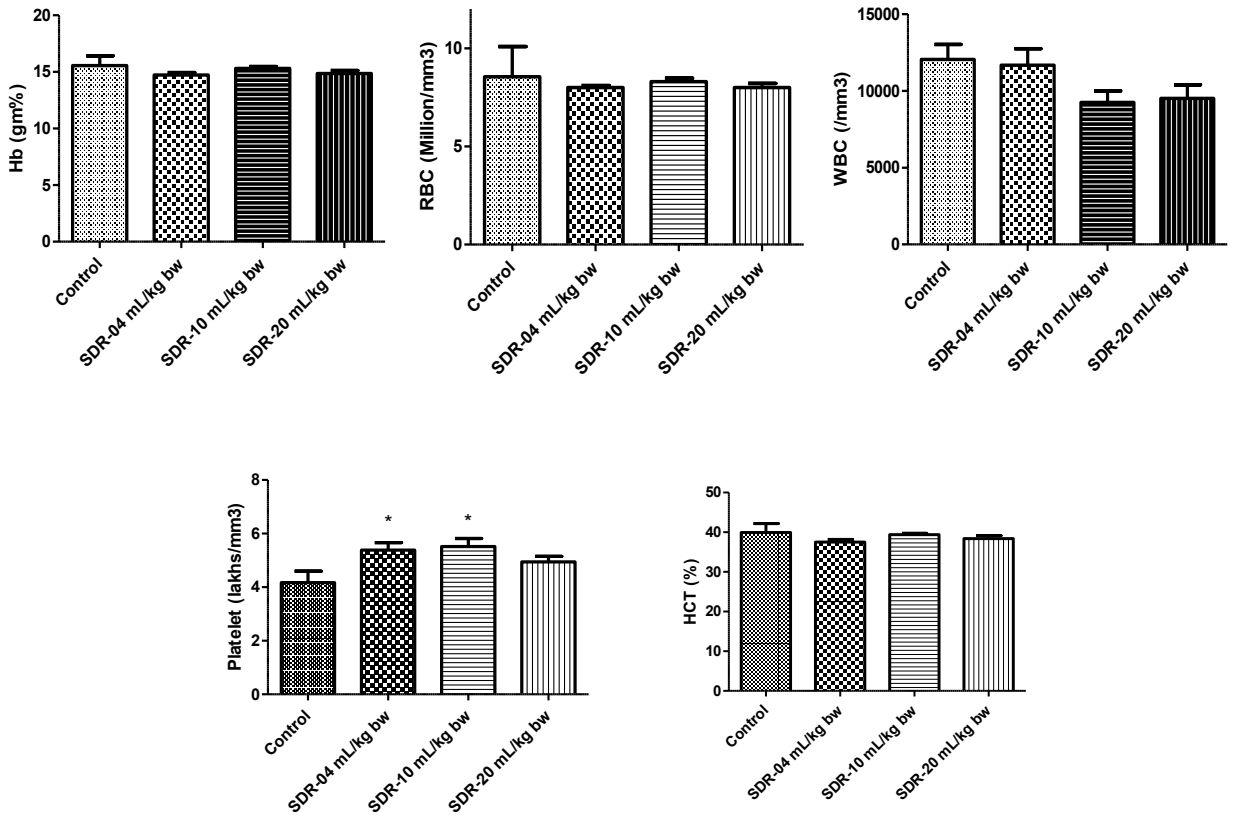

Figure 6: Effect of SDR on haematology in male rats; *$p < 0.05$ vs. control

Table 3: Effect of SDR on Clinical Chemistry in rats

Parameter	Female			
	Control	SDR		
		04 mL/kg bw	10 mL/kg bw	20 mL/kg bw
ALT (IU/L)	70.2±3.75	57.30±4.63	63.30±5.32	54.00±4.16
AST (IU/L)	144.20±6.01	159.70±13.48	181.90±5.75	183.70±15.10
ALP (IU/L)	141.20±19.96	170.50±19.11	104.10±5.95	163.20±23.50
Bilirubin (mg/dL)	0.1620±0.019	0.187±0.014	0.283±0.104	0.272±0.035
Total Protein (g/dL)	7.33±0.18	6.79±0.077**	6.50±0.06***	6.50±0.062***
Albumin (g/dL)	3.78±0.08	3.78±0.11	3.97±0.09	3.71±0.077
Globulin (g/dL)	3.55±0.19	3.01±0.15*	2.54±0.062***	2.79±0.071**
BUN (mg/dL)	20.19±0.73	21.44±1.1310	20.22±0.789	21.25±0.76
Creatinine (mg/dL)	0.69±0.018	0.784±0.016***	0.789±0.016***	0.761±0.011*
Glucose (mg/dL)	111.80±4.28	91.00±4.000*	87.50±5.53**	80.00±5.35***
Cholesterol mg/dL)	76.70±4.41	120.80±11.10**	124.20±8.76**	132.1±10.83***
Triglycerides(mg/dL)	74.90±7.33	121.80±10.52	118.40±22.75	172.1±19.15***
HDL (mg/dL)	48.20±4.81	52.70±4.45	58.80±2.91	62.20±2.76

Parameter	Male			
	Control	SDR		
		04 mL/kg bw	10 mL/kg bw	20 mL/kg bw
67.5±2.50	54.70±5.43	54.70±5.43	58.60±7.44	75.50±8.20
146.10±3.66	160.40±7.26	160.40±7.26	158.2±15.67	145.70±16.43
135.40±16.38	78.00±16.36	78.00±16.36	163.70±22.75	162.80±24.94
0.138±0.005	0.182±0.0138	0.182±0.0138	0.275±0.065	0.184±0.030
7.30±0.10	6.43±0.066***	6.43±0.066***	6.60±0.105***	6.34±0.069***
3.64±0.09	3.76±0.050	3.76±0.050	3.77±0.131	3.70±0.14
3.80±0.11	2.67±0.080***	2.67±0.080***	2.83±0.127***	2.64±0.149***
21.60±1.38	16.63±0.91*	16.63±0.91*	18.96±0.76	22.04±1.86
0.71±0.01	0.776±0.019*	0.776±0.019*	0.757±0.0165	0.785±0.016*
114.70±4.29	91.60±6.74	91.60±6.74	85.20±6.39*	93.30±8.293
65.80±4.59	146.0±11.01***	146.0±11.01***	99.10±11.15	109.30±16.68
84.20±11.00	152.6±21.44	152.6±21.44	126.30±13.59	110.30±24.99
36.40±2.73	49.60±1.507*	49.60±1.507*	49.00±3.59*	52.90±3.298**

(Values presented as Mean ± SEM; n=10/ sex; ANOVA; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs. control)

Histopathology

Heart, brain, kidneys, spleen, pancreas, adrenals, trachea, stomach, small intestine, sternum and bone marrow, testes/ uterus and ovaries of high dose SDR (20 mL/kg bw) and control group did not reveal any toxicologically significant finding following histological investigations. Changes of histological significance were observed only in the lungs and liver of the animals. Chronic interstitial pneumonitis of different grades was observed in both control and experimental animals and hence was not considered significant. Livers of most of the control group animals were observed to be normal. Foci of parenchymal inflammation and periportal inflammation were observed in 40% (4 animals) of females and 20% (2 animals) of males in high dose SDR group.

In the mid dose SDR group (10 mL/kg), liver of majority of animals (65%) were observed to be normal histologically. However, 20% animals showed microvacuolation of mild grade (5-33%) while 5% (1 animal) had microvacuolation of mild grade (5-33%) associated with focal portal tract inflammation and parenchymal inflammation. 5% (1 animal) had microvacuolation of severe grade (> 66%). Microvacuolation observed in high dose SDR group was also observed in the control animals and hence are not considered significant. Foci of parenchymal inflammation observed in the liver of high dose SDR may be attributed to SDR.

The representative histopathological images of major vital organs are shown in figure 9.

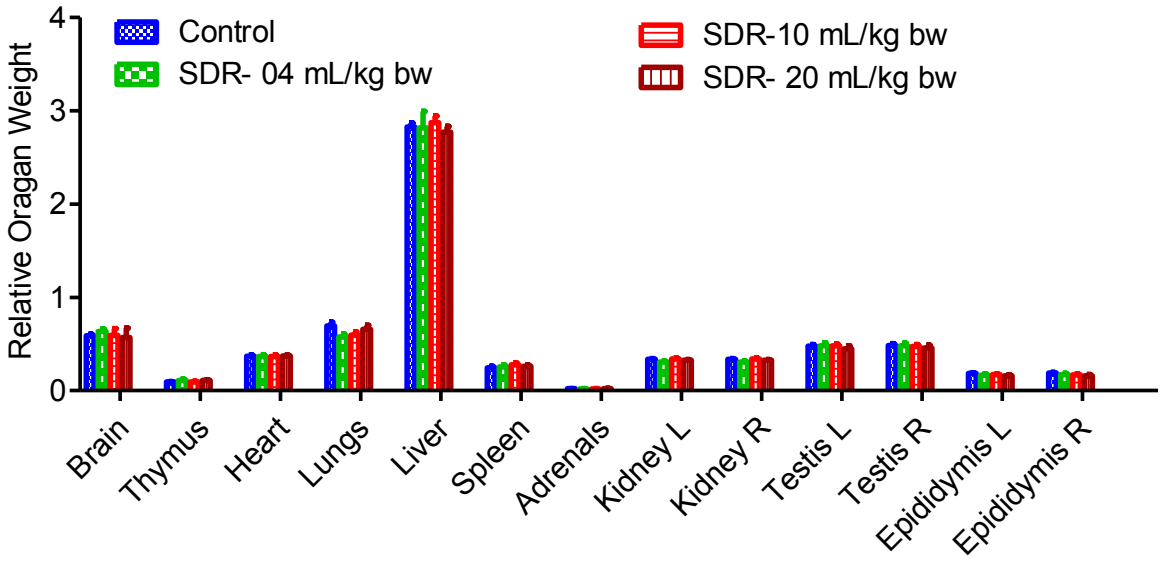

Figure7: Relative organ weight of control and SDR treated female rats

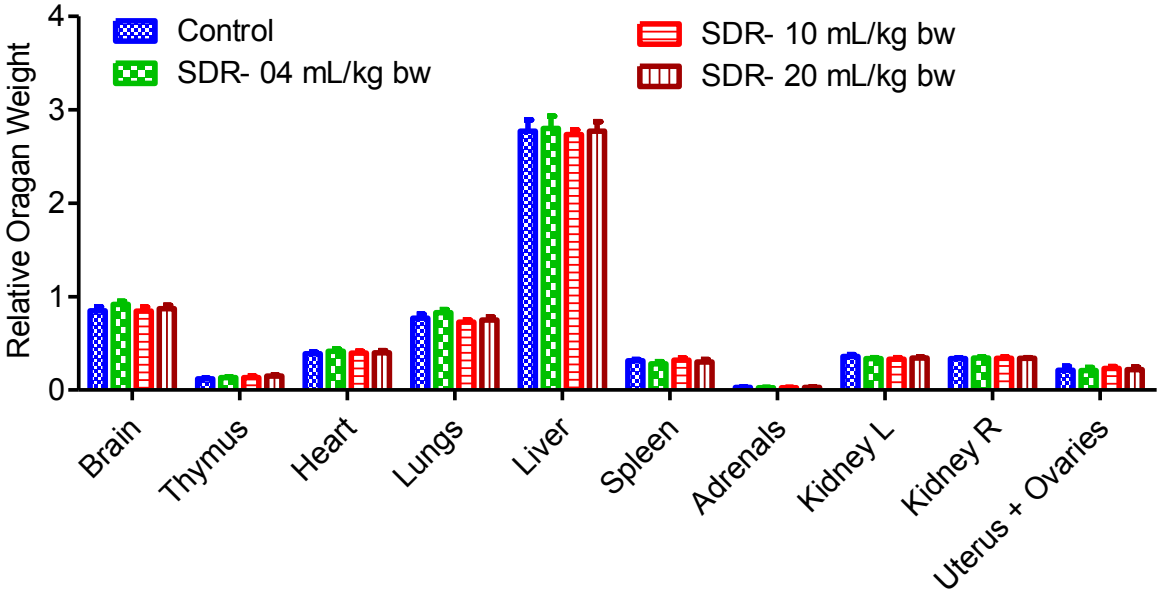

Figure 8: Relative organ weight of control and SDR treated male rats

Discussion

In the present study, no incidence of mortality was observed. No clinical indicator of systemic toxicity was observed in any group during the study duration. The data obtained for body weight which was recorded weekly showed pattern of weight gain in both SDR treated male and female rats as compare to control animals. However, there was significant decrease in body weight in SDR treated males at mid (10 mL/kg bw) and high dose (20 mL/kg bw) which was observed at 13th week. Similarly, a significant decrease in body weight was observed in SDR treated females at low dose (4 mL/kg bw) at

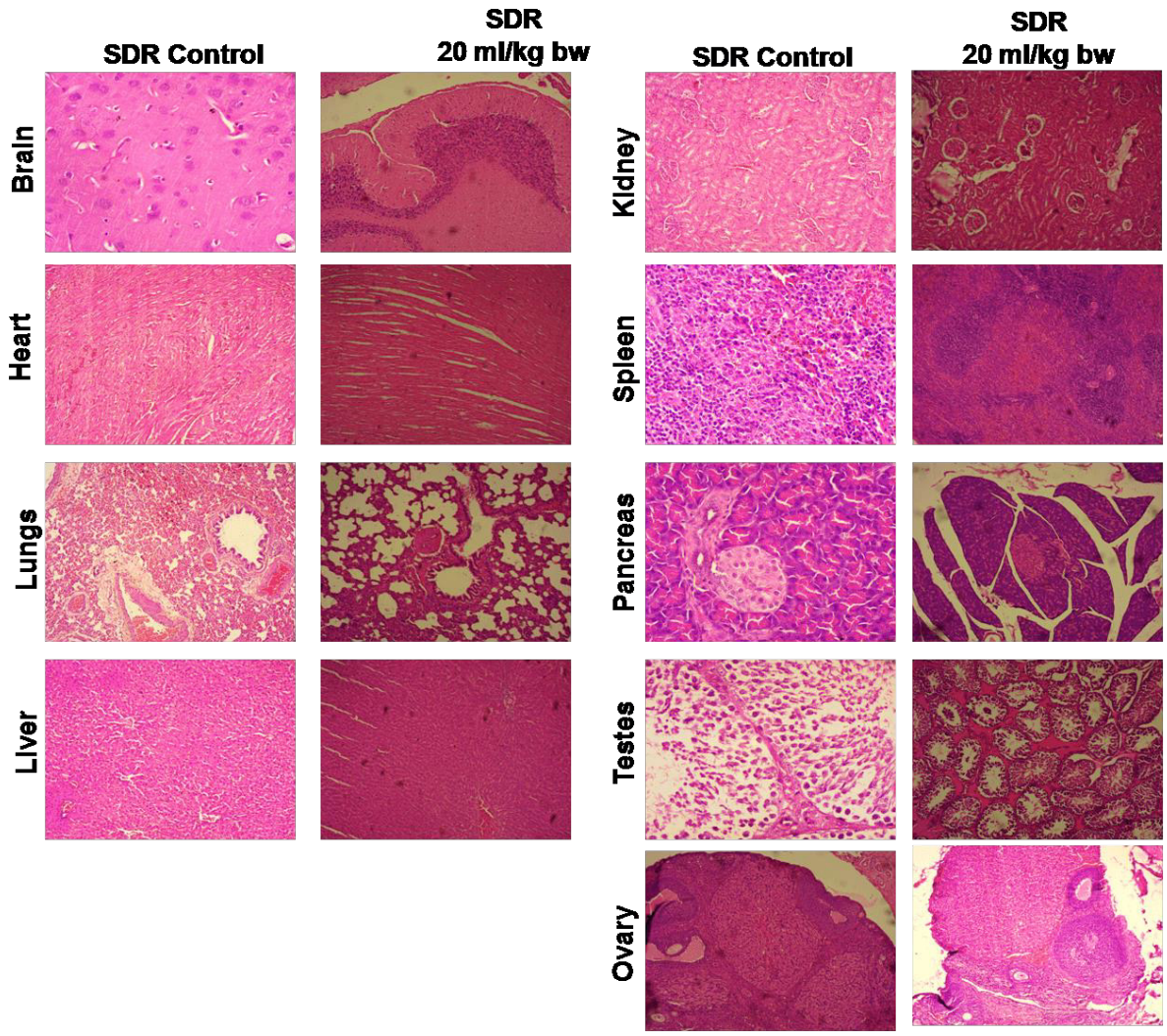

Figure 9: Histopathological sections of Control vs SDR treated (20 ml/kg bw) rats.

13th week of duration. Feed intake measured weekly throughout the study exhibited a trend of decrease in feed consumption compared to control group. Bulky test formulations may have an effect on the satiety of animals [11] and may reduce the feed consumption. The possible cause for reduction of feed intake in our study may be due to bulky nature of SDR. Further, the results indicated that long term administration of SDR may possibly interfere with gastrointestinal functions which inhibited the normal intake of food and lead to reduced body weight.

The values of globulin and total protein in the SDR treated groups were significantly low compared to respective control but remained within normal physiological limits and may not be considered toxicologically relevant. The fasting glucose level in SDR treated animals were significantly reduced in female at all tested dose level as compared to control. The glucose level in SDR treated males was significantly reduced in male at mid dose. Decrease in fasting blood sugar level may be due to presence of chicory seeds (*Cichorium intybus*) in SDR. The hypoglycemic action of chicory seeds extract at

the dose of 40 and 100 mg/kg bw was reported where results showed significant reduction of the fasting blood glucose level in early and late stage diabetic rats treated for 28 days [12]. Similar findings were reported in another study where chicory seed extract at dose of 125 mg/kg bw administered intraperitoneally for 21 days showed decline of fasting blood glucose level in diabetic rats [13]. The reduction in glucose level may be due to SDR causes increase insulin sensitivity, decrease intestinal absorption of glucose or hepatic glucose production in case of long term use.

Lipid parameters such as total cholesterol, triglycerides and HDL cholesterol in the SDR treated groups showed higher values compared to control group but the values were mostly within normal physiological limits. Changes in general metabolic events are reflected by alterations in cholesterol, triglycerides, or glucose. These alterations may not be served as indicators of specific target organ toxicity. Mild or moderate increases or decreases in serum cholesterol or triglyceride concentrations are relatively frequent findings in toxicology studies, although the exact mechanisms involved are often unknown. Several factors may be involved, including food consumption, body weight, physical activity, liver function, and hormone balance [14].

The haematopoietic system being a primary index of physiological and pathological status in human and animals is prone to the toxic effects of chemicals/drugs [15]. Presence of haematological alterations in animals has significant correlation to toxicity in human upon translation of preclinical data [16]. Based on haematological assay, all the observed parameter in treated group were found comparable to control and were found within normal physiological limits.

Liver is the first organ to be primarily exposed by portal circulation and it is well known target organ of toxic impact. The reason for liver toxicity is due to occurrence of major biotransformation and excretion of drugs through this organ [17]. The most common adverse effect of many clinically used drugs is hepatotoxicity which is characterized by alteration in ALT, AST, ALP and bilirubin levels. Elevation in ALT and AST levels shows their leakage in blood stream indicating damage of liver parenchymal cells [18,19]. The observation of hepatic profile in current study showed no significant alteration in ALT, AST, ALP or total bilirubin levels. Further there is no difference in absolute or relative liver weight of treated group and control animals and no lesion was observed in liver of treated or control animals indicating lack of any toxic effect on liver up to the highest tested dose. The crucial protection role using SDR may be due to presence of *Rosa damascena* which contains Phenolic compounds including gallic acid, syringic acid and quercetin as the major bioactive compounds. One of the studies reported the use of R. *damascena* as dietary supplement and alternative medicine in the management of non-alcoholic fatty liver disease. R. *damascena* showed effectiveness by reduction of hepatic enzymes and improvement of antioxidant status [20]. In the present study after administration of SDR high dose (20 ml/kg bw), histological

examination of liver (40% female and 20% male animals) showed foci of parenchymal inflammation and periportal inflammation which may be attributed due to administered drug. Similarly, certain histological changes were also observed at mid dose (10 mL/kg bw) treated animals. The histological alterations observed at mid and high dose which is 2.5 times and 5 times of therapeutic equivalent dose. The findings inferred that liver may be the target organ of toxicity if SDR used above therapeutic recommended dose in case of long term use.

Kidneys play a crucial role in drug excretion and detoxification which makes it important target for toxicological response. Exposure of kidney to high level of drug or/metabolites can cause cell damage primarily due to high blood flow, clearance and xenobiotics metabolism [21]. The major indicator for kidney damage are BUN and creatinine which were found within normal range and comparable to control (except minimal increase in creatinine; values within normal physiological limits). Furthermore, normal level of albumin in SDR and control animals supports normal renal functions. One of the major ingredient of SDR i.e. *Rheum emodi* (Rewand Chini) possess nephroprotective potential. Relationship between nephroprotective activity and antioxidant activity of *Rheum emodi* is well documented in literature. Anthraquinones, glycosides and tannins are the major constituents present in extract of *Rheum emodi*. Condensed tannins are found in alcoholic and aqueous extract of *Rheum emodi* which showed reno-protective action in rats possibly through elimination of active oxygen [22].

The evaluation of organ weights in toxicology studies is an integral component in the assessment of new drugs. [23]. Organ/body weight ratios (i.e., relative organ weight) were considered is an important tool which is useful in case when body weights were affected [24]. In the present study, relative organ weight data of male and female rats sacrificed at the end of the dosing period was found to be comparable with their respective controls.

Conclusion

The findings from this study, designed as per OECD guidelines, suggest that oral administration of SDR at the doses of 4, 10 and 20 mL/kg bw/day does not cause any adverse or otherwise toxic effects on the survival, body weight, feed consumption, haematology and biochemistry parameters in male and female rats. There were some histological changes in the liver of few animals at mid (2.5 times of therapeutic equivalent dose) and high dose (5 times of therapeutic equivalent dose) in SDR treated animals that may be attributed to SDR treatment, however, associated liver function parameters like ALT, AST and ALP did not show any alteration of liver function. Based on the results of this study, it may be indicated that liver may be the target organ for toxicity if SDR is used above recommended therapeutic dose for longer duration.

Conflict of Interest

None.

Acknowledgement

Authors acknowledge the financial and infrastructure support provided by Director General, CCRUM, Ministry of AYUSH, (Government of India), New Delhi. Additionally, the authors are thankful to National Institute of Nutrition for providing technical expertise for histopathological evaluation and Department of Pharmacy for manufacturing and supplying the study drug.

References

[1] National Formulary of Unani Medicine (NFUM), Part-I. Central Council for Research in Unani Medicine and for on behalf of Govt. of India. Ministry of Health & F.W. (Deptt. of AYUSH). 2006; pp 222-223.

[2] Shakya AK, Saxena M, Sharma N, Shrivastava S, Shukla S. Hepatoprotective efficacy of Sharbat-e-Deenar against carbon tetrachloride-induced liver damage. J Environ Pathol Toxicol Oncol 2012;31:131-141.

[3] Shakya AK, Shukla S. Protective effect of Sharbat-e-Deenar against acetaminophen-induced hepatotoxicity in experimental animals. J Trad Chin Med 2017;37:387-392.

[4] Moreira D, Teixeira SS, Monteiro MHD, De-Oliveira ACAX, Paumgartten FJR. Traditional use and safety of herbal medicines. Rev Bras Farmacogn 2014;24:248-257.

[5] Kabeeruddin M. Bayaz-e-Kabeer, Vol. 2. Hikmat Book Depot. Hyderabad; p 101.

[6] Khan MA, Urooj M, Thejaswini G, Ahmed SS, Kazmi MH, Husain GM. 180-Days repeated dose oral toxicity study of polyherbal unani formulation: Jawarish Shahi. J Clin Exp Tox 2017;1:21-29.

[7] Moreira D, Teixeira SS, Monteiro MHD, De-Oliveira ACAX, Paumgartten FJR. Traditional use and safety of herbal medicines. Rev. Bras. Farmacogn. 2014; 24(2):248-257.

[8] CPCSEA, Standard Operating Procedures (SOP) for IAEC. New Delhi: Committee for the Purpose of Control and Supervision of Experiments on Animals, 2010.

[9] OECD, Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Publishing 1998.

[10] Zillurehman. Dawasazi. Kutub khana Darul-uloom. Meerut 1985; p 136.

[11] EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA 201;9:2438.

[12] Ghamarian A, Abdollahi M, Su X, Amiri A, Ahadi A, and Nowrouzi A. Effect of chicory seed extract on glucose tolerance test (GTT) and metabolic profile in early and late stage diabetic rats. Daru 2012;20:56.

[13] Rezagholizadeh L, Pourfarjam Y, Nowrouzi A, Nakhjavani M, Meysamie A, Ziamajidi N, Nowrouzi PS. Effect of Cichorium intybus L. on the expression of hepatic NF-κB and IKKβ and serum TNF-α in STZ− and STZ+ niacinamide-induced diabetes in rats. Diabetol Metab Syndr 2016;13;8:11.

[14] Haschek WM, Rousseaux CG, Wallig MA. Clinical Pathology. In: Fundamentals of Toxicologic Pathology. Elsevier. 2nd ed. 2010; pp 43-65.

[15] Almanca CCJ et al. Toxicological evaluation of acute and sub-chronic ingestion of hydroalcoholic extract of Solanum cernuum Vell. in mice. J. Ethnopharmacol 2011;138:508-512.

[16] Olson H et al. Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. Regul Toxicol Pharmacol 2000;32:56-67.

[17] Afshar S, Farshid AA, Heidari R, Ilkhanipour M. Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. Toxicol Ind Health 2008;24:581.

[18] Singh A, Bhat TK, Sharma OP. Clinical Biochemistry of Hepatotoxicity. J Clin Toxicol 2011;S4:001.

[19] Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicol 2008;245:194-205.

[20] Rahimi R, Abdollahi M, Farzaei F, Farzaei MH, Memariani Z, Najafi F. Promising effect of Rosa damascena extract on high-fat diet-induced nonalcoholic fatty liver. J Tradit Complement Med 2017;7:508-514.

[21] Fuchs TC, Hewitt P. Biomarkers for drug-induced renal damage and nephrotoxicity-an overview for applied toxicology. AAPS J 2011;13:615-631.

[22] Alam MAA, Javed K, Jafri MA. Effect of Rheum emodi (Revand Hindi) on renal functions in rats. J Ethnopharmacol 2005;96:121-125.

[23] Sellers RS et al. Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies. Toxicol Pathol 2007;35:751-755.

[24] Michael B et al. Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. Toxicol Pathol 2007;35:742-750.