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## Original article

**Acute oral toxicity studies of antipsoriatic herbal mixture comprising of aqueous extracts of *Calendula officinalis*, *Momordica charantia*, *Cassia tora* and *Azadirachta indica* seed oil**

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**Abstract:**

Aqueous extracts of *Calendula officinalis*, *Momordica charantia*, *Cassia tora* and *Azadirachta indica* seed oil have been used individually for treating skin diseases such as psoriasis in traditional system of medicine. Though the individual herbal extracts and neem seed oil have been used safely there are no reports on the safety of these herbs and oil when used in combination. Hence a limit test at 5,000 mg/kg was carried out for the mixture comprising the extracts and the oil per Organization for Economic Cooperation and Development (OECD) guideline. Female rats received a single oral dose of 5,000 mg/kg body weight (b. wt.) of herbal mixture and three control female rats were orally given 2 ml. of distilled water. No concrete evidences of toxicities attributable to treatment with the herbal mixture were observed on behavioral pattern, hematology, clinical signs, serum biochemistry examination of liver and renal function and also histological evaluation of liver, kidney and heart. Therefore, single oral administration of herbal mixture up to 5,000 mg/kg b. wt. to rats under this study condition produced no significantly toxicological effects.

**Keywords:** Acute toxicity; Antipsoriatic herbal mixture; *Azadirachta indica*; *Calendula officinalis*; *Cassia tora*; Limit test; *Momordica charantia*

## Introduction

Herbal drug therapy is the most trusted system of medicine in countries like India, where people strongly believe in 'Ayurveda' as herbs are the part of rural Indian lifestyle. Most of the diseases which have no medicine in allopathic system can be cured successfully using traditional medicines. *Cassia tora* (Cesalpiniaceae), commonly known as 'Chakunda' is a road side weed found all over India, used as antibacterial, antifungal, antioxidant, hypolipidemic agent [1-5]. *Calendula officinalis* (Compositae) commonly known as 'Marigold' found in north India, has been used in more than 200 homeopathic formulations for its excellent wound healing activity apart from other medicinal uses [6-8]. *Momordica charantia* (Cucurbitaceae) commonly known as 'Bitter melon' is a common vegetable having potential antidiabetic activity [9]. *Azadirachta indica* seed oil or neem seed oil has been the most economical and potential agent for treating wide variety of skin ailments [10]. Apart from their popular uses these herbs have been reported individually for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis. [11-12]

Individually aqueous extracts of the herbs *M. charantia*, [13], *C. officinalis* [14], *C. tora* [15] and neem seed oil has been used safely for treating various ailments and skin diseases. Neem seed oil has also been used safely in various skin preparations [16]. As these herbs have been used individually for treating psoriasis, a combination comprising all of the above could have better efficacy against psoriasis. In view of this, an attempt was considered to develop a formulation comprising aqueous extracts of seeds of *C. tora* (CT), *M. charantia* (MC), flowers of *C. officinalis* (CO) and *A. indica* seed oil (AZoil).

Although individual herbal extracts and the neem seed oil have been used safely for treating various ailments since ages, safety of the combined herbal mixture intended to be used for developing formulation, comprising above herbal extracts and the neem seed oil has not been reported so far. As the intended combination is new there are no reports on its safety or toxicity upon oral ingestion.

To establish the safety of the intended combination to be used in formulation, acute toxicity studies upon oral ingestion was carried out in female Sprague-Dawley rats as per Organization for Economic Cooperation and Development guideline (OECD) 425 [17]. The method uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity.

## Materials and Methods

### Test material

Seeds of *C. tora* and *M. charantia* and *A. indica* seed oil were obtained from Natural Remedies, Veer Sandra industrial area, Bangalore. Dried flowers of *C. officinalis* was procured from Himalaya herb stores, Saharanpur. The crude drugs authenticated in Regional Research Institute, Bangalore, were used for the study. The apparatus used were reflux condenser, rotary flash evaporator Buchi type manufactured by Positive Infotech, vacuum oven manufactured by S.D (India corporation) and copper sieve of mesh size of # 10.

### Animals

Totally six healthy young adult nulliporous and non-pregnant female Sprague-Dawley rats, weighing 100-120 g (8-12 weeks old) at the start of the experiment procured from animal house of Al-Ameen College of Pharmacy (Registration No. 83 / 1999 / CPCSEA) were used. Animals were divided into 2 groups of 3 animals each.

The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature was 25 °C ( $\pm$  3 °C), humidity was 45-55% with a light period of 12 h (06.00 to 18.00). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow (Amrut Feeds, Bangalore, India) and unlimited supply of filtered drinking water.

### Preparation of the extracts and the herbal mixture

Dried seeds of *C. tora*, *M. charantia* and dried flowers of *C. officinalis* were powdered and passed through sieve #10. Thirty grams of the sieved powder was weighed accurately and subjected to extraction using reflux condenser and distilled water. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven, percentage yield of each extract was calculated (Table 1) and the dried extract was stored in air tight containers for further studies.

Aqueous extracts of seeds of *M. charantia*, *C. tora*, flowers of *C. officinalis* and neem seed oil were taken in the ratio of 2: 1.8: 2.9: 0.3 respectively. The extracts were weighed separately; 0.3 ml of neem seed oil was taken and triturated to get a uniform mixture. From this 5,000 mg was weighed accurately and dissolved in 5 ml

of distilled water. From the above stock solution, the required volume was calculated for each animal according to individual body weight.

### Administration of herbal mixture (Limit test at 5,000 mg/kg b. wt.)

Prior to dosing, animals were fasted overnight before being weighed, and the herbal mixture were orally administered in a single dose. The volume given was not more than 2 ml/100 gm body weight (b. wt.). Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the herbal mixture was administered, food was withheld for a further 3-4 hours. Control animals were administered with 2 ml distilled water. Limit test at 5,000 mg/kg was performed (Table 2) as per Paragraph 33(a) of OECD guidelines 425 [17].

**Table 1** Percentage yield of various extracts of *Calendula officinalis*, *Cassia tora* and *Momordica charantia*

Sl.No	Type of extract	Percentage yield*		
		<i>C. officinalis</i>	<i>C. tora</i>	<i>M. charantia</i>
1	Petroleum ether	10.52 ± 0.68	22.84 ± 0.66	18.69 ± 0.49
2	Methanol	18.69 ± 0.56	16.58 ± 0.85	20.49 ± 0.85
3	Ethanol	19.56 ± 0.49	19.76 ± 0.54	21.59 ± 0.68
4	Aqueous	25.82 ± 0.58	29.09 ± 0.46	26.42 ± 0.56

\*Values are in terms of Mean ± SEM of results done in duplicate.

**Table 2** Dose and frequency administration of herbal mixture for limit test at 5,000 mg/kg b. wt.

Agent	Diluent	Route of administration	Frequency of administration
Combined extract comprising of aqueous extracts of <i>Calendula officinalis</i> , <i>Momordica charantia</i> , <i>Cassia tora</i> and <i>Azadirachta indica</i> seed oil	Water	Oral route	Single dose

### **Clinical observations**

**Behavioral analysis:** Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

**Body weight analysis:** Individual weights of animals were recorded before the administration of drug on 1<sup>st</sup> day of the study and thereafter on the 7<sup>th</sup> and 14<sup>th</sup> day of the experiment before withdrawal of the blood from the individual animals. Changes in the weight were calculated and compared to that of the control animals.

**Hematological analysis:** Blood samples were collected by retro orbital puncture of all the test rats and the control rats. Blood smears were made on glass slides and stained with Leishman stain to perform total count, differential count and platelet count. [18] Estimation of hemoglobin percentage was done using haemocytometer. The tests were repeated on 7<sup>th</sup> and the 14<sup>th</sup> day.

**Biochemical analysis:** For the determination of blood sugar, total cholesterol, creatinine, urea, total and direct bilirubin, protein, SGOT, SGPT, alkaline phosphatase and acid phosphatase, blood samples were collected separately from each of the control and experimental rat by retro orbital puncture. The samples were then analyzed for the above biochemical parameters using standard procedures (blood sugar was determined by GOD/POD method, total cholesterol by CHOD-PAP method, creatinine by picric acid method, urea by UV kinetic/GLDH method, total and direct bilirubin by Jendrassik and

Grof method, total protein by biuret method, SGOT and SGPT by UV, kinetic acid phosphatase by kinetic color test and alkaline phosphatase by PNPP method) as described in the standard procedures and reagents using auto analyzer (Model:Biolis24i) [19].

**Organ weight analysis:** On completion of 14-day duration, both the test and the control animals were humanely sacrificed after collecting the blood for hematological and biochemical analysis. Vital organs like liver, kidney and heart from each animal were isolated after thorough perfusion of the organs with neutral saline, and pressed with help of tissue paper to remove any moisture. The isolated organs were observed for their morphology such as presence of any kind of lesions, etc., and the individual organ from each animal was weighed. The results were compared to that of the control animals. The results were statistically analyzed by unpaired 'student-t' test using GraphPad Prism version 5.0.

### **Histopathology of heart, liver and kidney:**

Heart, liver and kidney tissues isolated from individual animals were fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Paraffin sections (3  $\mu$ m) were cut on glass slides and stained with hematoxylin, eosin and periodic acid Schiff reagent and examined under a light microscope for pathological changes.

## **Results and Discussion**

The intended formulation comprises aqueous extracts of *M. charantia*, *C. tora*, *C. officinalis* and neem seed oil. The extracts and the neem seed oil have been used as medicinal agents traditionally since ancient time, and also in Indian system of medicine like Ayurveda. Aqueous extracts of these drugs have been reported to be safe both as internal and external dosage forms. Neem seed oil has also been used safely in various skin preparations [5,8,10,11].

The ratio of the aqueous extracts of all the three herbs and the neem seed oil was selected based on the dose of the individual extracts and the neem seed oil in the marketed formulations and the reported literature [1-14].

#### **Limit test 5,000 mg/kg body weight**

In principle, the method is not intended to allow the calculation of a precise LD<sub>50</sub>, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number to laboratory reporting consistency and repeatability. Limit test of higher dose i.e., 5,000 mg/kg b. wt. is mainly used in situations where the experimenter has information indicating that the test material is likely to be non-toxic [20].

Hence, the herbal mixture consisting of aqueous extracts of CT, MC, CO and AZoil was evaluated for oral toxicity in rats. Detailed behavioral, hematological, biochemical, histo-pathological, organ and weight analysis were also carried out and the results of test animals were compared to that of the control animals. Female rats have been selected because literature surveys and analyses conducted indicate that the two sexes usually respond similarly in acute oral toxicity tests, but when responses differ, females are generally more sensitive than males unless there is information suggesting that males are more sensitive for a given substance [17].

#### **Behavioral observations**

Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep and body weight of the treated as well as the control animal were found to be normal. Tremors, lethargy, diarrhea and coma were not observed (Tables 3 and 4).

**Table 3** Behavioral observations for control rats for the limit test at 5,000 mg/kg b. wt.

<b>Observations</b>	<b>30 min</b>	<b>4 hrs</b>	<b>24 hrs</b>	<b>48 hrs</b>	<b>1 wk</b>	<b>2 wks</b>
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioral pattern	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil

**Table 4** Behavioral Observations for test rats for the limit test at 5,000 mg/kg b. wt.

Observations	30 min	4 hrs	24 hrs	48 hrs	1 wk	2 wks
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioral pattern	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil

**Table 5** Effect of herbal mixture on the body weight of rats following single oral administration at concentration of 5,000 mg/kg b. wt.

Group	Dose level	Body weight (g) before treatment $M_1 \pm SD_1$ (N = 3)	Body weight (g) 14 days after treatment $M_2 \pm SD_2$ (N = 3)	Calculated 't' value	Remarks
Control	2 ml distilled water	135.00 $\pm$ 2.88	143.00 $\pm$ 1.78	t=2.359	NS
Treated	5,000 mg/kg herbal mixture	132.50 $\pm$ 2.50	140.00 $\pm$ 2.04	t=2.324	NS

$M_1$  and  $M_2$  = Sample mean value,  $SD_1$  and  $SD_2$  = Standard deviations of control and experimental group respectively.

N = number of rats, NS = Not significant.

### Body weight

The body weights of all the rats were increased after the oral administration of herbal mixture and the changes of the body weights were found to be statistically insignificant which are shown in Table 5. The gain in body weight of test animals indicates that the administration of the herbal mixture does not affect the growth of the animals.

### Organ weight analysis

Morphological observations of vital organs such as heart, kidney and liver indicated that there were no signs of any inflammation or toxicity in both control as well as in the test animals. The statistical results revealed that difference in the organ weights of control and the test was insignificant (Table 6).

### Hematological parameters

Hematological parameters of control and the test animals were found to be within the normal limits [18]. There was no significant change in all the tested hematological parameters in both controls as well as in test animals.

Hematological parameters tested such as total RBC, WBC, differential count and hemoglobin were estimated in the normal as well as in test animals on the 7<sup>th</sup> and the 14<sup>th</sup> day in order to study the effect of herbal mixture with respect of the composition of the blood. No significant changes in the hematological parameters of the test animals were observed with respect to control. This indicates that the mixture has no toxicity on the hematological parameters of the tested animals (Table 7).

**Table 6** Effect of herbal mixture on the organ weight of rats following single oral administration at concentration of 5,000 mg/kg b. wt.

Organs	Control organ weight (g)		Treated organ weight (g)	Calculated 't' value	Remarks
	$(M_1 \pm SD_1, n=3)$				
Liver	7.778 $\pm$ 0.107		7.778 $\pm$ 0.107	t=0.000	NS
Heart	20.120 $\pm$ 19.290		0.970 $\pm$ 0.011	t=0.993	NS
Kidney (right)	0.955 $\pm$ 0.057		0.815 $\pm$ 0.012	t=2.419	NS
Kidney (left)	1.023 $\pm$ 0.059		0.945 $\pm$ 0.024	t=1.208	NS

$M_1$  and  $M_2$  = Sample mean value,  $SD_1$  and  $SD_2$  = Standard deviations of control and experimental group respectively.  
n = number of rats. NS = Not significant

**Table 7** Effect of herbal mixture on hematological parameters of rat's blood after oral administration (5,000 mg/kg b. wt.)

Sl.No	Hematological parameters	Control rats $M_1 \pm SD_1$ (n=3)		Rats treated with herbal mixture		Calculated 't' value		Remarks	
		$M_2 \pm SD_2$ (n=3)							
		7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
1	Total RBC (Million/cc)	6.953 $\pm$ 0.0665	6.925 $\pm$ 0.334	7.025 $\pm$ 0.132	7.273 $\pm$ 0.169	t=0.0586	t=1.1580	NS	NS
2	Total WBC (Thousand/cc)	6.850 $\pm$ 0.250	7.025 $\pm$ 0.103	6.900 $\pm$ 0.265	7.000 $\pm$ 0.348	t=0.6472	t=0.2289	NS	NS
3	Differential leukocyte (%)								
	Neutrophil	66.750 $\pm$ 2.136	64.250 $\pm$ 2.323	67.250 $\pm$ 2.136	66.750 $\pm$ 1.250	t=0.7922	t=0.2020	NS	NS
	Lymphocytes	30.500 $\pm$ 3.403	34.000 $\pm$ 4.082	23.250 $\pm$ 1.702	27.000 $\pm$ 1.080	t=0.6585	t=1.8610	NS	NS
	Monocytes	2.250 $\pm$ 0.479	2.500 $\pm$ 0.289	3.000 $\pm$ 0.000	2.750 $\pm$ 0.250	t=0.4472	t=0.0000	NS	NS
	Eosinophil	2.500 $\pm$ 0.289	2.750 $\pm$ 0.479	2.500 $\pm$ 0.289	3.000 $\pm$ 0.408	t=0.4472	t=0.4472	NS	NS
4	Hemoglobin (%)	13.650 $\pm$ 0.206	14.100 $\pm$ 0.801	13.700 $\pm$ 0.208	14.530 $\pm$ 0.588	t=0.5440	t=1.5710	NS	NS

$M_1$  and  $M_2$  = Sample mean value,  $SD_1$  and  $SD_2$  = Standard deviations of control and experimental group respectively.  
n = number of rats. NS = Not significant

Analysis of hematological profile is an important parameter that can be useful in the detection of infection in the animal which may be due to various reasons including adverse effect or toxicity of the drugs. Increase in total cell count indicates bacterial infection. Neutrophil percentage increase is an indication of bacterial infection, while lymphocyte percentage increase indicates viral infection. Decreased hemoglobin suggests anemia, where as decreased WBC count suggests the decline in the functioning of immune system [19].

### Biochemical analysis

Biochemical parameters of both the test and the control animals were found to be within the normal limits [21]. There was no significant difference between the biochemical parameters of the control and test animals (Table 8).

These batteries of biochemical measurements can be used in the diagnosis of toxicity of the drugs on liver, heart and kidney, acid-base imbalance in the respiratory and metabolic systems, others involving lipid metabolism and various endocrine systems as well as other metabolic or nutritional disorders.



**Table 8** Effect of herbal mixture on biochemical parameters of rat's blood after oral administration (5,000 mg/kg b. wt.)

Sl.No	Biochemical parameters	Control rats $M_1 \pm SD_1$ (n=3)		Rats treated with herbal mixture $M_2 \pm SD_2$ (n=3)		Calculated 't' value		Remarks	
		7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
		1	Blood sugar (mg/dL)	62.000 $\pm$ 1.080	61.750 $\pm$ 4.008	61.500 $\pm$ 0.646	64.750 $\pm$ 1.652	t=0.3974	t=0.3856
2	Total cholesterol (mg/dL)	65.750 $\pm$ 2.839	53.500 $\pm$ 4.628	65.750 $\pm$ 2.839	56.750 $\pm$ 3.544	t=0.0000	t=0.5575	NS	NS
3	Creatinine (mg/dL)	0.800 $\pm$ 0.041	1.025 $\pm$ 0.095	0.800 $\pm$ 0.041	1.025 $\pm$ 0.132	t=0.0000	t=0.0000	NS	NS
4	Urea (mg/dL)	27.500 $\pm$ 1.500	43.25 $\pm$ 0.4787	29.00 $\pm$ 1.000	40.50 $\pm$ 1.708	t=0.8321	t=1.5500	NS	NS
5	Total bilirubin (mg/dL)	0.545 $\pm$ 0.160	0.975 $\pm$ 0.063	0.675 $\pm$ 0.048	0.850 $\pm$ 0.050	t=0.7771	t=1.5550	NS	NS
6	Direct bilirubin (mg/dL)	0.170 $\pm$ 0.006	0.9750 $\pm$ 0.063	0.175 $\pm$ 0.005	0.850 $\pm$ 0.050	t=0.6547	t=1.5550	NS	NS
7	Total protein (gm/dL)	6.600 $\pm$ 0.091	5.975 $\pm$ 0.111	6.450 $\pm$ 0.104	6.075 $\pm$ 0.138	t=1.0830	t=0.5657	NS	NS
8	SGOT (IU/L)	47.750 $\pm$ 8.750	44.250 $\pm$ 1.931	47.750 $\pm$ 8.750	50.000 $\pm$ 4.082	t=0.0000	t=1.2730	NS	NS
9	SGPT (IU/L)	43.250 $\pm$ 5.808	50.750 $\pm$ 2.175	54.500 $\pm$ 10.880	52.750 $\pm$ 4.423	t=0.9121	t=0.4058	NS	NS
10	Alkaline phosphatase (IU/L)	65.750 $\pm$ 1.493	65.000 $\pm$ 1.780	65.000 $\pm$ 1.780	71.000 $\pm$ 3.416	t=0.3229	t=1.5580	NS	NS
11	Acid phosphatase (IU/L)	44.750 $\pm$ 1.750	52.230 $\pm$ 2.687	47.480 $\pm$ 1.632	50.500 $\pm$ 4.193	t=1.1390	t=0.3464	NS	NS

$M_1$  and  $M_2$  = Sample mean value,  $SD_1$  and  $SD_2$  = Standard deviations of control and experimental group respectively.  
n = number of rats, NS = Not significant.

There was no significant difference in the levels of any of the tested hematological and biochemical parameters of test animals with respect to that of control animals. Hematological and the biochemical parameters of test and control animals were well within the normal limits. [18, 21] Hence, the study suggests that the herbal drug mixture has not altered the functioning of various systems and vital organs. Hence, the herbal drug mixture can be considered as non-toxic when given orally up to the dose of 5,000 mg/kg b. wt.

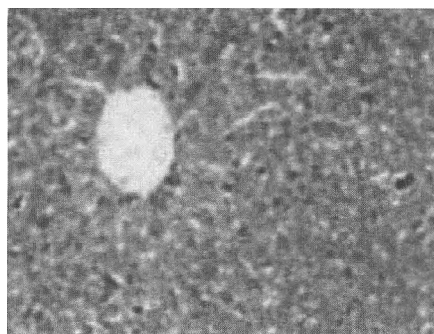
### **Histopathology of organs**

The histopathological studies of liver, kidney and heart of both control and experimental rats were performed after oral administration of the drugs for 14 days (Table 9). No detectable differences in the histopathology of these organs of control and the herbal mixture treated rats were observed when viewed under oil immersion objective. This indicates that the tested herbal mixture has no effect on cellular structure, i.e., the herbal mixture does not cause degeneration of the cells of these organs (Figures 1-3).

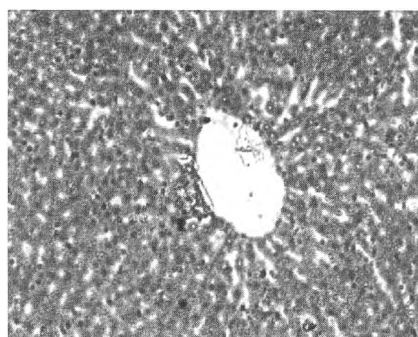
**Table 9** Effect of herbal mixture on histology of rat's heart, kidney and liver tissue after oral administration at dose 5,000 mg/kg b. wt.

Sl.No	Group	Drug	Dose (Oral)	Histo-pathological changes observed		
				Heart	Kidney	Liver
1	Control	Distilled water	2 ml/rat	NAD	NAD	NAD
2	Test	Herbal mixture	5,000 mg/kg	NAD	NAD	NAD

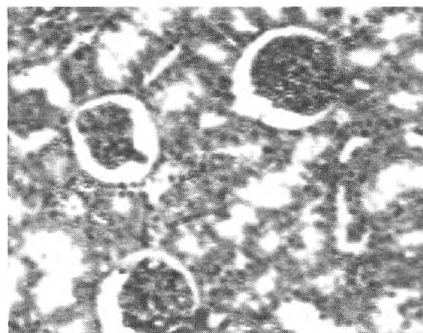
NAD = No abnormality detected.



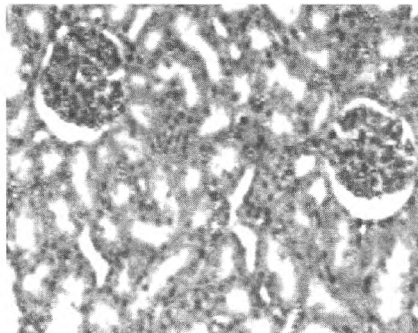
(a)



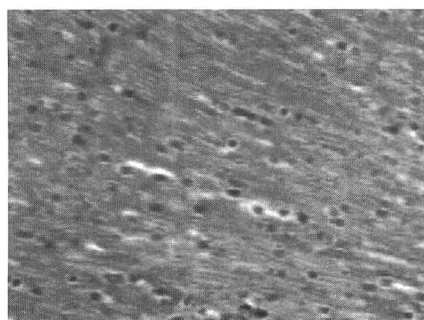
(b)

**Figure 1** Photomicrograph showing normal liver architecture (a) and liver of rat receiving herbal mixture at dose of 5,000 mg/kg b. wt. (b). (Magnification 10X).

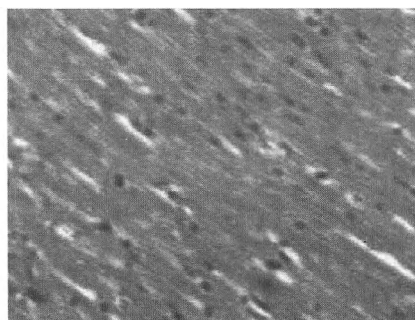
(a)



(b)

**Figure 2** Photomicrograph showing normal kidney architecture (a) and kidney of rat receiving herbal mixture at dose of 5,000 mg/kg b. wt. (b). (Magnification 10X).

(a)



(b)

**Figure 3** Photomicrograph showing normal heart architecture (a) and heart of rat receiving herbal mixture at dose of 5,000 mg/kg b. wt. (b). (Magnification 10X).

## Conclusion

Combined herbal extract comprising mixture of aqueous extracts of flowers of *Calendula officinalis*, seeds of *Momordica charantia* and *Cassia tora* and *Azadirachta indica* seed oil did not exhibit acute toxicity when given orally at concentration of 5,000 mg/kg body weight. This was evident by the normal behavior, hematological, biochemical and histological parameters evaluated to study the toxic potential of the herbal mixture. Hence the above said herbal mixture could be safe up to the dose of 5,000 mg/kg b. wt.

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