Research Article

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Toxicological Studies on Ayurvedic Formulation *Mersina* in Albino Rats

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Polyherbal formulations are an important Ayurvedic preparations widely used in Indian traditional system of medicine and also in many countries by different tribal. Mersina is an antidiabetic Ayurvedic formulation. Despite its wide use, no study has been published in the scientific literature about its toxicological profile. In present study the formulation was evaluated for the acute and repeated dose toxicity study. In the acute toxicity test, the albino rats were treated with Mersina (300mg/kg, 2000mg/kg and 5000mg/kg), orally. Animals were observed periodically during the first 24 hours and daily thereafter, for 14 days, after administration of the formulation. Mersina produced neither mortality nor changes in behaviour or any other physiological activities in rats, at all selected doses. In repeated dose toxicity study, Mersina (300mg/kg, 1000mg/kg and 2000 mg/kg per day) was administered orally for a period of 28 days in albino rats. The effects on body weight, food and water consumption, organ weight, hematology, clinical biochemistry as well as histology were studied. There were no significant differences in the body weight, organ weights and feeding habits between control and treated animals. Hematological analysis showed no marked differences in any of the parameters examined in either the control or treated groups. Except slight hypoglycemia there were no significant changes occurred in the blood chemistry analysis including cholesterol, triglycerides, blood urea nitrogen, and creatinine, AST, ALT and ALP in experimental animals. Pathologically, neither gross abnormalities nor histopathological changes were observed. The formulation Mersina was found safe in acute and repeated dose toxicity studies.

Keywords: Mersina; Poly herbal formulation; Traditional medicine; acute toxicity; repeated dose toxicity.

INTRODUCTION

Herbal medicines are popular remedies for diseases used by a vast majority of the world's population. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual. Presently there is growing interest in antidiabetic, nootropic, hepatoprotective and lipid lowering agents. Plant remedies have been and are being used by diabetic patient's throught the world. Studies suggest that using an antidiabetic plant in whole form or as complex extracts may offer many benefits due to the presence of multiple active components. Various herbal formulations such as Coagent db [1], Hyponidd [2] and Diasulin [3] are well known for their antidiabetic effects. The pharmacological effects of many plants have been studied in various laboratories, where as there are many limitations regarding the safety and efficacy of these formulations [4]. Mersina, a polyherbal antidiabetic formulation of ten ingredients is used in traditional medicine to treat type II diabetes, contains both antidiabetic and antioxidant principles (Table1). Gymnema sylvestre, Momordica charantia, Syzium cumini, are proven antidiabetic drugs that are alone or in combination act to control the hyperglycemia in diabetes [5-8]. Phyllanthus emblica, Trigonella foenum graceum, Coccinia indica, Tinospora cordifolia, Melia azadarichta and Javakhar are proven antioxidant drugs that protects from the oxidative stress of free radicals [9-12].

In the present study, the acute and repeated dose oral toxicity study of Mersina, a polyherbal formulation, were investigated in experimental animals.

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MATERIALS AND METHODS

Animals

Wistar rats (180 – 210g) of either sex were used in the study. They were maintained at a room temperature of 23 ± 2º C with 12- h light/dark

cycle and 45-55% relative humidity. The animals had free access to Amruth brand standard pellet diet (Nav Maharashtra Chakan Oil Mills Ltd. India) and water *ad libitum*. The animals were fasted overnight but were allowed free access to water. The experimental protocols were approved by Institutional Animal Ethics Committee (CPCSEA/SPTM/P-18/2008 and CPCSEA/SPTM/P-19/2008).

Table 1. Composition of Mersina

Botanical name	Common name	Family	Quantity/500 mg
Gymnema sylvestre	Ram's horn	Asclepiadaceae	75 mg
Momordica charantia	Bitter gourd	Cucurbitaceae	81 mg
Cassia auriculata	Tanner's cassia	Caesalpinaceae	63 mg
Syzium cumini	Jamun	Myrtaceae	96 mg
Phyllanthus emblica	Indian gooseberry	Euphorbiaceae	48 mg
Trigonella foenum gr.	Fenugreek	Fabaceae	50 mg
Coccinia indica	Little gourd	Cucurbitaceae	93 mg
Tinospora cordifolia	Gulancha	Menispermaceae	63 mg
Melia azadarichta	Neem	Meliaceae	30 mg
Javakhar	Yava-kashar	----	12 mg

Table 2. Sign of toxicity, mortality results of acute toxicity observations of Mersina in albino rats

Group	Dose (mg/kg)	Sign of toxicity (ST/NB)[a]	Mortality (D/S)[a]
Group I	0	0/3	0/3
Group II	0	0/3	0/3
Group III	0	0/3	0/3
Group IV	0	0/3	0/3

ST:sign of toxicity; NB:normal behaviour; D: died; S: survive.
[a]Values are expressed as animal numbers.

Drug and chemicals

Antidiabetic Ayurvedic formulation Mersina, developed by J & J De Chane Laboratories Private Limited, Hyderabad, is obtained as a gift sample.

Preparation of the herbal formulation

The herbal formulation was administered orally at various doses, as a suspension in 1% (w/v) Carboxyl Methyl Cellulose (CMC) prepared using mortar and pestle.

Acute Toxicity Studies

The acute toxicity of Mersina was evaluated in rat as per OECD Guideline 423 [13]. Three groups containing three female rats (weight: 180–210 g, age: 6–8 weeks) received Mersina at doses of 300 mg/kg, 2000mg/kg and 5000mg/kg body weight, orally by gavage. Animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Cage Side Observations

Observations included changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. A special attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Body Weight, Food and Water Intake

The change in body weight, food and water intake was recorded at two days interval.

Pathology

Overnight fasted surviving animals were weighed and humanely killed on day 15 using anesthetic ether. All test animals were subjected to gross necropsy.

Repeated Dose Toxicity Studies

Repeated toxicity studies were conducted as per OECD 407 Guidelines [14], on four groups of rats (0 mg/kg control, 300mg/kg low dose, 1000 mg/kg medium dose and 2000 mg/kg high dose), each containing five males and five females. Whilst Mersina was orally administered using gavage to test groups, distilled water was administered to control group for 28 days. The maximum volume administered was not greater than 2 ml/100 g body weight. All animals were supplied with standard food and water *ad libitum* during the testing periods. All rats were observed daily for toxic manifestations and mortality.

Body Weight, Food and Water Intake

Changes in body weight, water and food intake were measured once a week.

Table 4. Effects of 28 day oral administration of Mersina on [1]Hepatic function, [2]metabolism function and [3]kidney function tests in rats

	Female				Male			
	Control	Mersina (mg/kg)			Control	Mersina (mg/kg)		
	(1% CMC)	300	1000	2000	(1% CMC)	300	1000	2000
[1]SGPT	47.41	48.19	49.32	47.67	48.16	47.31	48.52	49.25
(IU/L)	±0.81	±1.74	±1.64	±1.38	±0.56	±1.40	±1.27	±1.43
[1]SGOT	68.25	66.19	67.23	67.16	68.51	67.25	68.32	68.65
(IU/L)	±0.16	±0.35	±1.71	±0.42	±0.22	±0.57	±0.68	±0.36
[1]ALP	134.64	132.11	133.73	133.42	135.75	134.16	135.41	134.25
(IU/L)	±1.37	±1.62	±1.91	±1.52	±2.21	±1.67	±1.29	±1.36
[2]Chole	91.3	90.5	90.7	91.12	92.34	91.47	92.31	91.15
(mg/dl)	±0.34	±0.81	±0.29	±0.15	±0.44	±0.30	±0.23	±0.25
[2]TG	57.1	58.3	56.7	57.5	59.2	58.4	58.7	59.3
(mg/dl)	±0.62	±0.48	±0.39	±0.77	±0.92	±0.53	±0.68	±0.73
[2]Glucose	68.19	64.38	66.76	67.25	65.21	68.35	64.22	64.51
(mg/dl)	±1.33	±1.23	±1.41	±1.15	±1.57	±0.83	±1.13	±1.06
[3]Creatinine	2.2	2.1	2.1	2.2	2.3	2.1	2.2	2.4
(mg/dl)	±0.25	±0.15	±0.55	±0.42	±0.13	±0.26	±0.34	±0.71
[3]BUN	18.31	17.60	17.29	17.73	18.14	17.12	19.41	18.35
(mg/dl)	±1.28	±1.66	±1.16	±1.09	±1.22	±1.62	±1.45	±1.21

Data are expressed as mean ± S.E., n = 5. No statistical difference between control and Mersina ($p < 0.05$)

a.Serum glutamate pyruvate transaminase , b.Serum glutamate oxalacetate transaminase c Alkaline phosphatase , a Cholesterol, bTriglyceride

Table 5. Effect of Mersina on Organ weights (g) in *rats* in repeated dose toxicity *study*

		Male				Female		
Organ	Control	Mersina (mg/kg)			Control	Mersina (mg/kg)		
		300	1000	2000		300	1000	2000
Brain	1.58	1.62	1.6	1.6	1.61	1.59	1.6	1.61
	± 0.12	± 0.15	± 0.13	± 0.14	± 0.18	± 0.16	± .014	± 0.18
Heart	0.62	0.61	0.61	0.63	0.62	0.62	0.63	0.64
	± 0.07	± 0.11	± 0.13	± 0.08	± 0.12	± 0.25	± 0.32	±0.15
Liver	10.12	10.14	10.18	10.15	10.14	10.15	10.16	10.18
	± 0.65	± 0.29	± 0.42	± 0.24	± 0.19	± 0.13	± 0.28	± 0.33
Kidney	1.5	1.49	1.49	1.51	1.52	1.52	1.53	1.54
	± 0.13	± 0.16	± 0.21	± 0.14	± 0.20	± 0.18	± 0.30	± 0.57
Spleen	0.71	0.72	0.71	0.7	0.71	0.72	0.72	0.73
	±0.12	± 0.16	± 0.30	± 0.26	± 0.38	± 0.29	± 0.22	± 0.23
Pancreas	1.3	1.33	1.32	1.31	1.33	1.32	1.33	1.34
	± 0.15	± 0.24	± 0.17	± 0.20	± 0.12	± 0.18	± 0.22	± 0.16
Testes	2.9	2.8	2.9	2.9	-	-	-	
	± 0.06	± 0.05	± 0.04	± 0.05				
Ovaries	-	-	-		0.14	0.13	0.13	0.14
					± 0.06	± 0.05	± 0.04	± 0.08

Data are expressed as mean ± S.E., n = 5. ($p < 0.05$).

Hematology

Hematological analysis was performed using an automatic hematological analyzer (Sysmex, Japan). Hemoglobin, hematocrit, total red blood corpuscles (RBC), total white blood corpuscles (WBC), platelets and red cell indices viz., packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of blood samples were recorded.

Clinical Biochemistry

Cholesterol, triglycerides (TGL), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), Blood urea nitrogen (BUN), creatinine, and glucose were analysed, using an autoanalyzer (Erba Chem 7, Germany).

Pathological Examination

Gross Necropsy

All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The brain, heart, liver, kidney, spleen and pancreas of all animals were removed and their wet weights were taken immediately after dissection to avoid drying.

Histopathology

Brain, heart, liver, kidney, spleen and pancreas were fixed immediately in 10% formalin for routine histopathological examination. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined under light microscope. Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provided with MOTIC IMAGES PLUS 2.0 software.

Statistical Analysis

The differences among experimental and control groups were determined using the SPSS 16 statistical software for Windows. Comparisons among different groups were performed by analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by student's *t*-test. All data are expressed as mean ± standard error of mean (S.E.M.); *p*-values less than 0.05 were considered to be significant.

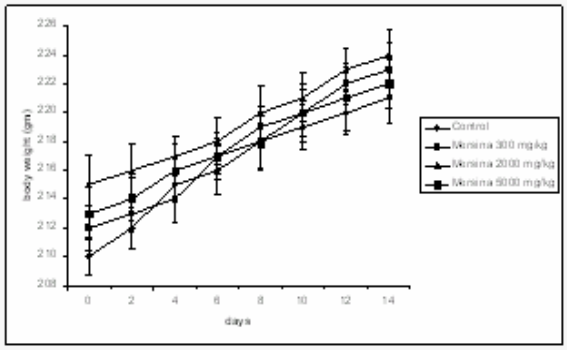

FIGURE 1. Effect of Mersina on body weight changes in rats.
Each point represents mean ± S.E.M n=3

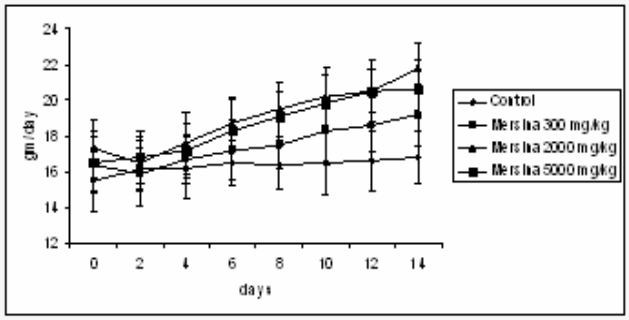

FIGURE 2. Effect of Mersina on food intake in rat.
Each point represents mean ± S.E.M,n=3

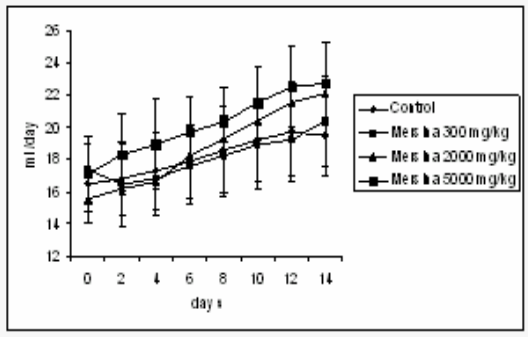

FIGURE 3. Effect of Mersina on water intake in rat.
Each point represents mean ± S.E.M,n=3

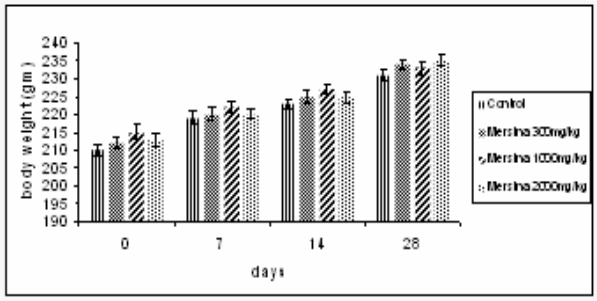

FIGURE 4. Effect of Mersina on body weight changes in male rat.
Each point represents mean ± S.E.M,n=5

RESULTS

Acute Toxicity Studies

There was no mortality or morbidity observed in animals through the 14-day period following single oral administration at all selected dose levels of Mersina (Table2). The LD_{50} value for oral administration of Mersina is larger than 5000 mg/kg body weight. The animals did not show any changes in the general appearance during the observation period. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviours such as self mutilation, walking backward and so forth were observed; gait and posture, reactivity to handling or sensory stimuli, grip strength were all normal. There was no significant difference in body weights between control and treatment groups (Figure 1). Food and water intake showed daily fluctuations within the range of control animals (Figure 2 and 3).

Repeated Dose Toxicity Studies

Body Weight, Food and Water Intake

There was no significant difference in body weights between control and treatment groups (Figure 4 and 5) of either sex. There was no significant difference between food and water consumption of treated animals compared with control (Figure 6 to 9).

Hematology and Clinical Biochemistry

The effect of repeated dose oral administration of Mersina on hematological parameters is presented in Table 3. The hematological analysis showed no significant changes in hemoglobin, hematocrit, red blood cells, and white blood cells in male and female treatment group compared to the control group.

The effect of repeated dose oral administration of Mersina on biochemical parameters is presented in Table 4. Except slight hypoglycemic effect there were no significant differences observed in any of the biochemical parameters examined in either the control or treated group of the male and female rats.

Pathological Examination

There were no significant differences between the control and treated groups in the organ

weights of male and female rats (Table 5). No alterations were detected in pathological examinations of the tissues during the microscopic examination of the internal organs. The findings were generally consistent with the expected pattern for Wistar rats of this particular age. Histopathological observations further support the safety of Mersina (Figure 10).

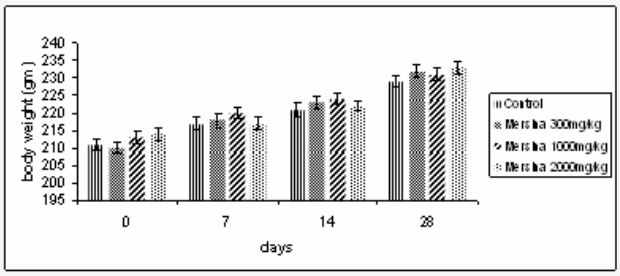

FIGURE 5. Effect of Mersina on body weight changes in female rat.
Each point represents mean ± S.E.M, n=5

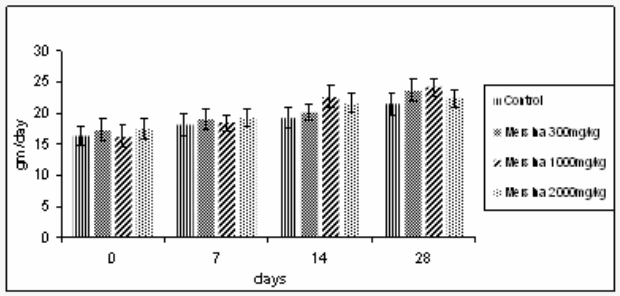

FIGURE 6. Effect of Mersina on food intake in male rat.
Each point represents mean ± S.E.M, n=5

DISCUSSION

To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in human. A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-

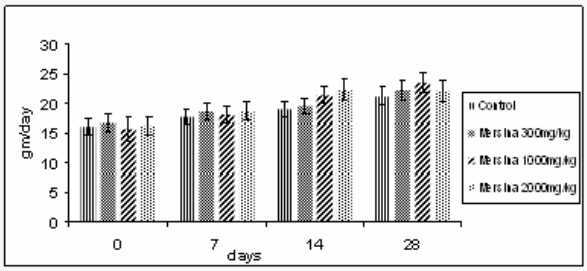

FIGURE 7. Effect of Mersina on food intake in female rat.
Each point represents mean ± S.E.M, n=5

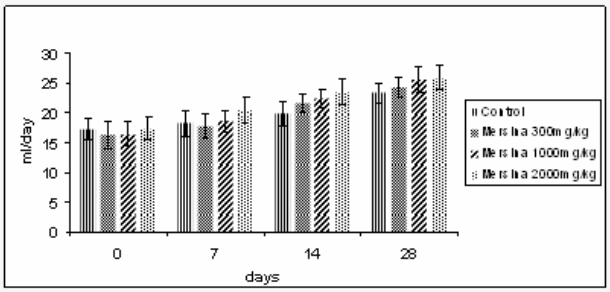

FIGURE 8. Effect of Mersina on water intake in male rat.
Each point represents mean ± S.E.M, n=5

conventional medicine, mainly of herbal source, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people [15]. Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for Mersina.

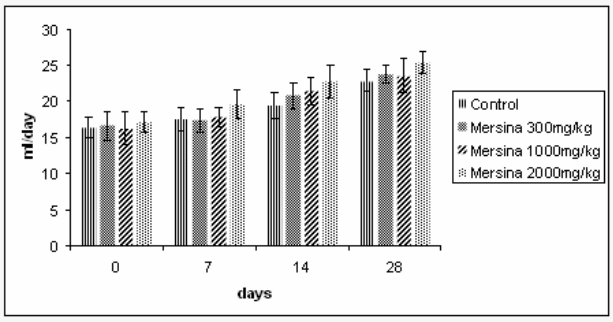

FIGURE 9. Effect of Mersina on water intake in female rat.
Each point represents mean ± S.E.M, n=5

Usually acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds. As there was no previous report on toxicity of Mersina, as per OECD guidelines three dose levels starting at 300mg/kg, 2000mg/kg and limit dose 5000mg/kg were selected for acute toxicity study. No mortality was observed in both control and in

groups of all selected dose levels. Animals in all groups did not exhibit any signs of adverse effect (NOAEL). Thus the LD_{50} of the formulation is greater than 5000mg/kg.

Dose selection for repeated dose toxicity studies was based on the results of acute toxicity studies. During single dose toxicity studies it was observed that Mersina was safe at dose of 2000 mg/kg. Therefore, this dose was used in repeated dose toxicity study as the highest dose.

In repeated dose toxicity study, the formulation was given orally at doses upto 2000mg/kg in rats. There was no change in animal behaviour and the changes in body weight were not significantly different in treated rats as compared to controls. Since, the changes in body weight have been used as an indicator of adverse effects of drugs and chemicals [16-18], the present results suggest that at the dose levels administered, Mersina is non-toxic in rats.

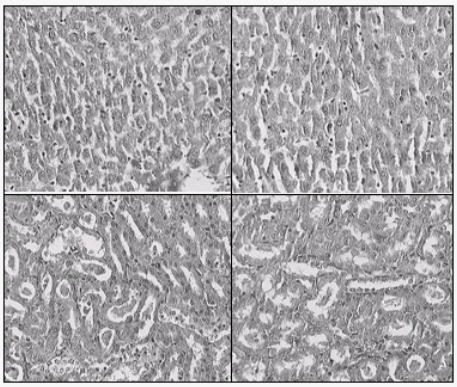

FIGURE 10. Histopathological analysis of organs treated with Mersina (Repeated Dose toxicity study): (a) liver from control group; (b) liver from Mersina 2000 mg/kg body weight treated group; (c) kidney from control group; (d) kidney from Mersina 2000 mg/kg body weight treated group.

In addition, determination of food consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients are essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional conditions [19]. In the present study, Mersina treated rats did not show significant differences in food consumption and also in water consumption when compared with rats from control group.

Analysis of blood parameters is relevant to risk evaluation as the changes in hematological

Table 3. Effects of 28 day oral administration of Mersina on Haematological parameters in rats

	Female				Male			
	Control	Mersina (mg/kg)			Control	Mersina (mg/kg)		
	(1% CMC)	300	1000	2000	(1% CMC)	300	1000	2000
RBC[a]	7.6± 0.29	7.51± 0.96	7.43 ± 0.88	7.32± 0.37	8.41± 0.41	8.52± 0.59	8.16 ± 0.32	8.24 ± 0.24
WBC[b]	7.4± 0.68	7.2± 0.70	7.5± 0.84	7.3± 0.55	7.7± 0.43	7.8± 0.31	7.4± 0.57	7.6 ± 0.42
Eos[c]	1.0± 0.51	1.0± 0.74	0.97± 0.49	1.0±0.37	1.0± 0.19	1.0± 0.26	0.9± 0.16	1.0± 0.21
Mono[d]	2.0± 0.45	2.1± 1.06	2.2± 0.83	2.190.62	2.34± 0.55	2.38± 0.74	2.41± 0.62	2.46± 0.35
Lymph[e]	70.39± 2.24	71.4± 2.5	72.11± 1.9	71.27± 2.3	73.52± 1.88	71.61± 1.43	73.23± 1.14	72.86± 1.47
Neut[f]	25.15± 2.36	23.24± 1.9	24.73± 1.66	22.36±1.84	22.28± 2.2	24.05± 1.43	23.28± 1.74	24.11± 1.52
PT[g]	11.09± 2.44	12.35± 0.42	11.5 ± 1.67	11.64± 1.28	11.08± 0.91	11.24± 0.52	11.73± 1.32	11.86± 1.06
Hb[h]	15.2± 0.15	15.1± 0.21	15.3± 0.19	15.2±0.33	15.5± 0.24	15.8± 0.20	16.1± 0.23	16.2± 0.40
HCT[i]	46.14± 0.29	47.25± 0.43	46.54± 0.39	46.85± 0.17	45.73± 0.65	45.32± 0.52	45.45± 0.25	46.02± 0.47
MCV[j]	52.32± 0.62	52.65± 0.48	52.11± 0.60	51.74±0.52	49.42± 0.81	50.65± 0.39	50.620.46	49.72± 0.67
MCH[k]	17.8± 0.38	17.5± 0.24	18.1± 0.41	17.6±0.52	18.4± 0.36	18.7± 0.69	18.5± 0.77	19.2± 0.46
MCHC[l]	32.5± 0.69	32.1± 1.21	32.7± 0.74	33.1 0.57	33.8± 0.86	33.4± 0.70	33.2± 0.42	34.3± 0.28
PLT[m]	1184± 36.41	1196±43.71	1164±24.14	1175±30.92	1216±29.61	1245±32.83	1239±40.26	1272±37.17

Data are expressed as mean ± S.E., n = 5 ($p < 0.05$). [a] Red blood cell (x 106 mm 3); [b] White blood cell (x 103 mm 3); [c] Eosinophil (%); [d] Monocyte (%);
[e] Lymphocyte (%); [f] Neutrophil (%); [g] Prothrombin time (in minutes); [h] Haemoglobin, [i] Hematocrit [j] Mean corpuscular volume (FL);
[k] Mean corpuscular haemoglobin (pg); [l] Mean corpuscular haemoglobin (%);concentration (g/dl); [m] Platelet (x 10^3 mm^{-3});

system have a higher predictive value for human toxicity, when data are translated from animal studies. After 28 days treatment, there were no significant changes in the hematological parameters between control and treated groups. Levels of white blood cells, red blood cells, and hemoglobin were not significantly different between control and test groups following repeated administration of Mersina. The results indicate that Mersina was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals [20]. Therefore, it possible to assume Mersina is not hematotoxic.

Significant changes in enzymes like ALP, AST and ALT represent liver impairment, since these are important indices of liver toxicity [21]. Serum cholesterol and proteins are mainly regulated via synthesis in the liver and increase or decrease in serum concentrations of constituents suggests some alterations in liver functions. As these enzymes and biochemical parameters were not altered by administration of Mersina after administration for 28 days indicating that there may be no severe liver damage.

Renal functions markers like urea and creatinine plasma levels [22-23] remained normal after administration of Mersina at all selected dose levels. Thus it can be stated that Mersina does not show any renal toxicity.

In conclusion, at the oral doses tested, the formulation was well tolerated and neither produced overt signs of clinical toxicity nor any signs of hepato-, nephro or haematotoxicity. Thus Mersina was found to be nontoxic when oral acute and repeated dose toxicities were performed. Overall, this study provides valuable data on toxicity profile of Mersina that should be useful for the planning of future preclinical and clinical studies of the formulation.

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