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ACUTE AND SUB-CHRONIC TOXICITY ASSESSMENT OF *CARALLUMA TUBERCULATA* PLANT COLLECTED FROM DISTRICT SHERANI OF BALOCHISTAN, PAKISTAN

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Abstract

World Health Organization revealed that almost 80% of rural people use herbal drugs as remedies for various diseases in humans and animals. Caralluma tuberculata, a member of the Asclepiadaceae family, has been used by local herbalists as an anti-ulcer, neuroprotective, antipyretic, anti-inflammatory, anti-nociceptive, and antioxidant drug. Keeping in view the importance and use of herbal medicines in remote areas and the scarce knowledge about these drugs, Methanolic crude extract of C. tuberculata (MCEC) was studied during the current study for a 20- and 90-day trial for acute and sub-acute toxic effects in mice and rabbits, respectively. The results revealed that the methanolic crude extract of C. tuberculata was safe at a dose up to 2000 mg/kg, which had no adverse effects on the experimental mice during 20 days, and up to 500mg/kg body weight for 90 days in rabbits as a sub-acute trial.

Keywords: *Acute and chronic Toxicity, Caralluma tuberculata, Crude Extract, Methanol, Sherani*

INTRODUCTION

World Health Organization reported that almost 80% of the rural people use traditional drugs for the prevention and treatment of various diseases of human beings and animals (1). The people of remote area, particularly in the developing countries, use traditional medicines as a safe natural source for their daily health care (2). The extract form of the herbal remedies has been used, usually, for the treatment of various health problems by these rural inhabitants (3).

The user of these herbal medicines are usually the traditional herbalists, having very little knowledge of new and modern health development, commonly they use their own skills and experience for the health recoveries (4). The assessment of some therapeutic plants obviously confirms the efficacy and consistency of ethno-medical information and old-fashioned practices of herbal plants in diseases cure (5). It is therefore needed to encourage the native healthcare knowledge, to strengthen the traditional therapists, and also improve the coordination between the new and old systems of healthcare (6). The current medication has gradually developed during the near fast due to the research efforts in this field, whereas this side is very poor in the traditional drugs (24). Although, the use of the traditional drugs is increasing day by day, with the concept of their naturalness and nontoxic or low toxic effects, but their dosages and efficacy still needs to be known (7).

The genus *C. tuberculata* belongs to the family Asclepiadaceae (also known as the milk weed family) which comprises some 200 genera and 2500 species (8). It is a plant species possessing a great therapeutic potential in folk medicine. The medicinal properties of *C. tuberculata* species include anti-hyperglycemic activity of *C. tuberculata.* This genus has been extensively explored for a variety of pharmacological activities

as compared to other species, but not all species were tested for their biological activity (9). While *C. tuberculata* also offered protection against mucosal damage of stomach. Several members of genus *C. tuberculata* have found various medicinal uses because of following properties such as antidiabetic, anti-hyperglycemic, anti-parasitic, anti-trypanosomal, anti-ulcer, neuroprotective, antipyretic, anti-inflammatory, antinocicepetive, antioxidant, antiobesogenic, and antiartherosclerotic (10). Locally known as "Daghmous," "Zakkum," or "Tikiwt", *C. tuberculata* is a plant species commonly used in traditional medicine for their presumed anticancer activity, thus, *C. tuberculata* has been studied for its chemical constituents (11).

The importance and use of *C. tuberculata* in the study area is almost common, but the efficacy, preparation and dosage regarding its toxic level is still needed to highlight therefore the present in vivo study was intended to estimate acute and sub- acute toxic effect of Methanolic Crude Extract of *C. tuberculata in mice and rabbits.*

METHODOLOGY

SELECTION OF PLANT MATERIAL

The aerial part of *C. tuberculata* was collected from Khotta village near the Sulaman range, and plant taxonomist authenticated at Pharmacognosy Department, (Pharmacy) University of Balochistan, Pakistan. A voucher specimen of the plant sample was deposited in the Pharmacy Faculty for reference.

EXTRACTION ASSAY

Aerial parts of *C. tuberculata* weighing 5 kg, were chopped and crushed by using the mixer and extracted with Methanol at room temperature, then concentrated in a rotating void evaporator at 40°C. The isolated material was stored at −20°C until used. The crude extract was dissolved in water at a desired concentration, for oral administration (gavages) (12).

ANIMAL HANDLING

Adult male mice and rabbits weighing from 21 to 25g (mice) and 1500 to 1700 g (rabbits) respectively were used in this study. Initially the animals were kept for one week as an adaptation period. They were kept at constant temperature and allowed for food and water ad libitum. They were nourished on grass and carrots (13).

EXTRACT PREPARATIONS

The dried extract was suspended in distilled water for pharmacological studies. The doses of C. tuberculata crude extract were determined according to the trial. It was prepared freshly before being used every day (14).

ORAL ACUTE TOXICITY STUDY IN MICE

The assessment of acute toxicity was performed according to the WHO and the Organization for Economic Cooperation and Development (OECD) guideline (15). To conduct the study approval of the Animal Ethics Committee (AEC) was received from the University of Balochistan.

The Methanolic crude extract of *C. tuberculata* (MCECT) was administrated in single oral dose of extract i.e., 100 to 2000mg/kg while the control group received distilled water (5 mice/group). Signs of toxicity and mortality have been recorded at the 1st, to 6th hours after oral administration and then once daily for 20 days (16).

STUDY OF CHRONIC (90 DAYS) ORAL TOXICITY IN RABBITS

Rabbits were divided into two groups (five animals per group) and kept in isolated cages during the study. The MCECT was given orally by pump to different groups daily at 500mg/kg body weight dose for 90 successive days, while the control group was on distilled water. Animals were closely watched daily for an overall conduct and toxicity signs during the trial period. After 90 days the rabbits were sacrificed, and blood was collected in anticoagulant containing tubes (17).

BIOCHEMICAL STUDY

WBC, RBC, Hb and Platelet Count (PLT) were calculated from the blood samples (18). Aspartate amino transferase, Alanine amino transferase, Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Creatinine Kinase Iso-Enzyme MB, Serum Albumin, Bilirubin, Total Protein, Glucose, Urea, Creatinine, Lipid Function Test and Lipid Profile were analyzed using Standardized diagnostic kits (Labkit)on a spectrophotometer (19).

STATISTICAL ANALYSIS

Results during the current study were shown as per mean ± standard error of mean (SEM), student t-test was performed for statistical analysis. Whereas p value was measured significant at $p<0.05$.

RESULTS AND DISCUSSION

ACUTE TOXICITY

A single oral dose of the MCECT extract did not cause any deaths neither did the mice show any signs of toxicity at dose levels used (250, 2000, mg/kg). The toxic dose (mice, oral) is therefore estimated to be beyond 2000mg/kg body weight MCECT (stem). The oral lethal median dose (LD50) of the extract was estimated to be greater (20).

BIO-CHEMICAL PROFILE OF MCECT

Blood Glucose (Random) was 110. + 1.307), were determined with an automated hematology (21) for control and 67.6+4.840 for extract treated rabbit, Urea was 0.46+0.152 for control and 56.8+1.322 for MCECT given group, Calcium serum was 11.1+0.190 for control and 9.036+0.0129 for MCECT given group, Phosphorus serum was 5.042+0.037for control and 5.036+0.1126 for MCECT given group, Uric Acid serum was 0.46+0.152 for control and 0.25+0.112for MCECT given group, Creatinine serum was 0.054+0.0375 for control and 0.1+0.044 for MCECT given group (Table I).

Table I. Chronic toxic activity and the effects of MCECT on Bio-Chemical Profile

	Blood Glucose Random	Urea	Uric Acid	Calcium serum	Phosphorus	Creatinine
Control	110.±1.307	0.46±0.152	0.46±0.152	11.1±0.190	5.042±0.037	0.054±0.0375
MCECT	67.6±4.840	56.8±1.322	0.25±0.112	9.036±0.0129	5.036±0.1126	0.1±0.044

Values are the mean number of activities ± standard errors mean, (n=5), ($P<0.05$)

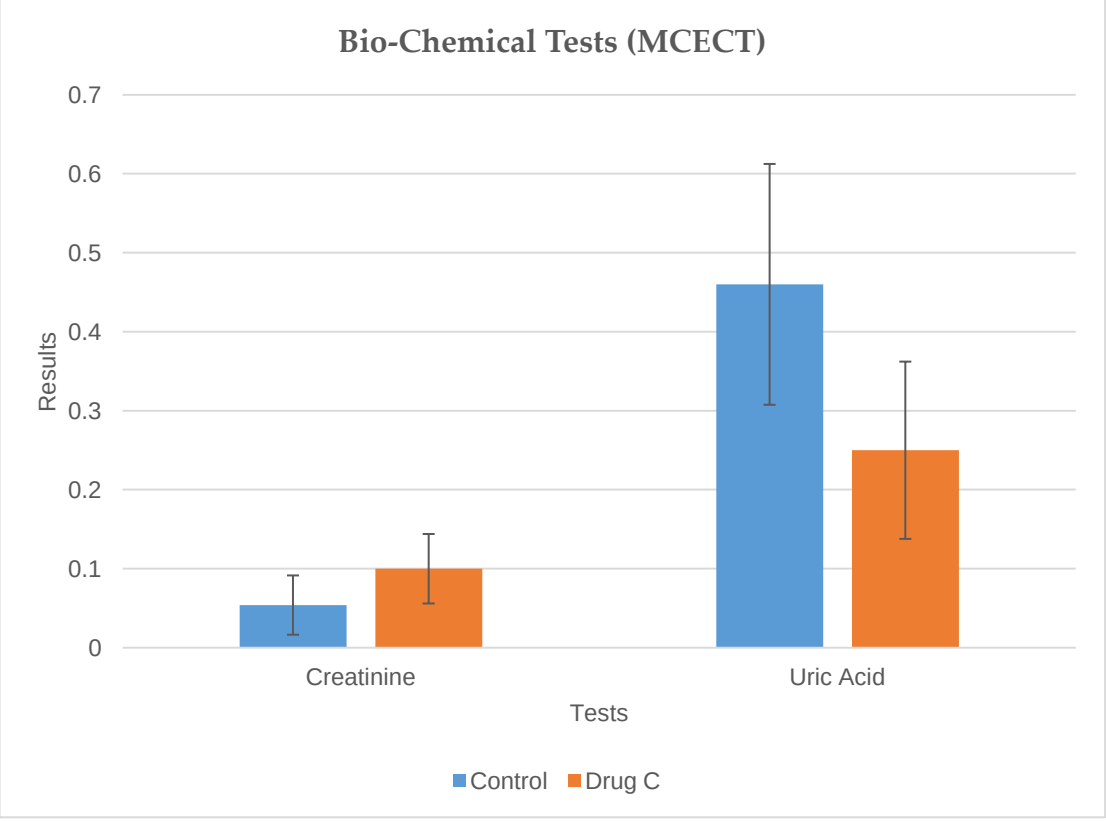

Fig. 1. Chronic toxic activity and the effects MCECT on Bio-Chemical Profile

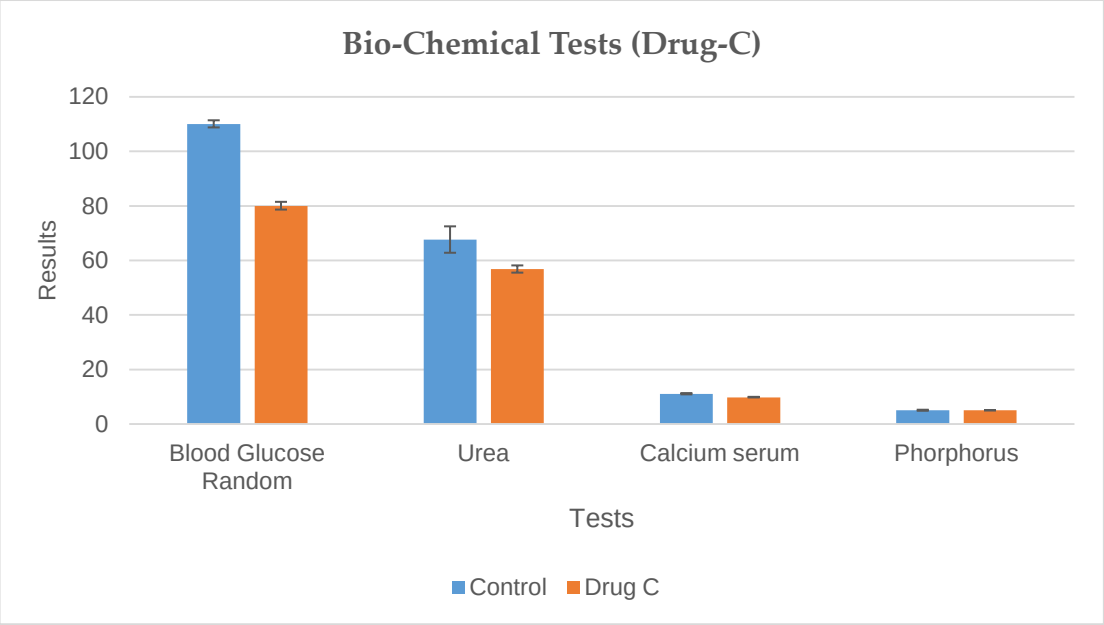

Fig. 2. Chronic toxic activity and the Effects of MCECT on Bio-Chemical Profile

MCECT EFFECTS ON CARDIAC ENZYME

For control LDH (U/L) level was 293± 6.620, and for extract treated rabbit 265.6±1.725. For control CPK (U/L) level was 320.6± 1.970, and for extract treated rabbit was 522.4±2.846. For control CK- MB level was 27.2± 1.284, and for extract treated rabbit was 27.14±0.366. For control SGOT level was 41.4± 2.385, and for extract treated rabbit was 53.6±0.874.

Table II. Chronic toxic activity and the Effects of MCECT on Cardiac Enzyme

	LDH	CPK	CK- MB	SGOT
Control	293+ 6.620	320.6+ 1.970	27.2+ 1.284	41.4+ 2.385
MCECT	265.6+1.725	522.4+2.846	27.14+0.366	53.6+0.874

LDH= Lactate dehydrogenase, CPK= Creatine phosphokinase, CK- MB= Creatine kinase myocardial band, SGOT= Serum glutamic-oxaloacetic transaminase

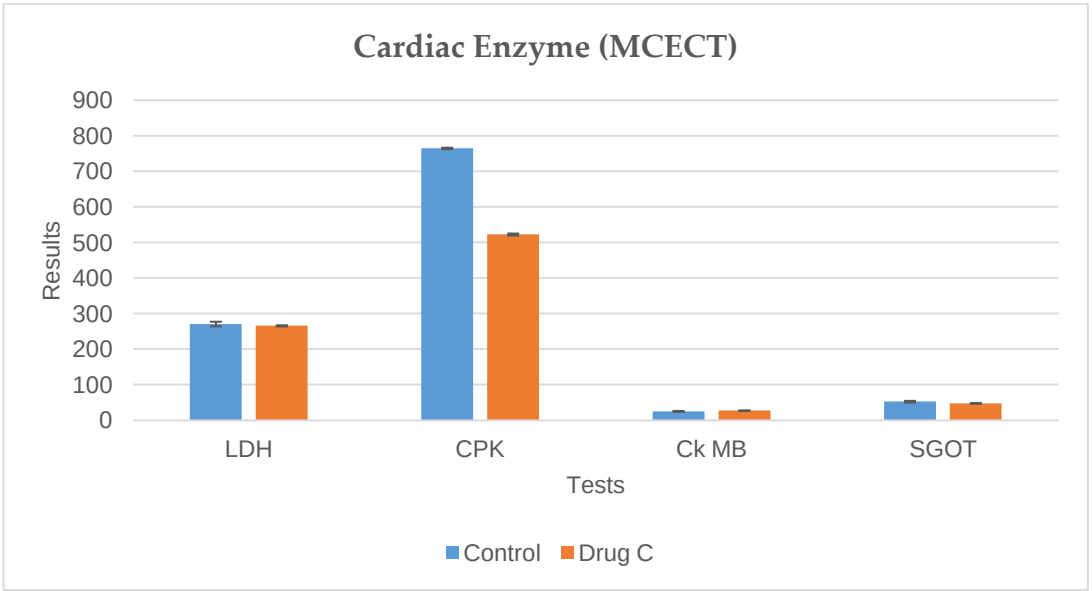

Fig. 3. Chronic toxic activity and the Effects of MCECT on Cardiac Enzyme

LIVER FUNCTION TEST (LFT)

TB Level in controlled group (CG) was 0.54± 0.143 (mg/dL), and in TG (treated group) value was 0.122±0.0196 (mg/dL). DB Level of CG was 0.056± 0.0095 (mg/dL), and TG value was 0.24±0.0401 (mg/dL). Level of AKP in CG was 195.4±4.018 (U/L), and in TG 104±1.585. Level of SGOT in CG was 60±0.951 (U/L), and in MCECT treated group value was 72±0.951 (U/L). GGT in CG was 7.2+ 0.972 (U/L), and in MCECT value was 4.24±0.0680 as shown in the Table III.

Table III. Chronic toxic activity and the Effects of MCECT on LFT

	TB	DB	SGPT	AKP	GGT
Control	0.54±0.143	0.05±0.0095	60±0.951	195.4±4.018	7.2±0.972
MCECT	0.122±0.0196	0.24±0.0401	72±0.951	104±1.585	4.24±0.0680

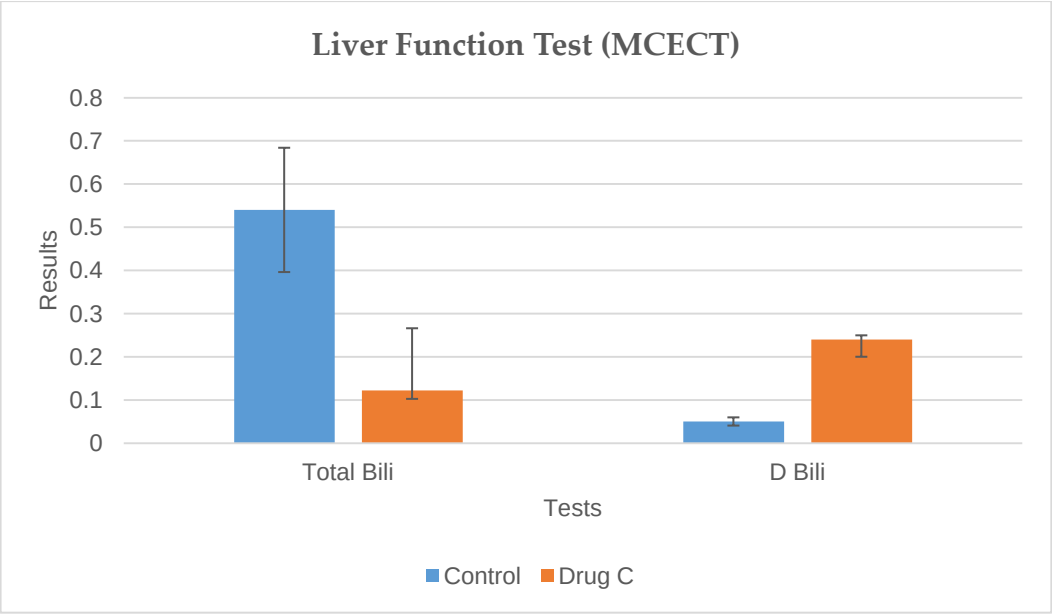

Fig. 4. Chronic toxic activity and the Effects of MCECT on LFT

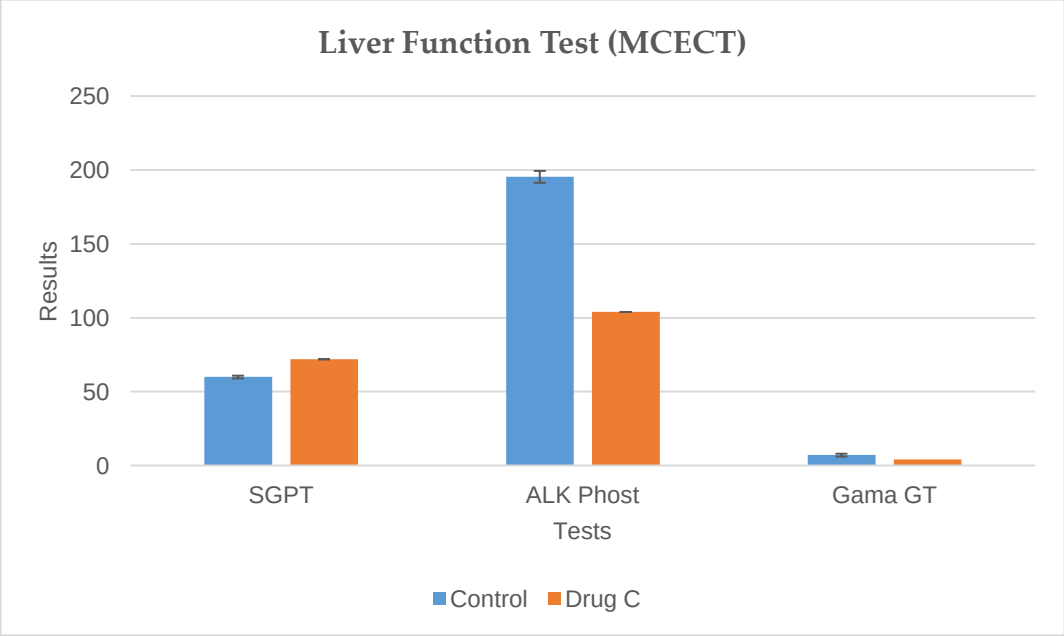

Fig. 5. Chronic toxic activity and the Effects of MCECT on LFT

LIPID PROFILE (LP)

During the LP test the Level of Cholesterol, Triglycerides, HDL, LDL, VLDL were found as 31.2±3.823, 202±1.520, 13.2±1.244, 21.8±1.36 and 28±1.585(mg/dL), respectively, in the controlled group. Similarly, the above parameters in the MCECT treated group, were, 17±1.677, 111.2±3.978, 12.182±0.111, 24.04±0.238 and 21.72±0.193, respectively as in Table IV.

Table IV. Chronic toxic activity and the Effects of MCECT Lipid Profile

	Cholesterol	Triglycerides	HDL	LDL	VLDL
Control	31.2+3.82	202+1.520	13.2+1.244	21.8+1.36	28+1.585
MCECT	17+1.677	111.2+3.978	12.182+0.111	24.04+0.238	21.72+0.193

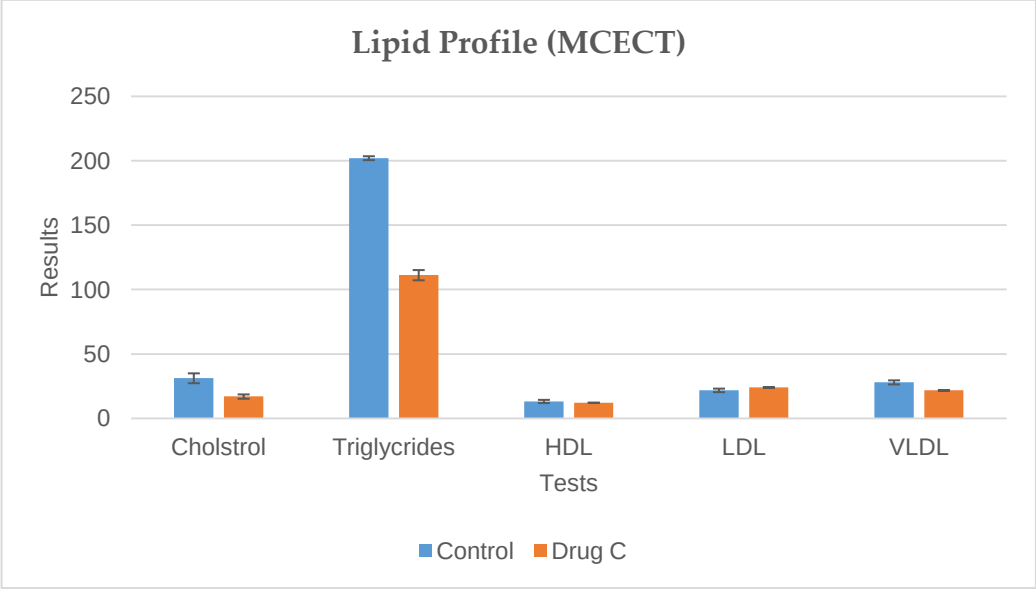

Fig. 6. Chronic toxic activity and the Effects of MCECT Lipid Profile

PROTEIN PROFILE (PP)

In-group I (Control) Total Proteins (g/dL) was 6.63±0.922, Albumin3.908±0.238 (g/dL) was and Globulin 0.56±0.143 (g/dL) was Al/Gb ratio 8.75±1.943 while MCECT treated group showing Total Proteins (g/dL) as 4±0.158, Albumin was 3.26±0.0814 (g/dL) and Globulin was 0.48±0.058 (g/dL) and Al/Gb ratio as 7.22±0.930.

Table V. Chronic toxic activity and the Effects of MCECT on PP

	TP	Al	Gb	Al/Gb ratio
Control	6.63±0.922	3.90±0.238	0.56±0.143	8.75±1.943
MCECT	4±0.158	3.26±0.0814	0.48±0.058	7.22±0.930

PP= Protein profile, TP = total protein, Al = Albumin, GB= Globulin

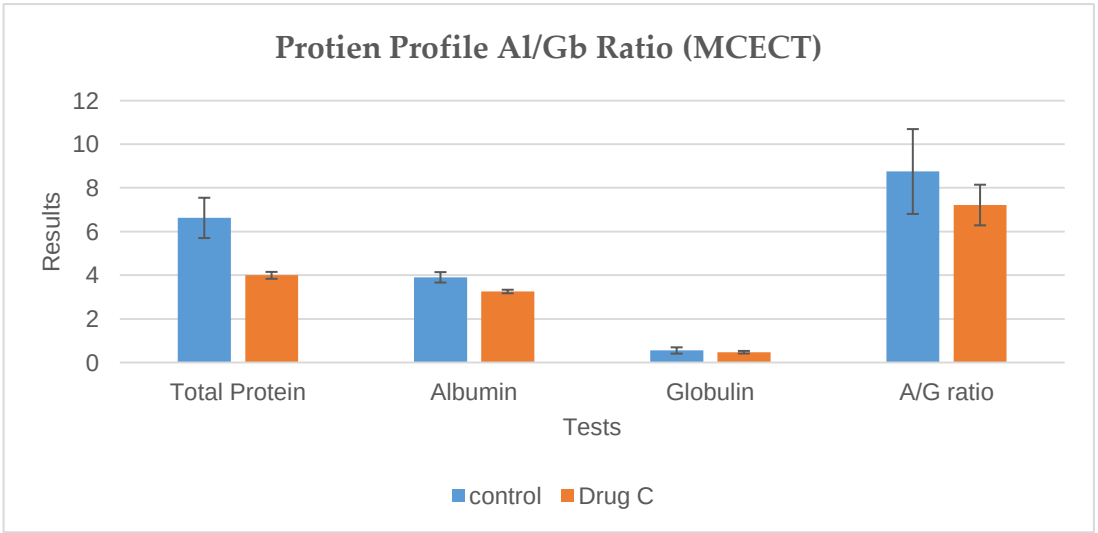

Fig. 7. Chronic toxic activity and the Effects of MCECT on PP

HEMATOLOGICAL PROFILE (HP)

The results during the current study in HP test of Hb, RBC, (HCT/PCV), MCV , MCH ,MCHC, total WBC count and Platelets count for the controlled group, were 11.94±0.300 (g/dl), 5.642±0.248 (million/ul), 35.9±7.287 %, 71.76±2.064 (fl),20.42±0.378 (pg), 31.2±6.328 (g/l), 9.2±0.986 and 190.2±3.579, while, MCCTE treated group showed values for the same parameters as 10.82±0.116 (g/dl), 5.79±0.0663 (million/ul), 42.06±0.517%, 82.3±0.182 (fl), 20.6±0.219 (pg) 27.7±0.378 (g/l), 6.04±0.0884 and 226±1.418, as in table 4.37.

Table VI. Chronic toxic activity and the Effects of MCECT Blood Tests

	Hb	RBC	HCT/PCV	MCV
Control	11.94±0.300	5.642±0.248	35.9±7.287	71.7±2.064
MCECT	10.82±0.116	5.79±0.0663	42.06±0.517	82.3±0.182
	MCH	**MCHC**	**WBC**	**Platelet Count**
Control	20.4±0.378	31.2±6.328	9.2±0.986	190.2±3.579
MCECT	20.6±0.219	27.7±0.378	6.04±0.0884	226±1.418

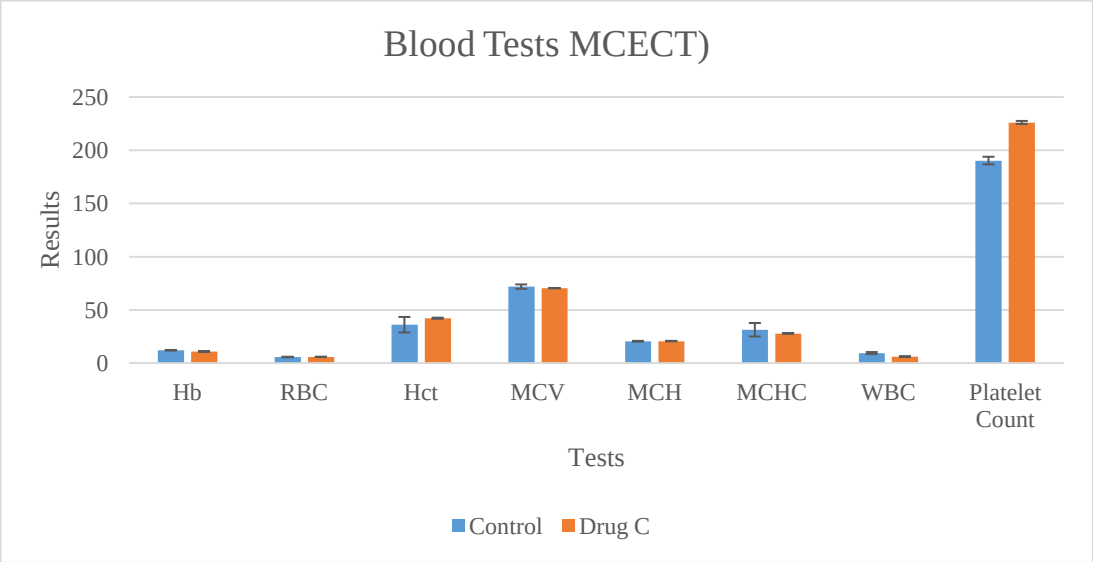

Fig. 8. Chronic toxic activity and the Effects of MCECT Blood Tests

DISCUSSION

In the current study Methanolic crude extract of *C. tubercullata* (MCECT) at a dose up to 2000 mg/kg had no adverse effect on the tested mice during 20 days of observation (22). The results indicated that MCECT at the dose of 2000 mg/kg didn't cause any adverse effect, whereas, the toxic dose might be greater than 2000 mg/kg, (23). For possible sub chronic toxic effects on kidney, heart and blood, *C. tuberculata* Methanolic crude extract (MCECT) was studied for toxic effects up to 90 days with a repeated dose of 500mg/bw concentration. The results showed that MCECT at 500 mg/kg bw dose had no sign of toxicity. However, a significant increase in urea and creatinine concentration was observed by comparing to the control group (24). Low blood creatinine levels may be a cause of muscle mass disease, severe liver, or kidney chronic disease (25). The increased level of serum creatinine of the experimental animals proposed that MCECT have antidiabetic property and may be beneficial in kidney and heart infections (26). The significant decrease in serum glucose level of the present study in the treated group is also an indication of anti-diabetic effect of MCECT (27).

Healthy heart is known by the cardiac enzyme's levels, such as CK-MB, LDH, AST and Lipid Profile, assessment in the serum (28). In the present study MCECT was given at 500 mg/kg body weight dose to the treated group, which showed a significant decrease in the above parameters. While the higher level of LDH in the blood pointed to acute or chronic cell damage (29).

In the group treated with MCECT, significant decrease in Cholesterol, HDL and LDL was observed, while, VLDL level was found with a significant increase, indicated the hyperlipidemia absence (30).

In calculation of Hematological profile MCECT treated group was given a 500mg/kg concentration showing no significant sign of toxicity (31), while a slight increase in the Platelets was detected. This may be due to the compound present in this extract which increases the capability to produce more platelets or may inhibit the destruction of platelets in the blood (32, 33).

CONCLUSION

It is concluded from the findings of the current study that methanolic crude extract of *C. tuberculata* was found safe at a dose up to 2000 mg/kg had no adverse effect on the tested mice during 20 days of observation during the acute an up to 500mg/kg bw for 90 days in rabbits as sub- acute trial.

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