

Anti-Diabetic and Toxicological Studied of Poly Herbal Compound –Mersina



Pharma

KEYWORDS : Mersina, aqueous extract, Antidiabetic activity, Alloxan-induced diabetes, Pancreas and Tolbutamide.

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ABSTRACT

The present study was carried out to evaluate the antidiabetic activity of Mersina (a poly herbal compound) in alloxan induced diabetic rats and rabbits for 14 days. A poly herbal compound (Mersina) at high dose (1000mg/kg) exhibited significant anti-hyperglycemic activity than (500mg/kg and 250mg/kg) which is compared with standard Tolbutamide in diabetic rats. A poly herbal compound (Mersina) showed reduction in glucose levels in normoglycemic and alloxan induced diabetic animals. Mersina showed significant regeneration of β cells of pancreas and hepatic cells in diabetic rats. Histopathological studies reinforce the healing of pancreas and liver by Mersina, as a possible mechanism of their antidiabetic activity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease, which is characterized by elevated blood glucose and resulted from either insufficient insulin and/or insulin resistance. The incidence of diabetes mellitus is tremendous increasing especially in developing countries. The disease causes substantial morbidity, mortality and long-term complications such as retinopathy, neuropathy and nephropathy. It is found to damage many parts of the body system, particularly the blood vessels and nerves [1].

Various medicinal plants have been used for years in daily life to treat disease all over the world. According to world health organization (WHO), medicinal plants are the best source to obtain a variety of newer herbal drugs. The use of plant extracts and phytochemicals, with known antioxidant, anti diabetic and antihyperlipidemic properties may be of immense importance in therapeutic treatment. [2,3]

Herbal medicines, containing active ingredients in complex chemical mixtures developed as crude fractions, extracted from aerial or underground parts of plant or other plant material or combination thereof, are widely used in health-care or as dietary supplements. One of the major drawbacks of these medicines is limited bioavailability, being poorly absorbed if taken orally [4-6].

Mersina is a poly herbal compound for the effective control of type 2 diabetes (non insulin dependent diabetes mellitus). Each capsule contains, Merasingi (*Gymnema sylvestre*) 75 mg, Karela (*Momordica charantia*) 81 mg, Tarwara (*Cassia auriculata*) 63 mg, Jamun (*Syzygium cumini*) 96 mg, Aunla (*Phyllanthus emblica*) 48 mg, Neem (*Melia azadirachta*) 30 mg, Methi (*Trigonella foenum graecum*) 15 mg, Kovai (*Coccinia indica*) 93 mg, Gulancha (*Tinospora cordifolia*) 63 mg, and Javakhar 12 mg [7].

Experimental Animals

Wistar albino rats (150–200 g) of both sexes were obtained from the animal house of Osmania medical college, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum.

Acute Oral Toxicity Studies

Mersina at the dose range of 100mg-2000mg/kg were administered orally to different group of rats comprising of ten rats in each group. Mortality was observed after 72h. Acute toxicity was determined according to the method of Litchfield and Wilcoxon [8].

Experimental design

Effect of mersina in normoglycemic animals

Five groups of animals, six in each received the following treatment schedule.

- Group I : Normal control
Group II : Standard Tolbutamide 50mg/kg
Group III : Mersina (250mg/kg)
Group IV : Mersina (500mg/kg)
Group V : Mersina (1000mg/kg)

The above study is undertaken in two different animal models. Wistar Rats of either sex weighing about 140-160gms and rabbit of either sex weight between 1.4 to 1.6 kg were selected for study.

The animal were divided into five groups of six each and fasted overnight. The first group taken as control group was given carboxymethyl cellulose 2gms, dissolved in 5ml of water for 14 days. The second group was given the standard drug Tolbutamide 50mg/kg for 14 days. The rest of the groups were given the test compound (Mersina) in dose of 250mg/kg, 500mg/kg and 1000mg/kg for 14 days respectively. The blood were collected from inner canthus of eye on 1st day, 7th day and 14th day and glucose levels were estimated by glucometer.

Effect of mersina in alloxan induced treated animals

Induction of diabetic in experimental animals:

Animals were made diabetic by a single intraperitoneal injection of alloxan monohydrate (100 mg/kg) [9]. Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

Six groups of animals, six in each received the following treatment schedule.

- Group I : Normal control
- Group II : Alloxan treated (100mg/kg.ip)
- Group III : Alloxan (100mg/kg.ip)+ Standard Tolbutamide 50mg/kg
- Group IV : Alloxan (100mg/kg.ip) + Mersina 250mg/kg)
- Group V : Alloxan (100mg/kg.ip) + Mersina 500mg/kg)
- Group VI : Alloxan (100mg/kg.ip) + Mersina 1000mg/kg)

It consists of observing the effect of the test compound (Mersina) in albino rats and rabbits treated with alloxan induced diabetic rats. Wisters Rats of either sex weighing about 140-160gms and rabbit of either sex weight between 1.4 to 1.6 kg were selected for study. All the animals were kept on the standewrd diet throughout the study, and were kept on fasting overnight before the study made next day. They were divided into six groups of six animal each.

Mersina and standard drug Tolbutamide were administered with the help of feeding cannula. Group I serve as normal control, which received saline solution for 14 days. Group II to Group VI are diabetic control rats (which previously received alloxan100mg/kg.ip)are given a fixed dose of Standard Tolbutamide 50mg/kg, Mersina (250mg/kg, p.o), Mersina (500mg/kg, p.o), and Mersina (1000mg/kg, p.o) for 14 consecutive days.

Collection of blood sample and blood glucose determination:

Blood samples were drawn from animal at weekly intervals till the end of study (i.e. 2 weeks). Fasting blood glucose estimation and body weight measurement ware done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

Histopathology of pancreas and liver of rats

The whole pancreas from each rat was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5µ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. Histopathology of rat liver was carried out by a modified Luna (Luna LG., 1999)[10]. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6h. Then the rats livers were paraffin embedded and 5 µ thickness microtone sections were made [11]. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/prottection.

Statistical analysis

All the values of were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and Dunnet’s t-test.

RESULTS

Table 1: Effect of marcina on basal blood glucose levels in rats

Groups	Treatment	Blood glucose level (mg/dl)		
		Day 1	Day 7	Day 14
Groups I	Normal control	81.1±3.1	80.1±4.3	80.5±2.6
Groups II	Standard Tolbutamide 50mg/kg	82.4±2.2	72.4±3.6**	60.8±2.8**
Groups III	Mersina 250mg/kg, p.o)	85.6±4.6	79.8±3.6*	72.4±4.2*
Groups IV	Mersina (500mg/kg)	80.3±2.4	74.5±3.2**	65.4±3.6**
Groups V	Mersina (1000mg/kg)	84.6±4.2	76.8±3.8**	62.1±0.2**

Values are mean ± SD n=6in each group

**p<0.01 as compare to control group.

Fig 1: Effect of marcina on basal blood glucose levels in rats

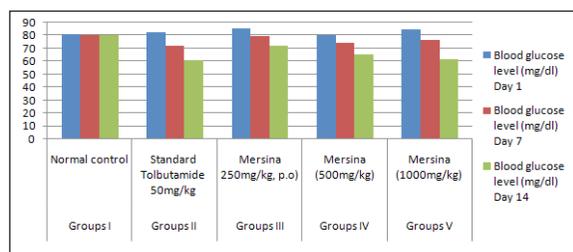


Table 2: Effect of marcina on basal blood glucose levels in rabbits

Groups	Treatment	Blood glucose level (mg/dl)		
		Day 1	Day 7	Day 14
Groups I	Normal control	81.3±8.3	82.2±6.4	82.4±6.5
Groups II	Standard Tolbutamide 50mg/kg	82.9±5.4	71.2±8.1**	60.9±5.4**
Groups III	Mersina 250mg/kg)	83.1±3.5	78.1±5.4*	72.1±3.5*
Groups IV	Mersina (500mg/kg)	85.1±7.6	76.1±7.6**	67.5±7.6**
Groups V	Mersina (1000mg/kg)	80.6±0.6	70.0±0.6**	62.0±0.6**

Values are mean ± SD n=6in each group

**p<0.01 as compare to control group

Fig 2: Effect of marcina on basal blood glucose levels in rabbits

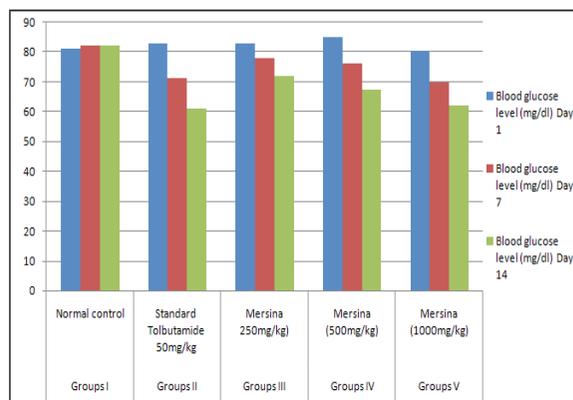


Table 3: Effect of marcina on blood glucose in alloxan treated rats

Groups	Treatment	Blood glucose level (mg/dl)		
		Day 1	Day 7	Day 14
Groups I	Normal control	65.5±3.1	67.1±4.3	67.5±2.6
Groups II	Alloxan (100mg/kg.ip)	216.8±8.2	222.4±6.6	268±2.8
Groups III	Alloxan (100mg/kg.ip)+ Standard Tolbutamide 50mg/kg	212.6±4.6	152.8±3.6**	66.4±4.2**
Groups IV	Alloxan (100mg/kg.ip) + Mersina 250mg/kg)	219.3±2.4	168.5±3.2*	82.4±3.6*
Groups V	Alloxan (100mg/kg.ip) + Mersina 500mg/kg)	220.4±1.7	164.5±1.3**	76.2±2.8**
Groups VI	Alloxan (100mg/kg.ip) + Mersina 1000mg/kg)	224.2±4.3	158.5±3.9**	68.4±2.2**

Values are mean ± SD n=6in each group

**p<0.01 as compare to control group

Fig 3: Effect of marcina on blood glucose in alloxan treated rats

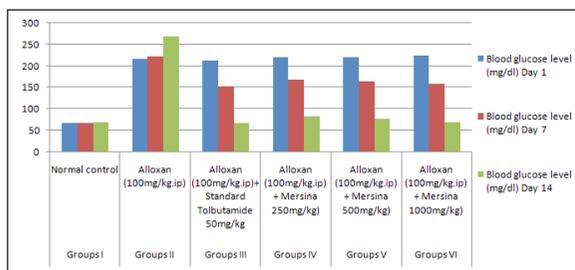


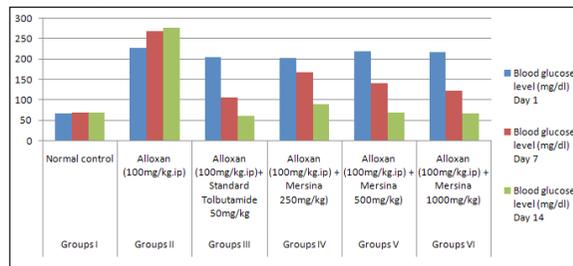
Table 4: Effect of marcina on blood glucose in alloxan treated rabbits

Groups	Treatment	Blood glucose level (mg/dl)		
		Day 1	Day 7	Day 14
Groups I	Normal control	65.5±3.1	67.1±4.3	67.5±2.6
Groups II	Alloxan (100mg/kg.ip)	225.4±2.2	267.4±3.6	275.8±2.8
Groups III	Alloxan (100mg/kg.ip)+ Standard Tolbutamide 50mg/kg	202.6±4.6	105.8±3.6**	60.4±4.2**
Groups IV	Alloxan (100mg/kg.ip) + Mersina 250mg/kg)	202.3±2.4	165.5±3.2*	87.4±3.6*
Groups V	Alloxan (100mg/kg.ip) + Mersina 500mg/kg)	218.4±1.7	140.5±1.3**	68.2±2.8**
Groups VI	Alloxan (100mg/kg.ip) + Mersina 1000mg/kg)	215.2±4.3	120.5±3.9**	65.4±2.2**

Values are mean ± SD n=6in each group

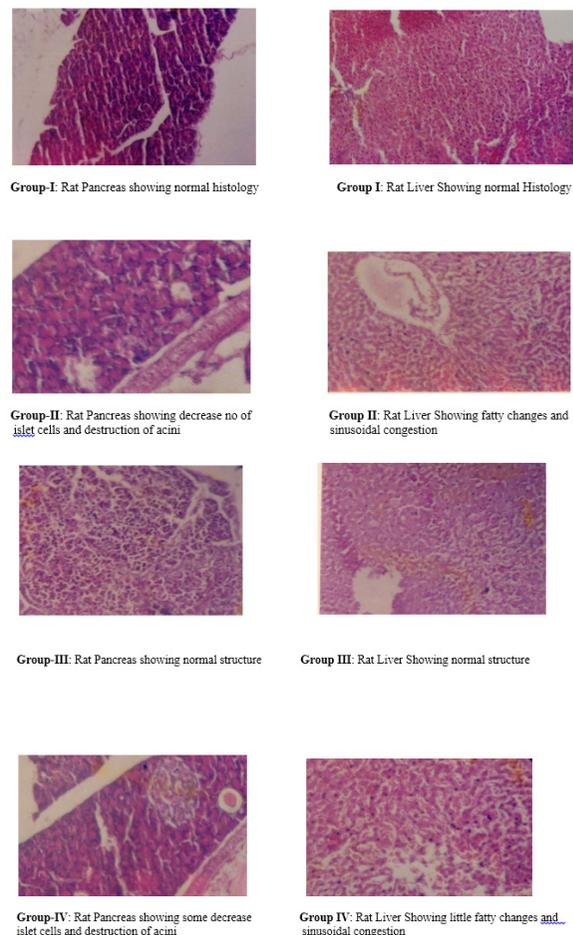
p<0.01 as compare to control group

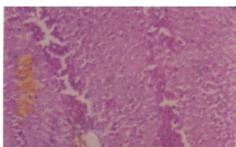
Fig 4: Effect of marcina on blood glucose in alloxan treated rabbits



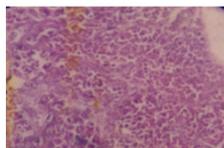
The acute oral toxicity study of Mersina showed no mortality upto 2000 mg/kg. The anti-hyperglycemic effect of the Mersina on the fasting blood sugar levels of diabetic animals is shown in Table1 and Table 2(Fig 1 & 2). Administration of alloxan (100 mg/kg, i.p.) lead to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Two weeks of daily treatment of Mersina lead to a dose-dependent fall in blood sugar levels by 25–50%. Effect was maximum till 14 days of treatment. Alloxan treated animals (Group II) showed increase in blood glucose levels. Standard Tolbutamide 50mg/kg + Alloxan treated (Group III) showed normal glucose levels. Alloxan treated and Mersina treated animals (Group IV-VI) reduction in glucose levels. Mersina1000mg/kg shown to be more effective than Mersina 500mg/kg & 250mg/kg in reducing the glucose levels as in Table 3 and Table 4(Fig 3 & 4).

Histopathology of Liver and pancreas

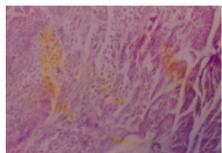




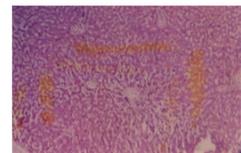
Group V: Rat Pancreas showing no decrease in islet cells and destruction of acini



Group V: Rat Liver Showing no fatty changes and sinusoidal congestion



Group VI: Rat Pancreas showing normal structure



Group VI: Rat Liver Showing normal structure

DISCUSSION

In light of the results, our study indicates that Mersina have good antidiabetic activity. Mersina exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic animals they can also improve the condition of Diabetic mellitus. The renewal of β cells in diabetes has been studied in several animal models. The total β cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug[12]. Mersina has been shown to act by β cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic[13], ephedrine[14], and *Gymnema sylvestre* leaf extracts[15]. In our studies, the damage of pancreas in alloxan-treated diabetic animals (Group II) and regeneration of β cells by Tolbutamide(Group III) was observed. The comparable regeneration was also shown by Mersina (Group III –Group VI). It is found that Mersina at high dose (1000mg/kg) is more effective than Mersina (500mg/kg and 250mg/kg) after 14 days of treatment. Hence from the above discussion it reveals that Mersina is more effective and shows similar curative effect as standard Tolbutamide (50mg/kg). This effect may be due to the presence of various poly herbal compounds. Histopathological studies reinforce the healing of pancreas, by Mersina, as a possible mechanism of their antidiabetic activity.

CONCLUSIONS

Mersina is a valuable antidiabetic drug. It can be used alone safely in the prescribed dose range for the effective control of maturity onset non-insulin dependent diabetic mellitus (Type II). It can also be combined with standard oral antidiabetic drugs with their reduced requirements for more effective antidiabetic control. It can be beneficial in the typical cases of maturity onset diabetics with body weight above normal range. It is safe and does not produce any serious side effect even on long term use in the prescribed dose range.

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