

ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF *CALENDULA OFFICINALIS*

Chakraborty G.S.*, Arora R, Majee C

Department of Pharmaceutical Technology, N.I.E.T., Greater Noida, Uttar Pradesh, 201306, India

*Dr. G.S.Chakraborty, Department of Pharmaceutical Technology, N.I.E.T., Greater Noida, Uttar Pradesh, 2010 306, India E-mail: phdgs77@indiatimes.com

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ABSTRACT

Calendula officinalis, belonging to the family of Asteraceae, commonly known as English Marigold or Pot Marigold is an aromatic herb which is used in Traditional System of Medicine. It is mainly used because of its various biological activities to treat diseases like analgesic, antidiabetic and anti-inflammatory. It is also used for ingastro-intestinal, gynecological, eye disease, skin injuries and in some cases of burn. The plant is rich in many pharmaceutical active ingredients like Carotenoids, flavonoids, glycosides, steroids and sterols. Thus the present study was designed to evaluate the antidiabetic and antihyperlipidemic effect of hydroalcoholic extract of *calendula officinalis* in alloxan induced diabetic rats. The extract was prepared by soxhlet extraction technique with a ratio of Water: Alcohol (70:30) for 36 hrs which ensured complete extraction of active constituents. Diabetes was induced by single intraperitoneal injection of alloxan (150 mg/kg) of body weight. Oral administration of hydroalcoholic extract of *Calendula officinalis* to diabetic rats, at a dose of 100 mg/kg body weight, resulted in a significant reduction in blood glucose, urine sugar and serum lipids in alloxan diabetic rats. The extract also increases the total haemoglobin lever. The extract effect was similar to that of insulin. Thus, the investigation clearly shows that hydroalcoholic extract of *Calendula officinalis* has both antidiabetic and antihyperlipidaemic effects.

KEYWORDS: *Calendula officinalis*, alloxan diabetic, Antidiabetic, Antihyperlipidaemic

INTRODUCTION

From the ancient times diabetes mellitus is called as madumeha. It is considered as a chronic metabolic disorder which is affecting 4% population worldwide¹. Eighty percent of the world's population relies primarily on traditional medicines for their health care needs. The characterization of this disorder (diabetes) is seen by abnormalities in metabolism related to carbohydrate, lipid and lipoprotein which results in hyperglycemia, hyperinsulinemia, hypertension and atherosclerosis². Control of diabetes mellitus involves exercise, diet and chemotherapy. Ayurveda is an ancient Indian System of medicine, which generally deals with plants and plant extracts. This form of medicines involves treating various diseases by the active ingredients present in plant³. However natural products derived from plant sources serves the best for the treatment as they are expected to have a similar degree of efficacy without the troublesome side effects associated with modern drug treatment. More than 400 plants with glucose lowering effect are known for their potent activity⁴. *Calendula officinalis*, (Asteraceae), commonly known as English Marigold or Pot Marigold, an aromatic herb which is used in Traditional System of Medicine. It is mainly used because of its various biological activities to treat diseases like analgesic, antidiabetic, anti-inflammatory, antiseptic, bactericide, in skin problems⁵ and as antifungal agent⁶. It is also used for in gastro-intestinal, gynecological, eye disease, skin injuries and in some cases of burn. The plant is rich in many pharmaceutical active ingredients like auroxanthin, carotenoids, flavonoids, flavoxanthin, glycosides, triterpenoids esters, steroids and sterols. The leaves and stems contain other carotenoids, mostly lutein (80%) and zeaxanthin (5%), and beta- carotene^{7, 8, 9}. Our Literature survey

showed that not much of the work has been carried out on these aspects of the following study. Its role on glucose and lipid peroxide metabolism in alloxan diabetic rats are studied in the present investigation.

MATERIALS AND METHODS

Calendula officinalis (Pot Marigold) leaves were collected fresh from the Herbal Garden of Jamia Hamdard, New Delhi, India and dried in shade. The plant was identified and authenticated at the Pant Anatomy Research Centre, Chennai. A voucher specimen (number Parc/H-101) was deposited in the Department of Pharmacognosy and Phytochemistry, Hamdard University. The dried leaves were blended with the help of Blending machine and were sieved through sieve No 60 and the powdered roots were kept separately in airtight containers until the time of use.

Preparation of alcoholic *Calendula officinalis* Leaf extract

The powdered leaves were extracted in soxhlet apparatus with ethanol and water (50:50) at a temperature of 60°C for 12-16 h. The resultant extract was filtered. The filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at a constant temperature of 40°C. The dried mass was stored in a refrigerator and considered as the extract. The yield of the extract was 8.66% (w/w, in terms of dried material).

Preparation of diabetic animals

Inbred Albino rats (Wistar strain) of either sex weighing (150-180 g) were procured from the Central Animal House Facility, Hamdard University, New Delhi. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12:12 h dark/light cycle) and had a free access to commercial pellet diet (Amrut rat feed, Mfd by: Nav Maharashtra Chakan Oil Mills Ltd, Delhi, India) and water *ad libitum*. The animal house was maintained at 25 ± 2°C and relative humidity was also maintained at (50 ± 15 %). Diabetes was induced in the rats by a single intraperitoneal injection of alloxan (150 mg/kg body weight). Alloxan is capable of producing fatal hypoglycaemia by a massive pancreatic insulin release; rats were treated with 20% glucose solution (15-20 ml) intraperitoneally after 6 h. The rats were then kept for 24 h on 5% glucose solution bottles in cages to prevent them from hypoglycaemia¹⁰. After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycaemia with blood glucose range of 200-270 mg/dl were used for the study. Blood was collected from the eyes (venous pool).

Experimental design

In the present investigation, a total of 36 rats (30 diabetic surviving rats+ 6 normal rats) were used. Diabetes was induced in rats, 2 weeks before starting the treatment. The rats were divided into six groups as follows, after the induction of alloxan diabetes and each containing six rats. Group I, control rats were given with 2ml of saline: Group 2, diabetic rats were given with 2 ml of saline; Group 3, diabetic rats were treated with hydro alcoholic extract of *Calendula officinalis* (25 mg/kg) suspended in saline daily using an intragastric tube for 42 days; Group 4, diabetic rats were treated with hydroalcoholic extract of *Calendula officinalis* (50 mg/kg) daily for 42 days Group 5, diabetic rats were given with hydroalcoholic extract of *Calendula officinalis* (100 mg/kg) daily for 42 days; Group 6, diabetic rats were given with insulin (6U/kg) intra-peritoneally daily for 42 days. During second, fourth and the sixth week of extract treatments, blood glucose and urine sugar of all the rats was determined. After 42 days of treatment, the rats were kept in metabolic cages and urine samples were collected. The urine sugar was estimated by Benedict's Method. Prior sacrifice, the rats were deprived of food for 12 h, but allowed free access to water. The rats were then sacrificed by /cervical dislocation. Blood was collected in separate tubes. One of the tube containing potassium oxalate and sodium fluoride was used for the estimation of glucose. The next tube containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for lipids estimation.

Haemoglobin

Fasting blood glucose and total haemoglobin were estimated by the methods described respectively^{11, 12}.

Estimation of cholesterol

Cholesterol in serum samples were estimated¹³.

Estimation of phospholipids

Phospholipids in serum were estimated by the prescribed method¹⁴.

Estimation of free fatty acids (FFAs)

Free fatty acids in serum were estimated by the employed method¹⁵.

Statistical analysis

All the grouped data were statistically evaluated and the levels of significance of various treatments were calculated using Dunnett's test. All the results were expressed as mean \pm S.D. from six rats in each group.

RESULTS

A significant dose dependent effect of hydroalcoholic leaf extract of *Calendula officinalis* on blood glucose and urine sugar in normal and experimental rats are cited in Table 1. The blood glucose and urine sugar were significantly elevated in diabetic rats as compared to normal rats. Upon oral administration of hydroalcoholic leaf extract of *Calendula officinalis* at 25 and 50 mg/kg body weight significantly lowered the blood glucose and the urine sugar as they were compared with the untreated group of diabetic rats. Hydroalcoholic leaf extract of *Calendula officinalis* at a dose of 100 mg/kg body weight restored the blood glucose and urine sugar to the normal levels. The 100 mg/kg body weight of hydroalcoholic leaf extract of *Calendula officinalis* showed the highest blood glucose lowering effect, thus it was thought worthwhile to undergo the studies with the desired dosage. The levels with respect to the total haemoglobin and change in the body weight in normal as well as in the experimental rats are mentioned in Table 2. Both of the parameters showed a significant lowering in diabetic rats when they were compared with normal rats. On oral administration of hydroalcoholic leaf extract of *Calendula officinalis* a significant increase in total haemoglobin and body weight was observed. The levels of serum cholesterol, phospholipids and free fatty acids are described in Table 3. The levels of serum cholesterol, phospholipids and free fatty acids were significantly higher in diabetic rats as compared to the normal rats or untreated group. On administration of hydroalcoholic leaf extract of *Calendula officinalis* the levels were lowered. The hydroalcoholic leaf extract of *Calendula officinalis* at a dose of 25, 50, 100 mg/kg showed a significant blood glucose lowering effect. The hydroalcoholic leaf extract of *Calendula officinalis* at a dose of 100 mg/kg was found to be highly significant as it restored all the parameters to the normal levels. Thus the effect of hydroalcoholic leaf extract of *Calendula officinalis* was found to be similar to that of insulin administration.

DISCUSSION

Induction of alloxan causes severe damage to the insulin secreting cells of the pancreas which thereby leads to hyperglycaemia. In our investigation, we have found that on administration of hydroalcoholic leaf extract of *Calendula officinalis* to the diabetic rats reversed their blood glucose which was reflected in their urine sugar levels. The mechanism by which the extract brings about the action may be due to the potentiation of the insulin effect of plasma by increasing the secretion of insulin from the pancreas mainly from the β - cells of islets of Langerhans or it may be from the bound form. A significant decrease in body weight was observed with alloxan diabetic rats. When hydroalcoholic leaf extract was administered to the alloxan treated rats, the loss of weight was reversed. The total haemoglobin levels were lowered with the treated dose of alloxan in diabetic rats. This has been proven with many researchers also that haemoglobin levels are generally lowered in diabetic rats. Elevated serum lipid levels were also observed in alloxan diabetic rats, since lipids play a major role in the patho physiology of diabetes. If the levels are raised it may lead to the risk factor of coronary heart diseases¹⁶. The high concentration of serum lipids in diabetes is due to the increase in the mobilization of free fatty acids which are released from the peripheral depots. Thus it can be concluded that hydroalcoholic leaf extract of *Calendula officinalis* has shown a potential antidiabetic and antihyperlipidaemic effects with a dose of 100 mg/kg body weight which restored all the parameters in the alloxan diabetic rats and its effects are similar to that of insulin.

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Table 1: Dose dependent effect of Hydro-alcoholic extract on blood glucose and urine sugar in Diabetic rats

Group	Blood Glucose (mg dl ⁻¹)		Urine Sugar
	Initial	Final	
Normal	74.8 ± 4.1	84.8 ± 3.8	+
Diabetic+ Control	259.8 ± 5.1	326.8 ± 5.5 ^{###}	+++
Diabetic+ CRHAt (25 mg)	256.4 ± 7.2	207.6 ± 3.4 ^{***}	+
Diabetic+ CRHAt (50 mg)	264.8 ± 4.2	134.7 ± 4.1 ^{***}	+
Diabetic+ CRHAt (100 mg)	266.3 ± 4.0	85.5 ± 3.7 ^{***}	-
Diabetic + Insulin	267.6 ± 3.1	84.8 ± 4.1 ^{***}	-

Values are mean ± SEM (n=6), *** p< 0.001, # P, 0.05, (+) sugar 0.25%, (+++) Sugar more than 2%, ANOVA calculated by Dunnett's Multiple Comparison Test.

Table 2: Effect of Hydro-alcoholic extract on total hemoglobin and change in body weight in Diabetic rats

Group	Total Hb (g dl- l)	Change in Body Weight (g)
Normal	13.5 ± 1.1	35.2 ± 1.4
Diabetic+ Control	11.3 ± 0.6 [#]	17.3 ± 0.3 [#]
Diabetic+ CRHAt (100 mg)	14.4 ± 0.7 ^{**}	11.4 ± 0.2 ^{**}
Diabetic + Insulin	14.2 ± 0.8 ^{**}	10.4 ± 0.6 ^{**}

Values are mean ± SEM (n=6), *** p< 0.001, # P,0.001, ANOVA calculated by Dunnett's Multiple Comparison Test

Table 3: Effect of Hydro-alcoholic extract on cholesterol, phospholipids and free fatty acids in Diabetic rats

Group	Cholesterol	Serum (mg dl-1) Phospholipids	Free Fatty Acids
Normal	80.2 ± 1.2	03.5 ± 2.2	77.5 ± 1.1
Diabetic+ Control	165.2 ± 4.7 [*]	153.3 ± 0.6 [*]	171.3 ± 0.6 [*]
Diabetic+ CRHAt (100 mg)	83.4 ± 4.2 ^{**}	104.4 ± 3.4 ^{**}	78.4 ± 0.7 ^{**}
Diabetic + Insulin	264.2 ± 0.8	105.2 ± 0.8 ^{**}	79.2 ± 0.8 ^{**}

Values are mean ± SEM (n=6), * p< 0.001, ** P,0.001, ANOVA calculated by Dunnett's Multiple Comparison Test.

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