Effects of Portulaca Oleracea (Kulfa) and Taraxacum Officinale (Dhudhal) in Normoglycaemic and Alloxan-Treated Hyperglycaemic Rabbits

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Abstract
Blood glucose levels of normal and alloxan-diabetic male albino rabbits were determined after oral administration of various doses of the whole, dried and powdered plants of the Portulaca oleracea, Linn. (Fam. Portulacaceae) and Taraxacum officinale, Wiggers (Fam. Compositae). It was concluded that the powders of both plants produced significant hypoglycaemic effects in normal rabbits but not in alloxan-treated rabbits. Acute toxicity and behavioural changes were not observed in the doses tested. It is conceivable that both the indigenous medicinal plants contain some hypoglycaemic principles which act probably by initiating the release of insulin from pancreatic beta-cells of normal rabbits. (JPMA 35 : 2071985).

Introduction
Portulaca oleracea and Taraxacum officinale, locally known as Kulfa and Dudhal respectively, have been used in traditional medicine for treating diabetes, liver diseases and some metabolic disorders1-4. The present investigations were undertaken to study the effects of powdered Portulaca oleracea and Taraxacum officinale plants on blood glucose levels following oral administration to normoglycaemic and alloxan-treated hyperglycaemic male albino rabbits. Acute toxicity and behavioural patterns were also studied to determine the safety of these herbs.

Material and Methods

Plant Material
Shade-dried T. officinale plant was provided by the Pakistan Forest Institute, Peshawar while fresh P. oleracea plant purchased from the vegetable market of Faisalabad was washed with tap-water and dried under the shade. Dried plants were milled in an electric grinder and stored in well-closed cellophane bags at 4°C.

Chemicals
Alloxan-monohydrate and a-d-glucose (anhydrous) were obtained from B.D.H. Laboratories (Chemical Division), Poole, England, Glacial acetic acid, benzoic acid (sublimed), 0-toluidine, thiourea and trichloracetic acid were procured from E. Merck, Darmstadt, West Germany. Gum tragacanth was purchased from the local market. Tolbutamide (Rastinon) was supplied by Hoechst (Pakistan) Ltd., Karachi.

Animals
Male, adult, healthy° albino rabbits of a local strain weighing between 1000-1200 g were used. They were kept in an air-conditioned animal room of the Physiology and Pharmacology department at the University of Agriculture, Faisalabad. They were given a balanced rabbit feed prepared by Nutrition Department of this University and allowed tap-water ad libitum. The effects of Portulaca oleracea and Taraxacum officinale were studied on blood glucose levels of the normal and diabetic rabbits. For acute
toxicity studies and effect on behavioural pattern, albino rabbits and Sprague Dawley rats of either sex were used.

**Preparation of Diabetic Rabbits**
A group of rabbits were made diabetic by injecting intravenously, 150 mg/kg body weight of alloxan-monohydrate\(^5\). Eight days after injection, the blood glucose levels of all surviving rabbits were determined by the 0-toluidine method. Rabbits with blood glucose level of 250-500 mg/100 ml were considered as diabetic and were employed for further studies.\(^5,6\)

**Grouping of Rabbits**
Rabbits were randomly divided in 11 groups (I-XI) of six animals each. Group I served as control and received 15 ml of 2% gum tragacanth solution in water orally. The animals of groups II, III, IV and V were treated orally with 0.5, 1.0 and 1.5 and 2 g/kg body weight of P. oleraceae powder suspended in 2% gum solution, respectively. Group VI was given tolbutamide 500 mg/kg body weight. Animals of groups VII, VIII, IX and X were alloxan-induced diabetics and they received 15 ml of gum solution and 1, 1.5 and 2 g/kg body weight of P. oleraceae powder orally, respectively. The diabetic rabbits of group XI were treated with tolbutamide, 500 mg/kg body weight.

Similar to above, 11 groups of 6 rabbits each were made to study the effects of T. officinale powder (0.5, 1.0, 1.5 and 2 g/kg body weight) and a control hypoglycaemic drug, tolbutamide, (500 mg/kg body weight) and a control hypoglycaemic drug, tolbutamide (500 mg/kg) on blood glucose of normal and diabetic rabbits.

**Preparation and Administration of Drug Suspensions**
P. oleraceae and T. officinale powders were administered on body weight basis after suspending in 10 ml of 2% gum tragacanth solution, with the final volume being 1.5 ml. They were administered to each animal by using a stainless steel feeding needle attached to a syringe. The needle was inserted into the stomach through the oesophagus and the plunger was pressed slowly and steadily. Immediate sneezing and coughing indicated injection into the lungs and in this case the needle was at once withdrawn and another animal was taken.

**Collection of Blood**
fter administration of the drug suspensions the animal was held in a wooden rabbit holder and immediately 0.2 ml of blood was collected from an ear vein. Similar samples of 0.2 ml were collected at 4, 8, 12 and 24 hour intervals. After collecting blood, absolute alcohol was applied on the pricked site to protect the ear against infection.

**Determination of Blood Glucose**
Blood glucose was determined by the method of Fings et al\(^7,8\) using 0-toluidine reagent. This method is very sensitive and can detect small amounts of blood glucose and the results obtained are comparable to the glucose oxidase method\(^5\).

**Acute Toxicity and Behavioural Pattern Study**
To study any possible toxic effect or change in normal behavioural pattern of the animals, different doses of both the plants were administered orally to groups of 6 male albino rabbits and Sprague Dawley rats. The symptoms including awareness, mood, motor activity, CNS excitation, posture, motor incoordination muscle tone, reflexes, autonomic responses\(^8\) were recorded. Animals were closely watched for 6 to\(^8\) hours after the dose for any of the toxic symptoms, and possible mortality. They were kept under observation for a total of seven days.

**Statistical Analysis**
Mean blood glucose levels were expressed in mg/100 ml ± SEM in all experiments and Student’s ‘t’ test was used to check their significance.

**Results**
Standard Curve for Glucose Determination
Standard curve for glucose estimation was linear up to 300 mg/l00. ml and blood glucose levels of samples found to be above 300 mg/100 ml were re-determined after dilution.

Effects of Portulaca Oleraceae
Gum tragacanth used as a control had no effect on the blood glucose of normal and diabetic rabbits at any time interval normal animals administered 0.5 g/kg and 1 g/kg of p. oleraceae showed a non-significant decrease in blood glucose at 4, 8 and 24 hours interval (P > 0.05) except that the latter dose at a 12 hour period produced a significant reduction (P < 0.05). The animals treated with 1.5 g/kg and 2.0 g/kg of P. oleraceae had a significant fall in blood glucose at 8 and 12 hours interval (P < 0.05 and P < 0.001) where as the 2 g/kg group showed this effect after 24 hours (Fig. 1). Similar doses of portulaca oleraceae given to alloxan diabetic rabbits caused no change in blood sugar. Thus the maximum fall of blood glucose with all the doses of portulaca oleraceae in normals was produced at 12 hours. The dose response curve attained with percent decrease in blood glucose as ordinate and the dose as abscissa, was linear.

Effects of Taraxicum Officinale
Taraxiciuni officinale given in a dose of 0.5 g/kg body weight to normal rabbits showed no change in blood glucose levels the 1g/kg, 1.5 g/kg and 2 g/kg doses produced a significant lowering of blood glucose at 8 and 12 hours with the 2 g/kg group having a fall at 4 hours also. The 24 hour level was same as the 0 hour value. Similar doses given to diabetic rabbits failed to show any significant results. The maximum decrease in blood glucose with all the doses of taraxacum officinale was produced at 8 hours interval and the dose response curve was again linear.

Effect of Tolbutamide
The normal animals treated with tolbutamide (500mg/kg orally) showed a significant fall in blood glucose with the maximum being after 4 hours. The diabetic rabbits had no significant hypoglycaemic effect with tolbutamide.

Acute Toxicity and Behavioural Pattern Studies
Rabbits treated with 3, 4, 5 and 6 gram/kg body weight of the whole dried and powdered P. oleraceae and T. officinale plants remained alive upto 7 days and did not show any visible signs of acute toxicity e.g. restlessness, respiratory distress, convulsions, coma and death. Moreover, the behavioural pattern records studies in rats also revealed no prominent change in the awareness, mood, motor activity, CNS excitation, posture and motor incoordination, autonomic responses, food consumption and body weight.

Discussion
The data obtained confirms the findings of Marquis et al,9 that 2% gum tragacanth solution does not produce any significant change in blood glucose of rabbits. However, as is shown in Figures 1 and 2,
Fig. 1. Blood glucose levels of normal rabbits (Means ± SEM mg/100 ml) at various time intervals after oral administration of 2% gum tragacanth solution and Portulaca oleracea powder (0.5, 1.0, 1.5 and 2.0 g/kg) body weight, orally suspended in 2% gum solution.

NS : Non-significant decrease as compared to zero level (P > 0.05).

* : Significant decrease as compared to zero level (P < 0.05).

** : Highly significant decrease as compared to zero level (P < 0.001).

Number of animals in each group : 6
the administration of different doses of the indigenous medicinal plants P. oleraceae (Kulfa) and T. officinale (Dudhal) have caused a decrease in the blood glucose of normal rabbits. These crude drugs produced a significant and consistent hypoglycaemic response in these rabbits. For comparison, tolbutamide (500 mg/kg) was administered orally to the normal rabbits. Similar to results obtained by
Akhtar ct al.\textsuperscript{6} and Augusti\textsuperscript{10} these doses of tolbutamide produced a significant decrease of blood glucose levels at 4, 8 and 12 hours. However the administration of P. oleracea (2 g/kg) produces a significant fall in blood sugar even at a 24 hr interval (Fig. 1), showing that this plant possesses a longer duration of action than tolbutamide, which produces hypoglycaemia for 6 - 12\textsuperscript{11} hrs. Further, calculations also show the maximum decrease in blood glucose produced by P. oleracea (2 g/kg) and T officinale (2 g/kg) are respectively 63 and 65 percent of the maximum decrease produced by the 500 mg/kg of tolbutamide.

Sulphonylureas as tolbutamide produce hypoglycaemia by stimulating pancreatic beta-cells to release more insulin into the blood stream and by increasing glycogen deposition in the liver. Thus it may be postulated that hypoglycaemic principle(s) in P. oleracea and T. officinale plants also exert an hypoglycaemic effect in rabbits by causing the release of insulin from pancreatic beta-cells. A similar mechanism has been proposed to explain the hypoglycaemic effects of Momordica charantia Euphorbia pro-strata and Fumaria Parviflora\textsuperscript{5,6,12} Sulphonylureas, however, do not decrease blood glucose level in alloxan-diabetic animals.

P. oleracea and T. officinale could not significantly lower blood glucose levels in diabetic animals. Similarly, tolbutamide did not produce any significant change in the glucose levels of the alloxan-diabetic rabbits. It is, therefore, conceivable that hypoglycaemic principle(s) in P. oleracea and T. officinale exert a hypoglycaemic effect in rabbits with intact pancreatic beta-cells by triggering insulin release. However, it remains to be established whether or not these plant drugs actually act by stimulating the secretion of insulin. Nevertheless, the results do suggest that their active principles do not seem to possess insulin-like activities are non toxic and have no effect on behavioural pattern of animals.

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References

10. Augusti, K.T. and Benaim, M.E. Effect of essential oil of Onion (APDS) on blood glucose, free