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ANTI-DIABETIC ACTIVITY OF ROOTS OF *QUERCUS INFECTORIA* OLIVIER IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The methanolic extract of roots of *Quercus infectoria* Olivier at a dose of 250 mg/kg and 500 mg/kg body weight respectively was tested for anti-diabetic activity in Alloxan-induced hyperglycaemic rats. The blood glucose levels were measured at 0, 2h, 4h and 6h after the treatment. The methanolic extract reduced the blood glucose Alloxan- induced diabetic rats from 285.52 to 206.57mg/dl, 6h after oral administration of extract ($P<0.01$). The antidiabetic activity of methanolic extract of *Quercus infectoria* Olivier was compared with glibenclamide, an oral hypoglycaemic agent (3mg/kg).

INTRODUCTION: Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world [1]. Diabetes is one of the leading causes of death in humans and animals. The presence of diabetes confers increased risk of many devastating complications such as cardiovascular diseases (CVD), peripheral vascular diseases (PVD)[2], complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations and blindness [3].

Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureasa are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and in some cases diminution of response after prolonged use [4]. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world, especially in countries where access to the conventional anti- diabetic agents is inadequate. Although several medicinal plants have gained importance for the treatment of diabetes, many remain to be scientifically investigated [5]. Laboratories are conducting research on these medicinal plants in a scientific manner for the development of alternative drugs and strategies for better management of diabetes.

Quercus infectoria Olivier (Fagaceae) is a small tree found in India, Greece, and Iran. In Asian countries, the whole plant parts of *Q. infectoria* have been used for centuries in oriental traditional medicines for treating various diseases [6]. The plant *Q. infectoria* have also been pharmacologically documented to possess astringent, antidiabetic [7], antitremorine, local anaesthetic [8], antiviral [9], antibacterial [10], antifungal [11], larvicidal [12] and antiinflammatory [13] activities. The main constituents found in the roots of *Q. infectoria* are tannin (50-70%), terpenoids and small amounts of free gallic acid and ellagic acid [14-16].

The present study was aimed to investigate the anti-diabetic activity of an alcoholic extract of *Quercus infectoria* Olivier roots in Alloxon induced diabetic rats.

MATERIAL AND METHODS: All the chemicals and reagents used were from C.H.D. and Ranchem. Glassware used from Borosil.

Plant Materials: The roots of *Quercus infectoria* Olivier used in this study were obtained from the local market of Uttarakhand and were identified based on its physical characteristics. The roots were dried and crushed to small pieces using pestle and mortar and powered in an electric grinder.

Preliminary Phytochemical Screening: The powder of the roots of *Quercus infectoria* Olivier was subjected to successive extraction with different solvents in increasing order of polarity of solvents. The dry extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents [17].

Physical Evaluation Parameters: In physical evaluation total ash, acid insoluble ash and water soluble ash were found to be 4.27% w/w, 0.87% w/w and 1.29% w/w respectively. The water soluble extractive value alcohol soluble extractive value was found to be 25% and 30%. Loss of drying was found to be 3.62% w/w (**Table 1**).

Preparation of the Plant Extract: The roots were collected locally, dried and coarsely powdered. The petroleum ether, chloroform, methanol extract was prepared by Soxhlet extraction at 80°C, 60°C, 78°C for 6 hours, then the solvent was recovered at 80°C, 60°C, 78^{o}C respectively.

Animals: Adult albino rats weighing about 100-150 g were used in the present investigation. All the rats were given a period of acclimatization for 15 days before starting the experiment. They were fed *ad libitum* everyday with standard chow diet and were given free access to water. Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water.

Induction of Diabetes: Diabetes was induced in the albino rats by administering Alloxan monohydrate. Animals were allowed to fast for 24 hr and were injected with freshly prepared Alloxan monohydrate (120mg/kg, i.p.) in normal saline. After 72 h, blood was collected from tail vein of the rats under ether anesthesia and blood glucose levels were estimated using a glu-oxidase peroxidase reactive strips and glucometer one touch basic plus. The animals were considered to be diabetic if the blood glucose values were above 250 mg/dl, and those animals alone were used for the study. Control rats were injected with normal saline alone.

Experimental design for Anti-Diabetic Activity: The rats were divided into five groups comprising 6 animals in each group as follows

Group I: animals served as normal control, which received normal saline.

Group II: untreated diabetic control (Alloxan, 120 mg/kg body weight).

Group III: Diabetic rats treated with glibenclamide (3mg/kg body weight)

Group IV and V: Diabetic rats treated with methanolic extracts of *Quercus infectoria* Olivier (250 mg/kg and 500 mg/kg body weight, respectively)

The blood glucose levels of experimental animals were determined at 0, 2, 4 and 6 h after feeding the plant extract by using glu-oxidase peroxidase reactive strips and glucometer (one touch basic plus).

Statistical Analysis: Values are expressed as mean S.E.M. (n=6). Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's *t* test 15. $P<0.01$ and $P<0.05$ were considered statistically significant when compared with diabetic control.

RESULTS AND DISCUSSION: Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is physical evaluation of the powdered root and next is the *in vitro* anti-diabetic activity assay[18]. In physical evaluation total ash, acid insoluble ash and water soluble ash were found to be 4.26% w/w, 0.87% w/w and 1.29% w/w respectively. The water soluble extractive value alcohol soluble extractive value was found to be 25% and 30%. Loss of drying was found to be 3.62% w/w (**Table 1**).

The present work has detected the anti-diabetic activity of *Quercus infectoria* Olivier root extract in Alloxan-induced hyperglycemia in rats. Alloxan-induced diabetes mellitus and insulin deficiency lead to increased blood glucose level. When *Quercus infectoria* Olivier root extract was administered to diabetic rats, hypoglycaemia was observed after 2 hrs, with the maximum effect being seen at 6 hrs.

Oral treatment with the methanolic extract of *Quercus infectoria* Olivier roots (250 and 500 mg/kg body weight) to Alloxan- induced diabetic albino rats produced dose dependant reduction of blood glucose levels particularly 6 h after treatment (n=6, p<0.01) (-59.33 to –78.05 g/dl, respectively) compared to diabetic control group (**Table 2**). The methanolic extract of the plant at a dose of 250 mg/kg body weight and 500 mg/kg body weight reduced the elevated level of blood glucose from 279.67 to 220.34 and 285.52 to 206.57 g/dl, respectively) 6 h after treatment. Glibenclamide (3 mg/kg body weight) used as a standard drug and also produced a significant reduction in blood glucose levels compared to control group (288.27 to 186.67 g/dl, P<0.01).

TABLE 1: DIFFERENT STANDARDIZATION PARAMETER OF ROOTS OF *QUERCUS INFECTORIA* OLIVIER

PARAMETER	VALUES OBTAINED
Total ash	4.27% w/w,
Acid insoluble ash	0.87% w/w
Water soluble ash	1.29% w/w
Water soluble extractive	25%
Alcohol soluble extractive	30%
Loss on drying	3.62% w/w

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF ROOTS OF *QUERCUS INFECTORIA* OLIVIER ON PLASMA GLUCOSE LEVELS IN ALLOXAN-INDUCED DIABETIC RATS

Group	Treatment	Blood glucose level in mg/dl after			
		0	2hr	4hr	6hr
I	Normal control	75.68±3.17	76.53±2.18	74.48±2.13	73.21±2.48
II	Diabetic control	286.65±8.14	282.34±6.42	276.8±5.40	280.32±7.18
III	Diabetic control + Standard (3mg/kg)	288.27±7.54	240.32±5.15	206.65±6.84	186.67±8.14
IV	Diabetic control + Extract (250mg/kg)	279.67±6.14	263.32±9.14	242.11±3.14	220.34±9.14
V	Diabetic control + Extract (500mg/kg)	285.52±6.17	239.56±8.74	224.38±7.19	206.57±5.57

Values are mean S.E.M. (n=6). *P<0.01; **P<0.05 Vs Diabetic control. One way analysis followed by Dunnet's *t* test.

From the results it is assumed that the root extract of the plant could be responsible for stimulation of insulin release and observed restoration of blood glucose level. Further, the decreased blood glucose lowering effect of the methanolic extract in Alloxan-induced diabetic rats could also possibly due to increased peripheral glucose utilization. It has been reported that using medicinal extract to treat the Alloxan-induced diabetic rats results in activation of *β*-cells and insulinogenic effects.

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