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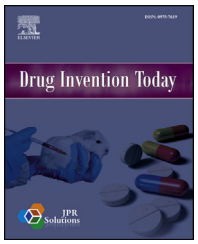

Original Article

Antioxidant and protective effect of *Curculigo orchioides* on liver, pancreas and kidney tissue in alloxan induced diabetic experimental rats

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ABSTRACT

Background: The aim of this study was to investigate the antioxidant activity of *Curculigo orchioides* in alloxan induced diabetic rats.
Methods: Diabetes was induced experimentally in 12-h fasted rats by intraperitoneal injections of alloxan (120 mg/kg b.w.) and *C. orchioides* (200 mg/kg b.w.) was administered orally for 21 days.
Results: Untreated diabetic rats in comparison with normal rats showed significantly lower mean activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), lower mean levels of non-enzymatic antioxidants (reduced glutathione, vitamin C, vitamin E), elevated mean levels of pancreatic malondialdehyde (MDA), elevated mean activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Following oral administration of *C. orchioides* (200 mg/kg b.w./day) to diabetic rats for 21 days exhibited significant improvement of the above parameters. Histopathological studies showed significant changes like cytoplasmic vacuolization of hepatocytes, leukocytic infiltration and edema in the liver and kidney of alloxan-induced diabetic rats. These histopathological abnormalities were found to be normalized after treatment with *C. orchioides* extract.
Conclusion: These results suggest that the methanolic extract *C. orchioides* enhanced the antioxidant defense against reactive oxygen species produced under hyperglycemic conditions, hence protecting the liver, pancreatic and kidney tissue injuries.

1. Introduction

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia. Till date the pharmaceutical drugs administered for diabetes are either too expensive or have undesirable side effects. Treatment with sulphonylureas and biguanides is associated with adverse side effects. However, complementary medicine has grown in popularity in recent

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years owing to its minimal side effects and appropriate action. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine were used commonly in India. Many indigenous Indian medicinal plants have been found to be useful in successful management of diabetes and some of them have been tested for their active ingredients. The World Health Organization (WHO) has also recommended the evaluation of the plant's effectiveness and conditions against chronic ailment in place of chemically synthesized drugs.

In recent years, much attention has been focused on the role of oxidative stress that plays a key role in the pathogenesis of secondary diabetic complications.[1] Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems that has increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides.[2] In addition to reduced glutathione (GSH), there are other defense mechanisms against free radicals, such as the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals respectively.

Many of the complications of diabetes mellitus, including retinopathy and atherosclerotic vascular disease contribute to the leading cause of mortality in diabetes mellitus that are correlated with oxidative stress.[3] Plants often contain substantial amounts of antioxidants, flavonoids, tannins etc. The present study suggests that antioxidant action of the methanolic extract of *Curculigo orchioides* may be an important property associated with the hypoglycaemic effect on diabetes mellitus. *C. orchioides* Gaertn., is one of the well known medicinal plant belonging to the family *Hypoxidaceae* (*Amaryllidaceae*). It is distributed widely in the southern parts of Japan, China, India and Australia, generally used as a tonic in traditional Chinese medicine to treat decline in physical strength.[4] Its rhizomes are used as an alternative for demulcent, diuretic, restorative and for the treatment of jaundice.[5] Curculigoside, an active compound isolated from *C. orchioides* can improve cognitive function and is developed as a new drug for the treatment of Alzheimer's disease.[6,7] Therefore, this study was designed to investigate the protective effect of *C. orchioides* on tissues lipid peroxides, enzymic and non-enzymic antioxidants in diabetic rats.

2. Materials and methods

2.1. Chemicals

Alloxan monohydrate was purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Preparation of methanolic extract of C. orchioides

The root parts of *C. orchioides* were collected, shade-dried and then finely powdered (collected from the Bharathidasan University, Tamil Nadu). 500 g of powder was extracted with methanol using a Soxhlet apparatus. The solvent was then evaporated under reduced pressure at 40 °C and dried in vacuum dessicator.

2.3. Experimental animals

Adult male albino rats of the Wistar strain (170–190 g) used in the present study were obtained from Madras Veterinary College, TANUVAS, Chennai, India. The animals were housed in clean polypropylene cages under conditions of controlled temperature (25 ± 2 °C) with a 12/12-h day–night cycle, they had free access to food and water *ad libitum*. Animal experimentation was carried out as per the rules and protocols approved by the Institutional Animal Ethical Committee (IAEC).

2.4. Induction of experimental diabetes mellitus

Rats were fasted overnight (12 h) and diabetes was induced by single intraperitoneal injection of alloxan (120 mg/kg b.w.). Induction of diabetes was confirmed by measuring blood glucose level 6 days after the administration of alloxan. Rats with blood glucose level >280 mg/dl were considered as diabetic and preferred for further experimentation.

2.5. Experimental design

The rats were divided into five groups of six animals in each group ($n = 6$).

Group I: normal control rats, received vehicle alone.
Group II: diabetic control rats received 120 mg/kg of alloxan [intra peritoneal injection (*ip*)].
Group III: normal rats received methanolic extract of *C. orchioides* orally (200 mg/kg/day) for 21 days.
Group IV: diabetic rats received methanolic extract of *C. orchioides* orally (200 mg/kg/day) for 21 days after alloxan treatment.
Group V: diabetic rats received glibenclamide orally (10 mg/kg b.w./day) for 21 days.

On 21st day, the animals were anaesthetized and sacrificed by decapitation. Liver, kidney and pancreatic tissues were collected and stored at −20 °C for antioxidant assays. A small portion of kidney and liver were excised and fixed in 10% formalin for histopathological analysis.

2.6. Estimation of lipid peroxidation

The level of lipid peroxidation was determined in tissue homogenate of liver and kidney by the method of Ohkawa et al, 1979.[8]