Research Article

In vitro Antioxidant and *in vivo* Antidiabetic Potential of *n*-butanol Extract of *Chrysanthemum fuscatum* in Streptozotocin Induced Diabetic Rats.

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ABSTRACT

Free radicals and lipid peroxide, easily formed in the diabetic state, play an important role in the development of diabetic complications. The present study designed to investigate the *in vitro* antioxidant activity of *n*-butanol extract of *Chrysanthemum fuscatum.* Also, its hypoglycemic and antilipid peroxidative properties in streptozotocin induced diabetic rats. The *n*-butanol extract of *C. fuscatum* was administered orally (200 mg/kg, for 15 days) to streptozotocin-induced diabetic rats. Hypoglycemic effects, lipid peroxidation and lipid profile in diabetic rats treated with *n*-butanol extract were assessed and compared to control (normal and diabetic rats). Diabetic rats treated with extract caused a significant decrease in blood glucose and lipid peroxidation in liver, kidneys and pancreas. *Chrysanthemum fuscatum* extract exhibits *in vitro* antioxidant activity with IC_{50} (4.36 µg/mL) slightly higher than quercetin (3.85 µg/mL). Results indicated that *n*-butanol extract of *Chrysanthemum fuscatum* might be beneficial for diabetes and its complications.

Keywords: *Chrysanthemum fuscatum*, Diabetic rats, Streptozotocin, Oxidative stress, DPPH test.

INTRODUCTION

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem; it is the fourth main cause of death in most developed countries. The prevalence of diabetes estimated to reach 330 million by the year 2025, according to International Diabetes Federation[1]. It is could be a bunch of metabolic disorders portrayed via hyperglycemia following imperfections in insulin emission, insulin activity, or both. The chronic hyperglycemia identified with stretched pathology and damage, which distress multiple organs. It additionally incorporates a bigger chance of getting dyslipidemia, high blood pressure, and obesity[2]. Various complications develop including both macro- and micro vascular dysfunctions[3].

Diabetes mellitus is classified into two main types, type 1 and type 2, with type 1 resulting from the body's failure to produce insulin, and requires one to be injected with insulin. Type 2 diabetes mellitus describes a condition of fasting hyperglycemia that occurs despite the availability of insulin[4].

Diabetes invariably accompanied by oxidative stress, which has been postulated to be the unifying pathogenic mechanism mediating the appearance and progression of chronic diabetic complications. The increased levels of lipid and DNA peroxidation products found in diabetic patients are the result of an imbalance between free radicals and antioxidants which directly caused by hyperglycaemia[5].

The oxidative stress impairs various cellular functions and plays important roles in the pathophysiology of many diseases. In type 1 diabetes, reactive oxygen species generated by macrophages and participate in the toxic actions that lead to necrosis or apoptosis of the insulin-producing cells. In type 2 diabetes, chronic hyperglycemia leads to overproduction of reactive oxygen species and the high reactivity of reactive oxygen species caused cellular injury through nonspecific modification and disruption of proteins, phospholipids and nucleic acids[6].

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species can initiate degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids are commonly found in plants have been reported to have multiple biological effects, including antioxidant activity[7].

Flavonoids are a group of polyphenolic substances, very widespread in nature, which are found in plants predominantly in the form of glycosides. Especially aglycones are pharmacologically effective. Many of them show hepatoprotective, diuretic, vasodilation, antibacterial, anti-inflammatory, antidiabetic, antiallergic and chemoprotective effects. In recent years, increased attention has paid to study their antioxidative activity and capability to extinguish or absorb free radicals[8].

Also, plant part with hypoglycemic effect are still prevalent in developing countries, were thy have used to alleviate the symptoms of diabetes for many centuries[9].

In our present study we examined the anti-diabetic properties of *n*-butanol extract of *Chrysanthemum fuscatum*, a member of compositae family, we also screened the antioxidant activity of *n*-butanol extract *in vitro*.

MATERIAL AND METHODS

Plant material

Chrysanthemum fuscatum Desf., growing abundantly in Sahara, was collected from the Dayas of Touggourt region in the South East of Algeria in 2001 and authenticated by Pr. N. Khalfallah (Department of Nature and Life Sciences, Brother Mentouri University, Constantine, Algeria). A voucher specimen was deposited in the Herbarium of the Department of Nature and Life Sciences, Brother Mentouri University, Constantine under the code: CCF 05/01/01.

Extraction procedure

Air-dried leaves: (1500 g) were powdered and macerated at room temperature with EtOH–H_2O (8:2 v/v) for 48 h three times. After filtration, the filtrates were combined, concentrated under vacuum (up to 35°C), diluted with 600 ml H_2O, filtered to remove chlorophyll and successively extracted with (3x400 ml), chloroform, ethyl acetate and *n*-butanol. The organic solutions were dried with sodium sulfate (Na_2SO_4), filtered using common filter paper and concentrated in vacuum (up to 35°C) to obtain the following extracts: $CHCl_3$ (5g), EtOAc (28g), *n*-butanol (42 g) resulted in final extracts.

Experimental animals

Male *Wistar Albino* rats weighing (230-260g) used in the present study. The animals were kept in 12h light/dark cycles and maintained in an air-conditioned room at 22 to 25 °C, with free access to food and water ad *libitum* for two weeks. The general guidelines for the use and care of living animals in scientific investigations were designed according to the ethical standards for animals use and approved by the local ethical committee of animal use and followed[10].

Animals were divided into 4 groups, each consisting of 7 animals:

T: Control rats received citrate buffer.

EXT200: Non- diabetic control rats received by gavage 200mg/kg/day of *n*-butanol extract for 15 days.

D: diabetic control rats.

D+EXT: diabetic rats treated by *n*-butanol extract plant (200mg/kg/day) for 15 days[11].

Induction of experimental diabetes

Diabetes was prompted through single intravenous injection of freshly prepared streptozotocin (STZ) (55 mg/ kg b.w.) in 0.1 M citrate buffer (pH = 4.5) to overnight starved rats[12]. Diabetic rats were permitted to drink 20% glucose solution overnight to overcome the initial drug induced hypoglycemic death. The blood glucose level was measured after three days, and rats with glucose levels >250 mg/dL were considered as diabetic.

At the end of the experimental period, animals were sacrificed by cervical dislocation and livers, pancreas and kidneys were isolated to measure the level of MDA. The blood is collected through the retro-orbital sinus at the eye of rats for analysis of biochemical parameters. Levels of glucose, total cholesterol and triglycerides were estimated using commercial kits (Spinreact, SPAIN).

Estimation of lipid peroxidation

Lipid peroxidation In different organs (liver, kidney and pancreas), was evaluated by measuring malondialdehyde (MDA) according to the method of Uchiyama and Mihara, 1978[13]. Organs were removed and homogenized in cold KCl 1,15% to make a 10% homogenate. 3 ml of 1% phosphoric acid and 1ml of 0,67% thiobarbituric acid (TBA) aqueous solutions were added to 0.5 ml of 10% homogenate pipetted into 10 ml centrifuge tube. Then, the mixture heated for 45 min in a boiling water bath. The mixture cooled at room temperature. After that, 4 ml of *n*-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and absorbance was measured at 532 nm.

DPPH radical scavenging activity

Hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay used stable l,l-diphenyl-2-picrylhydrazyl (DPPH) radical as a reagent. Various concentrations of the samples in methanol were added to 3 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period in the dark and at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I %) was calculated as follows:

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate[14].

Statistical Analysis

Data are expressed as the mean ± SD. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical interferences were based on student's test for mean values comparing control and treated animals. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves[15, 16].

In this study, STZ (55 mg/kg) was utilized as diabetogenic operator to prompt the diabetes in *albino Wistar* rats. Heavy loss in body weight was observed in STZ induced diabetic rats compared to control group (*P< 0.001*) (Fig. 1). Diminishing body weight in diabetic rats plainly affirms a corruption of basic proteins because of diabetes[16]. The deficit in body weight noticed in STZ instigated diabetic control rats may be due to muscle squandering[18,19]. Oral administration of *n*-butanol extract of *Chrysanthemum fuscatum* result in weight gain in STZ induced diabetic rats.

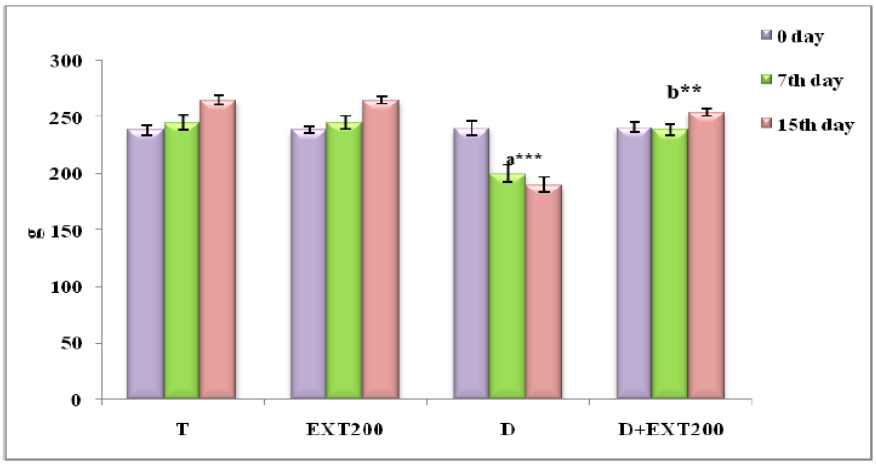

Figure 1: Effect of *n*-butanol extract of *Chrysanthemum fuscatum* on the body weight in STZ- induced diabetic rats.
**P<0.05; **P<0.01; ***P<0.001, compared to control group. a: compared to Citrate group;b: compared to diabetic control group; T: rats received citrate buffer; Ext.200: extract at the dose 200mg/kg; D: diabetic control rats; D+EXT: diabetic rats treated by extract plant (200mg/kg).*

In our present study, we have observed in diabetic rats significant increase of blood glucose (*P< 0.001*) cholesterol *(P< 0.01*) and triglycerides *(P< 0.01*) compared with control rats (Fig.2, 3). The level of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited action of lipolytic hormones on the fat depots. We observed earlier that the hypercholesterolemia and hypertriglyceridemia occurs in STZ –induced diabetic rats. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia[20].

Streptozotocin caused a massive reduction in insulin release, by the destruction of β-cells of the islets of Langerhans and inducing hyperglycemia[21]. Streptozotocin enters the β-cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induced activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP.

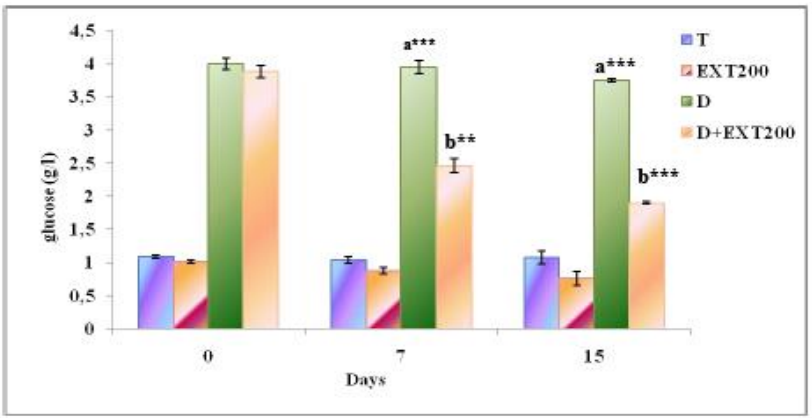

Figure 2: Effect of *n*-butanol extract of *Chrysanthemum fuscatum* on serum glucose level in STZ- induced diabetic rats.
*P<0.05; **P<0.01; ***P<0.001, compared to control group. a: compared to Citrate group;b: compared to diabetic control group; T: rats received citrate buffer; Ext.200: extract at the dose 200mg/kg; D: diabetic control rats; D+EXT: diabetic rats treated by extract plant (200mg/kg).

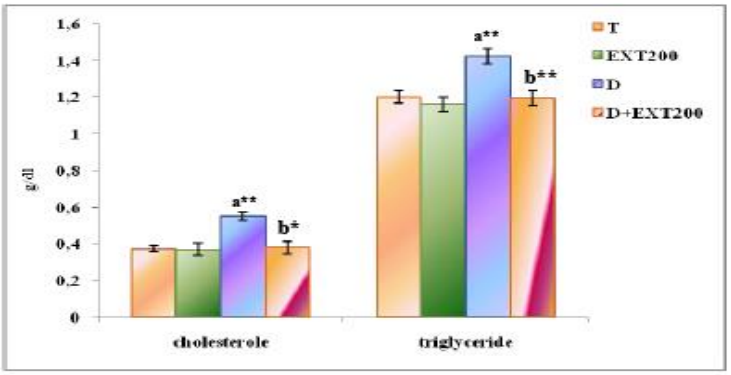

Figure 3: Effect of *n*-butanol extract of *Chrysanthemum fuscatum* on serum cholesterol and triglyceride level in STZ- induced diabetic rats.

*P<0.05; **P<0.01; ***P<0.001, compared to control group. a: compared to Citrate group;b: compared to diabetic control group; T: rats received citrate buffer; Ext.200: extract at the dose 200mg/kg; D: diabetic control rats; D+EXT: diabetic rats treated by extract plant (200mg/kg).

Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, β cells undergo the destruction by necrosis[22].

Administration of *n*-butanol extract of *Chrysanthemum fuscatum* to diabetic rats caused significant reduction in blood glucose, triglycerides and cholesterol. The possible mechanism by which *n*-butanol extract brings about its hypoglycaemic action may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β- cells of islets of Langerhans or its release from the bound form[23].

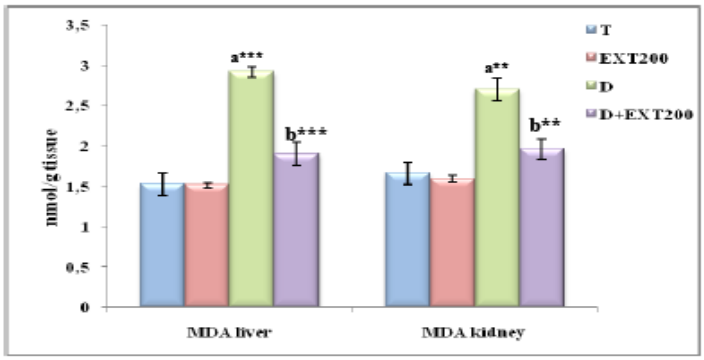

Figure 4: Effect of *n*-butanol extract of *Chrysanthemum fuscatum* on MDA liver and kidney level in STZ- induced diabetic rats.

*P<0.05; **P<0.01; ***P<0.001, compared to control group. a: compared to Citrate group;b: compared to diabetic control group; T: rats received citrate buffer; Ext.200: extract at the dose 200mg/kg; D: diabetic control rats; D+EXT: diabetic rats treated by extract plant (200mg/kg).

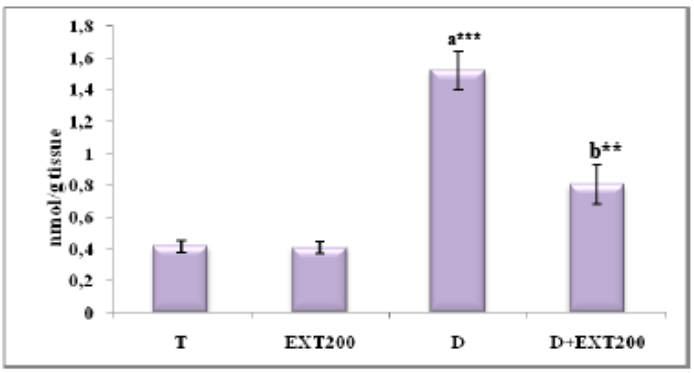

Figure 5: Effect of *n*-butanol extract of *Chrysanthemum fuscatum* on MDA pancreas level in STZ- induced diabetic rats**.**

*P<0.05; **P<0.01; ***P<0.001, compared to control group. a: compared to Citrate group;b: compared to diabetic control group; T: rats received citrate buffer; Ext.200: extract at the dose 200mg/kg; D: diabetic control rats; D+EXT: diabetic rats treated by extract plant (200mg/kg).

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The LPO concentration increase significantly in the liver (*P< 0.001*), kidneys *(P< 0.01)* and pancreas *(P< 0.001*) of diabetic rats compared to control rats. Treatment of STZ diabetic rats with *Chrysanthemum fuscatum* (200 mg/kg) resulted in significant decrease in level of MDA.

There has been considerable recent debate regarding the extent to which increased oxidative stress contributes towards the development of diabetic complications. The fact that the role of antioxidant compounds in both protection and therapy of diabetes mellitus were also emphasized in previous scientific researches. Hyperglycaemia results in the generation of free radicals which can exhaust antioxidant defences thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid peroxidation[24].

Lipid peroxide-mediated tissue damage has been observed in the development of both diabetes type 1 and type 2. It has been observed that insulin secretion is closely associated with lipoxygenase-derived peroxides. Increased concentration of lipid peroxide in the liver can result in decreased activity of cytochrome P 450 and cytochrome b5 and this may affect the drugs metabolising-activity in chronic diabetes[25]. Our study shows that administration of *n*-butanol extract of *Chrysanthemum fuscatum* tends to bring the liver, kidney and pancreas TBARS back to near normal (Fig.4, 5).

DPPH$^{\bullet}$ is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract[26]. Scavenging of DPPH$^{\bullet}$ radical is related to the inhibition of lipid peroxidation. DPPH$^{\bullet}$ is usually used as a substance to evaluate the antioxidant activity[27]. Antioxidants either transfer an electron or a hydrogen atom to DPPH$^{\bullet}$, thus neutralizing its free radical character. DPPH$^{\bullet}$ test, which is based on the ability of DPPH$^{\bullet}$, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH• assay has been largely used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts. The reducing capacity of compounds could serve as indicator of potential antioxidant property [25, 28, 29]. In the present study, The IC_{50} values of *n*-butanol extract of *Chrysanthemum fuscatum* and Quercetin were 4.36 µg/mL and 3.85 µg/mL, respectively (Fig.6, 7). The experimental results show that *n*-butanol extract possess a significant antioxidant activity *in vitro*.

CONCLUSION

Based on our present experimental data, it can suggest that *n*-butanol extract of *Chrysanthemum fuscatum* may prevent hyperglycemia and hyper lipidemia in STZ induced diabetic rats, which can be due to various mechanisms. These results suggest that extract treatment possesses anti-diabetic activity in STZ diabetic rats. These data imply that the treatment as antioxidant therapy may be beneficial in preventing diabetic complications due to lipoperoxidation and free radicals in diabetic rats.

The present series of tests provided evidence that the extract used in this study contained active substances which they produced a reduction of diabetic blood glucose levels. Further investigation is in necessary to determine the exact the constituents responsible for antidiabetic effect.

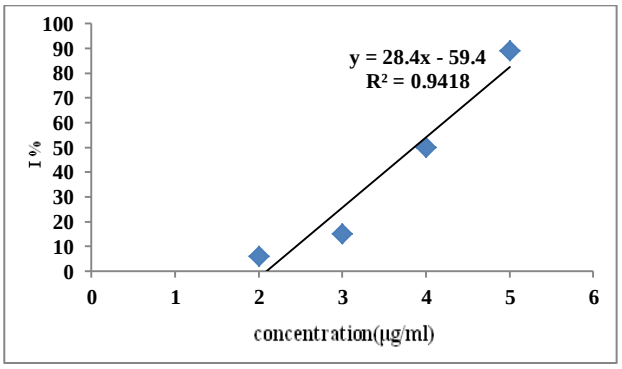

Figure 6: Free radical scavenging activity (DPPH method) of Quercetin.

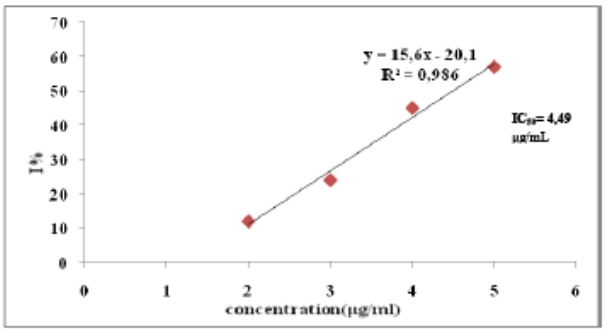

Figure 7: Free radical scavenging activity (DPPH method) of *n*-butanol extract of *Chrysanthemum fuscatum*

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