Full Length Research Paper

Anti-diabetic activity of aqueous root extract of Anacyclus pyrethrum L. in streptozotocin-induced-diabetic rats

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The present work is to study for the first time the anti-diabetic properties of aqueous extract of roots of Anacyclus pyrethrum L. in normal and streptozotocin (STZ)-induced diabetic rats and to achieve a primary pharmacological screening contained in the aqueous extract. A total of 20 rats including 10 diabetics and 10 normal rats were used for this study. The anti-diabetic activity of aqueous extract of roots was evaluated by using normal and STZ induced diabetic rats at a dose of 250 mg/kg p.o daily for 21 days. Blood glucose levels were measured using GOD-POD. Screening for major classes of phytochemical was done using standard chemical tests. Per oral administration of the aqueous extract of the roots (250 mg/kg body weight) to streptozotocin-induced diabetic rats exhibited a significant antihyperglycemic activity in STZ-induced diabetic rats, whereas in normal rats no hypoglycemic activity was observed. Phytochemical screening showed a wealth in compounds: Tannins, saponins, alkaloids, amino acids, steroids and terpenoids. Aqueous extract of roots exhibit attractive properties and can therefore, be considered a promising candidate for future application as alternative therapeutic agents, particularly in the development of anti-diabetic drugs.

Key words: Anacyclus pyrethrum L, anti-diabetic activity, streptozotocin, aqueous root extract, phytochemical screening.

INTRODUCTION

Diabetes is one of the most prevalent chronic diseases in the world. It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Considerable attention has been placed on understanding the pathophysiology of diabetes mellitus because of its importance in human health. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 2008). Current treatment includes insulin therapy, although this provides good glycemic control, it can do little to prevent secondary complications. Besides, these drugs are associated with side-effects or diminution in response after prolonged use (Mahadeva and Subramanian, 2009). Moreover, providing modern medical healthcare across the world is still a far-off goal due to economic constraints. Thus, it is necessary that we continue to look for new and, if possible, more efficacious drugs, and the vast reserves of phytotherapy may be an ideal target (Mahadeva and Subramanian, 2009). Plants have played a significant role in maintaining human health and improving quality of life for thousands of years. The use of medicinal plants is increasing because of their widespread use and for their curative effects on various diseases (Bhavsar et
In order to discover other hypoglycaemic agents, many investigations have been performed in traditional medicine testing eventual hypoglycaemic plants (Girija et al., 2011; Meliani et al., 2011; Salahuddin et al., 2010; Kumar et al., 2010; Solati et al., 2010; Adeneye et al., 2009). *Anacyclus pyrethrum* L. (Compositeae, Asteraceae), commonly known as "African pyrethrum" and referred to as "Tigenthast", is a perennial, procumbent herb, which is native from Northern Africa and is cultivated in the Mediterranean (Bellakhdar, 1997). The root of *A. pyrethrum* is being widely used in the local traditional medicinal treatment system for a variety of several diseases: it is used in the treatment of some rheumatic and neuralgic affection of the head, the tooth and face. A local ethnobotanical survey carried out showed its possible anti-diabetic activity (Allali et al., 2008). Previous chemical studies indicate that the plant possesses immunomodulatory properties (Bendjeddou et al., 2003; Ching et al., 2007). Everywhere in Algeria, the root is used as sternutatory, sialagogue and diaphoretic. It is regarded as a tonic to the nervous system. It is also used in respiratory infections and in the treatment of liver disease (Bellakhdar, 1997; Bendjeddou et al., 2003). In some other countries, the roots are also considered aphrodisiac and sexual stimulant. In Indian medicine, the plant is widely recognized as tonic and rejuvenator (Singh et al., 1998). It has been reported that the *A. pyrethrum* root has antibacterial and anti-inflammatory activities and is known for its insecticidal properties (Bellakhdar, 1997). However, the literature indicates that there is no scientific evidence to support the anti-diabetic effect of *A. pyrethrum* in spite of its ethnobotanical usage. The aim of the present work was evaluate for the first time the anti-diabetic activity of *A. pyrethrum* to ascertain the scientific basis for the use of this plant in the treatment of diabetes and to do the phytochemical preliminary screening of roots.

**MATERIALS AND METHODS**

**Plant material**

The roots of *A. pyrethrum* were harvested from the mounts of Tlemcen (north-west of Algeria), in April 2009. The plants collected were identified by Pr. Nouy Benabadj, "Laboratory of Ecology and Ecosystem Management", University of Tlemcen (Algeria). A voucher specimen was deposited in this laboratory. Plant samples were dried in the share and conserved for future use.

**Preparation of aqueous extract**

Fresh roots were dried at room temperature. Aqueous extract was obtained as follows. In brief, 100 g of dried roots were cut into small bits and extracted by refluxing with distilled water for one hour. Thereafter, it was decanted and filtered with filter paper and followed by centrifugation for 30 min at 5000 rpm. The supernatant was filtered to eliminate any residues. The filtrate was dried in the oven at 40°C to make a powder yielding 2.3% (w/w). The solid residue was stored in dessicator prior use for subsequent experiments.

**Phytochemical analysis**

**Alkaloids**

Five milliliters of the crude extract were added to 2 mL of hydrochloric acid. One milliliter of Dragendorff's reagent was added to this acidic medium. An orange or red precipitation was produced which indicates the presence of alkaloids (Kokate, 2001).

**Amino acids**

One milliliter of the crude stock extract was added a few drops of Ninhydrin reagent. The purple colour appearance shows the presence of amino acids (Harborn, 1998).

**Saponins**

The crude extract solution was diluted with 20 mL of distilled water and it was agitated in a graduated cylinder for 15 min. The formation of 1 cm foam layer showed the presence of saponins (Kokate, 2001; Harborn, 1998).

**Flavonoids and tannins**

To one milliliter of the crude stock extract, 10 mg magnesium turnings were added into 1 mL of the filtrate, followed by the addition of 0.05 mL concentrated sulphuric acid. The presence of magenta red observed within 3 min confirmed the presence of flavonoids. The presence of blue-black precipitates resulting from the addition of ferric chloride (0.01 g/mL) reagent indicated the presence of tannins (Harborn, 1998).

**Steroids and terpenoids**

Screening the presence of steroids and terpenoids was performed as described by Kumar (Kumar et al., 2009). 0.2 g of extract was dissolved in 10 mL methanol and filtered. 1 mL of chloroform and 1 mL of concentrated sulphuric acid were then added into 1 mL of filtrate by the side of the tube and the presence of yellow with green fluorescence at the sulphuric acid layer indicated the presence of steroids. For the detection of terpenoids, 1 mL of acetic anhydride and 2 mL of concentrated sulphuric acid were added into 1 mL filtrate. Presence of reddish brown on interface indicated the presence of terpenoids.

**Animals**

Male wistar rats between 12-13 week of age and weighing 250-300g were procured from Mascara University Animal house, Algeria. The animals were under standard conditions and fed with rodent diet and water *ad libitum*.

**Induction of diabetes**

Diabetes was induced in rats by intravenous (i.v) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1M cold citrate buffer (pH = 4.5) (Sancheti et al., 2010). Blood glucose level was measured using GOD-POD (Kit spinreact). Blood samples were withdrawn from retro-orbital plexus under light ether
anaesthesia before. A fortnight later, diabetes was confirmed.

**Acute toxicity**

Acute toxicity study on *A. pyrethrum* roots extract was performed in experimental rats. Graded doses of the aqueous extract of roots (100, 250, 500 and 1000 mg/kg body weight) were administered p.o and the animals were observed for 2 weeks following administration (Olagbende-Dada et al., 2010). The dosage schedule was fixed as 250 mg/kg body weight/rat/day for 21 days.

**Experimental protocol**

The normal rats and those with hyperglycemia (blood glucose 200-360 mg/100 mL) were divided into four groups of five animals each and treated by per oral as shown below.

- **Group 1**: Normal control given only saline (9 g/L) (NC).
- **Group 2**: Control treated with aqueous extract at dose of 250 mg/Kg (NTAE).
- **Group 3**: Diabetic control given only saline (9 g/L) (DC).
- **Group 4**: Diabetic rats treated with aqueous extract at dose of 250 mg/Kg (DTAE).

Blood samples and body weight were measured at weekly intervals on days 0, 7, 14 and 21, till the end of study. Blood glucose levels were measured using GOD-POD (Kit spinreact) (Trinder, 1996). At the end of the experiment, an oral glucose tolerance (OGTT) test was practised. Animals (four groups) were loaded with glucose (3 g/kg). Blood glucose level was determined at 0, 60 and 120 min after glucose loading.

**Statistical analysis**

All the values of body weight, fasting blood sugar, and OGTT were expressed as mean ± SEM. Data were analyzed statistically using the student’s t-test. In all cases, p<0.05 was used as the criterion of statistical significance.

**RESULTS**

**Preliminary phytochemical screening**

Table 1 shows the presence of various compounds such as tannins, amino acids, alkaloids, saponins steroids and terpenoids. However, flavonoids are completely absent. Through phytochemical prospecting of the extract, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial (Todkar et al., 2010), antioxidant (Benhammou et al., 2009) and anti-diabetic (Debbab et al., 2010).

**Acute toxicity study**

In the present study, all animals that were fed with amounts of 100, 250, 500 and 1000 mg/kg body weight of *A. pyrethrum* aqueous extract have reacted well after treatment and showed no signs of toxicity or behavioral changes at any doses selected until the end of study.

**Changes in body weight**

Figure 1, shown the change in body weight gain in control rats and control treated with aqueous extract. The body weight was decreased in diabetic rats while oral administration of root extract improves the body weight gain in STZ-induced diabetes.

**Oral glucose tolerance test in normal rats (OGTT)**

Oral glucose tolerance test in diabetic and normal rats showed an increasing in blood glucose level one hour after glucose administration. A significant reduction was marked in diabetic treated (P< 0.05) with aqueous extract 2 h (Figure 2) after treatment and suppressed the rise in blood glucose compared with standard group.

**Antihyperglycaemic effect of aqueous extract**

The effect of the aqueous extract of roots on the fasting blood glucose levels of both normal and diabetic rats are given in Table 2. The fasting blood glucose levels of diabetic untreated rats (Group 4) were significantly higher than those of normal untreated rats (Group 1). The extract of roots at a dosage of 250 mg/kg produced the

| Table 1. Phytochemical prospection of aqueous extract roots of *A. pyrethrum*. |
|-----------------|---|---|---|---|---|---|---|
| Metabolites     | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Aqueous Root    | + | - | + | + | + | + | + |

Table 2. Effect of 3-week treatment with aqueous extract of roots of *A. pyrethrum* on blood glucose level in STZ (50 mg/Kg ip),-induced diabetic in rats (Mean±SEM).

<table>
<thead>
<tr>
<th>Group (n=4)</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>1</td>
<td>NC</td>
<td>119.7 ± 5.1</td>
<td>120.0 ± 4.5</td>
</tr>
<tr>
<td>2</td>
<td>NTAE</td>
<td>250</td>
<td>107.7 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>DC</td>
<td>226.7 ± 2.4*</td>
<td>297.7 ± 2.9*</td>
</tr>
<tr>
<td>4</td>
<td>DTAE</td>
<td>250</td>
<td>221.0 ± 2.0</td>
</tr>
</tbody>
</table>

STZ single dose of 50 mg/kg p.o on day 0; * p < 0.05 when compared with normal control, ** p < 0.001 when compared with diabetic control.

Figure 1. Change in body weight in the treatment of aqueous extract in STZ induced diabetic rats.

Figure 2. Effects of aqueous extract of *A. pyrethrum* on OGTT in normal rats (Mean±SEM).
maximum fall of 62.12\% in the blood glucose levels of diabetic rats after 21 days of treatment. However any hypoglycemic effect in normal treated rats was observed.

**DISCUSSION**

Blood glucose is a key marker for diagnosis and prognosis of diabetes mellitus. Insulin deficiency causes drastic elevation in levels of blood glucose as a result of excessive production of endogenous glucose and also causes a drastic change in body weight (Ramachandran et al., 2011), which may be due to excessive breakdown of tissue proteins and lipids caused by insulin insufficienty. Various anti-diabetic plants and herbs are found through traditional use but their introduction into modern therapy needs testing of the compounds by modern research methodology. In the present study, oral treatment with *A. pyrethrum* roots extract appreciably lowered the level of blood glucose and improved the insulin level in STZ-induced diabetic rats. The anti-hyperglycemic effect of aqueous extract may be due to stimulatory effect on the remnant β-cells to secrete more insulin or from regenerated β-cells (Mahadeva and Subramanian, 2009). Another observation arising from this study is the effect of the aqueous extract of roots on the body weight in the treated rats. The improvement in body weight in diabetic rats treated may be due to improvement in metabolic activity of the system to maintain glucose homeostasis. These results suggested that root contained some biological principle(s) that posses insulin protective or insulin-like activity (Maqsood and Tanveer, 2009) but again this hypothesis would require experimental validation and further experiments are required to elucidate the exact mechanism of action as well as on the isolation of bioactive principles. The phytochemical screening of root revealed the presence of tannins, saponins, alkaloids, amino acids, steroids and terpenoids that are known to be bioactive antidiabetic principles (Meliani et al., 2011). Amino acids have been reported as antihyperglycemic agents, because that absolute or relative lack of insulin leads to defective amino acid/protein metabolism (Capraró et al., 2010). Phenolics are found to be effective antihyperglycemic agents (Abdelmoaty et al., 2010; Hossam et al., 2011). Alkaloids and tannins have been reported to possess hypoglycemic activity (Sharma et al., 2010; Nkriote et al., 2011). The saponin is known to elicit serum cholesterol lowering activity and may be classified as a direct hypoglycaemic agent, in contrast to the indirect agents such as the sulphonylureas that act by stimulating the pancreatic beta cells to release more insulin (Chang-yong et al., 2010; Abdel-Hassan et al., 2000).

**Conclusion**

It can be stated, that the aqueous root extract of *A. pyrethrum* has beneficial effects, in reducing the elevated blood glucose level of STZ-induced-diabetic rats, but has no effect on normal rats. Further studies will be focussed on determination of the mechanism(s) of action, as well as on the isolation of bioactive principles for contribute toward the development of a potent anti-diabetic drug.

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