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Full Length Research Paper

Antioxidant potential of aqueous leaf extract of *Ageratum conyzoides* Linn. in diabetic rats

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The antioxidant activity of aqueous extract of leaves of *Ageratum conyzoides* (AC) in the serum of male diabetic rats was evaluated using Ferric Reducing Antioxidant Power (FRAP) assays, by determination of malonadehydes, lipid hydroperoxydes and protein thiol groups. The plant extract was tested at doses of 100, 200 and 300 mg/kg on diabetic rats during three weeks of treatment; glibenclamide (10 mg/kg) was used as positive control. Glycaemia of diabetic rats was also determined, at the beginning and at the end of the experimentation. The results showed that *A. conyzoides* did not have an incidence in serum protein thiols and serum malonaldehydes level. Nevertheless, the aqueous extract induced lowering of lipid hydroperoxides in the groups treated with 100 mg/kg (p 0.01) and 200 mg/kg (p 0.01) when compared to the negative control group. Power (FRAP) was also higher in the 100 mg/kg group. In addition, glycaemia was decreased at the ferric reducing antioxidant the third week in the group receiving 200 mg/kg (p 0.01) and 300 mg/kg (p 0.01). *A. conyzoides* had a positive effect on the oxidation-reduction system on streptozotocin induced diabetic rats and improved glycaemia of diabetic rats.

Keys words: *Ageratum conyzoides*, antioxidant, antihyperglycemic, streptozotocin, diabetic rats.

INTRODUCTION

Ageratum conyzoides is widely utilized in traditional medicine systems wherever it grows, although applications vary by region. Traditional communities in India use this plant as a bacteriocide, antidysenteric, and antilithic (Okunade, 2002), and in Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide (Okunade, 2002). In Central Africa it is used to treat pneumonia, but the most common use is to cure wounds and burns (Okunade, 2002). *A. conyzoides* is also utilized to treat fever, rheumatism, headache and colic (Okunade, 2002., 1993) . In Cameroon aqueous extracts of leaves or the whole plant are used as an anti-diabetic (Tsabang et al., 2001).

In diabetes mellitus, hyperglycemia may depress the natural antioxidant system (Pavana et al., 2009). Several reports indicate that modified oxidative stress is due to chronic hyperglycemia (Bhor et al., 2004). Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus (Baynes, 1991).

Several pharmacological investigations have been conducted to determine the efficacy of this plant (Okunade, 2002). The chemical composition shows that *A.* conyzoides contain many bioactive compounds including flavonoids, alkaloids, cumarins, essential oils, chromenes, benzofurans, terpenoids and tannins (Okunade, 2002).

However, we have not found any studies on the antihyperglycemic and antioxidant effects of *A. conyzoides* in experimental diabetes mellitus. Thus, in the present study, we evaluated the antihyperglycemic and antioxidant effects of *A. conyzoides* in streptozotocin induced diabetic rats.

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MATERIALS AND METHODS

Collection and preparation of plant material

Mature *A. conyzoides* was collected during the month of February, 2006 in Yaoundé, Centre province, Cameroon. Botanical identification was performed at the National Herbarium of Yaoundé, in comparison with the voucher specimen N°19050/SFR/Cam. The leaves were shade-dried and ground into powder.

Preparation of the extracts

138 g of the powdered leaves was boiled in distilled water (2.25 L) for 30 min. The decoction was taken and allowed to cool for 30 min at room temperature (24 ± 5°C). This decoction was filtered twice and the filtrate was dried in an oven (55°C) for 3 days. The yield after extraction was about 29% (w/w).

Drugs and chemicals

Glibenclamide (Glib) was purchased from Strides Arcolat Ltd. Bangalore, India and Streptozotocin from Sigma-Aldrich Co Ltd, United Kingdom.

Animals

Male albino *Wistar* rats (180 - 220 g) were maintained under standard laboratory diet and tap water *ad libitum* in the Animal House of the Institute of Medical Research and Medicinal Plants Studies, Cameroon.

Prior to the experiment, the rats were divided into 5 experimental groups of 6 animals each. The animals were subjected to fasting for 16 h (before the determination of glycaemia and at the end of the experiment when collecting blood sample) but they had free access to water. The study was carried out with the approval by the Institutional Animal Ethics Committee.

Induction of diabetes

Animals were rendered diabetic by an intravenous injection of a freshly prepared streptozotocin (STZ) solution at a dose of 55 mg/kg body weight in acidified saline solution (0.9%; pH 4.5), as described by Szkudelski (2001). In this case, the control animals received only the acidified saline solution (pH 4.5). After 72 h, when the condition of diabetes was stabilized, the animals with blood glucose levels above 200 mg/dL were selected for the study and divided into 5 groups.

Experimental design

Groups I, II and III were given the aqueous extract of leaves of *A. conyzoides* (suspended in distilled water 10 ml/kg) orally daily at doses of 100, 200 and 300 mg/kg respectively during three weeks. Animals of group IV received glibenclamide at a dose of 10 mg/kg as positive control while those of group V served as a negative control and received appropriate volumes of vehicle (distilled water) orally.

Determination of antihyperglycemic activity

Blood samples for glucose determination were obtained from the tip of the tails of the rats before administration of drugs at the initial week, and at the third week thereafter. Blood glucose level was determined using a glucometer, Glucotrend®2 (An Accu-Chek system of the Roche Group Germany, Roche diagnostics GmbH D-68298 Mannheim, Germany) in all animals.

Determination of antioxidant activity

At the end of the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on serum of control and experimental animals in each group.

FRAP (Ferric Reducing Antioxydant Potential) were estimated by the method of Benzie and Strain (1996), while protein thiol level was assayed by the method of Ellmann (1959). Serum malonaldehydes (Yagi, 1976) were assayed and lipid hydroperoxides (Wolf, 1994) was estimated.

Statistical analysis

All values were expressed as mean ± S.D. The data were statistically analysed by the classical student's paired *t*-test. Data of treated groups were compared to those of negative control.

RESULTS

Effect of A*. conyzoides* on blood glucose level after multiple administrations of *A. conyzoides* (AC) in hyperglycemic rats

After three weeks of treatment, there was a significant decrease (P 0.01) in blood glucose in groups that received 200 and 300 mg/kg of aqueous crude extract, while glibenclamide was ineffective in diabetic rats as represented by Figure 1.

Antioxidants effect of *A. conyzoides* (AC) in hyperglycemic rats

The groups receiving aqueous crude extract of AC at 100 mg/kg (P 0.05) and 200 mg/kg (P 0.01) showed a significant decrease of lipid hydroperoxides respectively as demonstrated in Figure 2. Serum malonaldehydes in different groups of diabetic rats was similar to that of control as represented in Figure 3. At the end of the experiments, the protein serum thiol was not significantly different in the groups treated with AC when compared to the control group. The group receiving glibenclamide showed a thiol level slightly higher than the control group as seen in the Figure 4. Ferric reducing antioxydant potential increased in the group receiving 100 mg/kg (P 0.05) of *A. conyzoides* and treated with glibenclamide (P 0.01) compared to control. The level of FRAP in the two groups were comparable as represented in Figure 5.

DISCUSSION

The aim of the present study was to evaluate the possible

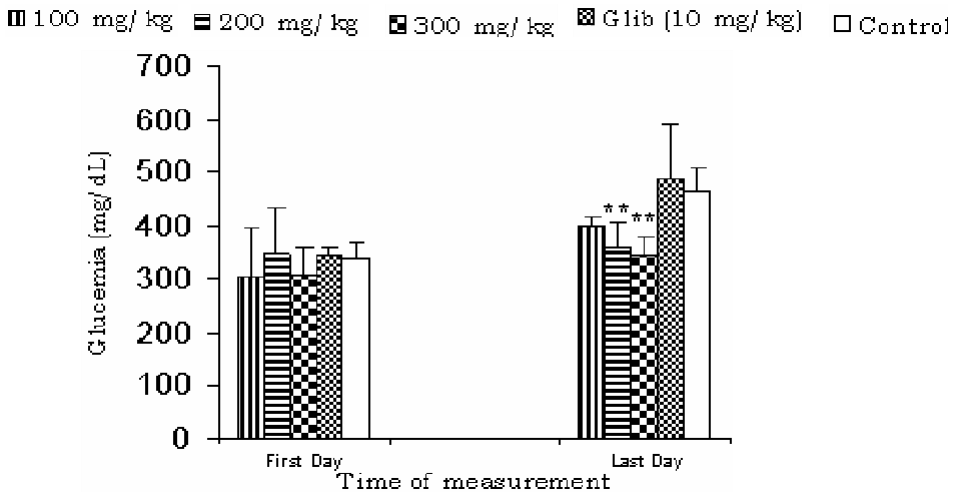

Figure 1. Glycaemia level after 3 weeks of administration of 100, 200 and 300 mg/kg of aqueous extract of *A. conyzoides* and glibenclamide (10 mg/kg). Results (mol/L of serum) are presented as mean ± standard deviation of the mean. n = 6, number of rats. **P 0.01 group vs. control.

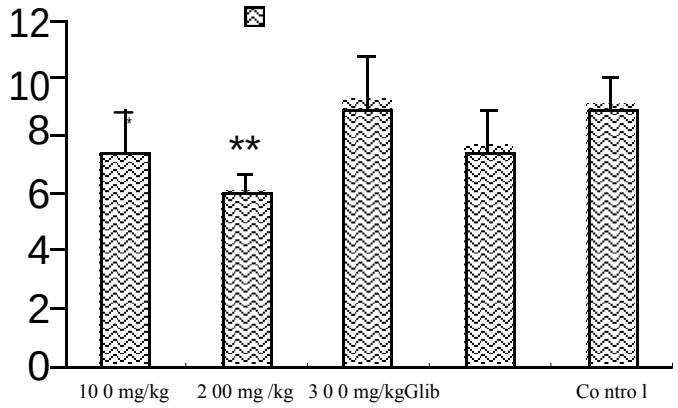

Figure 2. Serum lipid hydroperoxide level after 3 weeks of administration of 100, 200 and 300 mg/kg of aqueous extract of *A. conyzoides* and glibenclamide (10 mg/kg). The results (µmol/L of serum) are presented as mean ± standard deviation of the mean. n = 6 number of rats, *P 0.05. **P 0.01 vs. control group.

protective effects of aqueous extract of leaves of *A. conyzoides* on glucose level serum and antioxidant defense systems of plasma in streptozotocin induced diabetes rats. The levels of glucose in blood FRAP, protein thiol, lipid hydroperoxide and malonaldéhyde were estimated in serum of control and experimental groups of streptozotocin (STZ) induced diabetic rats.

The present study showed that oral administration of *A. conyzoides* extract decreased the blood glucose level in diabetic rats. The result suggests that the extract produces an antidiabetic action mediated by an increase in peripheral glucose uptake in diabetic rats, especially at a concentration of 200 and 300 mg/kg (Figure 1). In this study, the antihyperglycemic effect of the extract was more efficacies in lowering blood glucose level than that of glibenclamide, a standard antidiabetic drug that act by stimulating insulin secretion from pancreatic -cells (Tian et al., 1998). Taking into consideration the mechanism of action of glibenclamide which is the stimulation of insulin liberation, glibenclamide is only effective in moderate diabetic condition and has little or no effect in a severe diabetic condition where the -cells of the pancreas are totally destroyed (Suba et al., 2004). Thus, the antihyperglycemic effect of the extract might be due to a increase in peripheral uptake glucose. The percentage fall in blood glucose levels was effective in treated extract

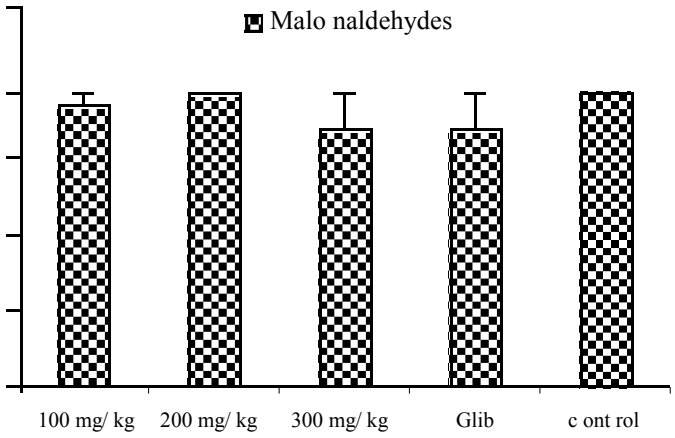

Figure 3. Serum malonaldehyde level after 3 weeks of administration of 100, 200 and 300 mg/kg of aqueous extract of *A. conyzoides* and glibenclamide (10 mg/kg). The results (µmol/L of serum) are presented as mean ± standard deviation of the mean. n = 6 number of rats.

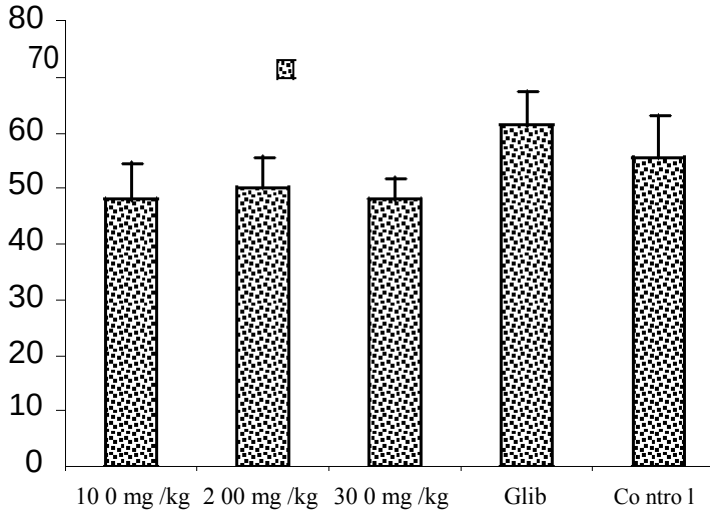

Figure 4. Proteinic serum thiols after 3 weeks of administration of 100, 200 and 300 mg/kg of aqueous extract of *A. conyzoides* and glibenclamide (10 mg/kg). The results (µmol/L of serum) are presented as mean ± standard deviation of the mean. n = 6, number of rats.

diabetic's rats and not with glibenclamide (Figure 1); it implies that the antihyperglycemic effect of AC is not dependent on the degree of -cell destruction. From the results of the present study, it may be suggested that the mechanism of action of AC is different to glibenclamide action.

Hyperglycemia induces the generation of free radicals which can affect antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation (Giugliano et al., 1996). In addition, an increased of lipid peroxidation has been reported both in clinical and experimental diabetes (MacRury et al., 1993). In this aim, we chose to work on a model of severe experimental diabetes.

Treatment with plant extract at 100, 200 and 300 mg/kg showed lowering hydroperoxides (p 0.05 and p 0.01) and increase in ferric reducing antioxidant potential in rat's serum comparable to that of glibenclamide. The extract had no effect on proteinic serum thiols and on

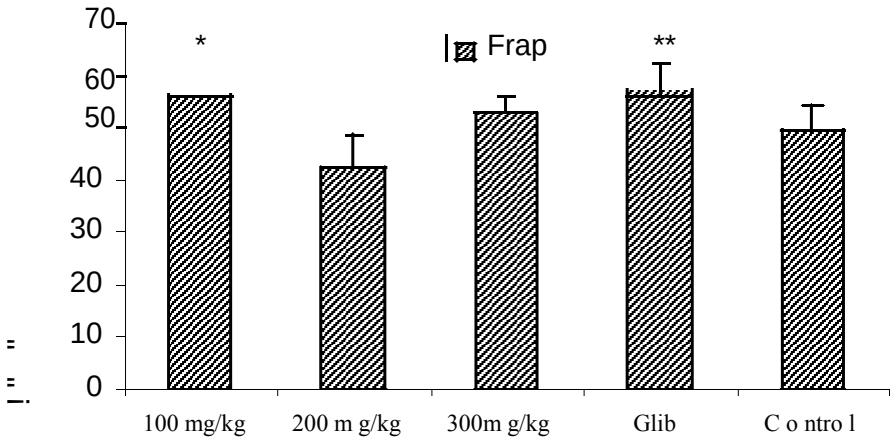

Figure 5. Serum ferric reducing antioxidant power (FRAP) level after 3 weeks of administration of 100, 200 and 300 mg/kg of aqueous extract of *A. conyzoides* and glibenclamide (10 mg/kg). The results (µmol/L of serum) are presented as mean ± standard deviation of the mean. n = 6 number of rats. *P 0.05. **P 0.01 vs control group.

serum malonaldehyde level (Figure 3 and 4). The hydroxyl radical scavenging activity of the aqueous extract of AC is shown in Figure 2, at 100 and 200 mg/kg, the aqueous extracts exhibited significant scavenging activity. The ability of those above doses to quench hydroxyl radicals seem to be directly related to the prevention of propa-gation of the process of lipid peroxidation. It is known that hydroperoxide are primary products of lipid-peroxidation, the lowering effect of lipid hydroperoxide level may be due to an antioxidant activity of *A. conyzoides* crude extract. Many plants like mulberry fruit extracts showed their ability effect to scavenge hydroxy radical (Song- Hwan and Hyung-Joo, 2007). In this study the level of hydroperoxide in treated extract groups may be related to scavenging lipid hydroperoxide radical's activity of one or more of its components, suggesting its potent antilipid peroxidative like previously observed by Pavana et al. (2009).

We reported an increase of frap serum level which is synonym of potentialization of antioxidant potential (Figure 5). Many studies showed increase in antioxidant activity proportionally to the polyphenol content. A linear relationship between FRAP values and total polyphenol, tannin, proanthocyanidin and flavonoid contents was well established (Maksimovic et al., 2005).

Thus, the saponins or/and polyphenols in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defence contributing to the protection against oxidative damage in streptozotocine induced diabetes.

Hence from our results, it is suggested that AC has a significant protective effect against streptozotocin-induced diabetes in rats, and this can be attributed to the combined effect of various chemical constituents of this plant. Saponines, flavonoids and tannins were reported to be the main constituent of this plant (Okunade, 2002). They are known to have antioxidant activities in many diseases. Their role in the treatment of diabetes (Yoshikawa et al., 2001; Rhemann and Zaman, 1989) and antioxidant activity (Yoshiki et al., 1998) is well established. Presence of saponins, tannins and flavonoïdes in the aqueous extract was confirmed through our preliminary phytochemical screening also. Therefore the improving role of AC aqueous extract in diabetes may be attributed at least to those components which can act with other miscellaneous compound to produce these antioxidant and antihypeglycemic effects.

In conclusion, the present study showed that AC leaves possess potent antioxidant activity, which may be directly or indirectly responsible for then hypoglycaemic property. These potent antioxidant properties may contribute towards preventing peroxidative damage. Further studies are in progress to identify the active components in AC and their role in controlling diabetes.

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