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Caralluma flava N.E.Br extract reduces plasma glucose level and improves plasma antioxidant enzymes in hyperglycemic rats

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Abstract
The hypoglycemic effect of the aqueous extract of *Caralluma Flava N.E.Br* leaves in streptozotocin-induced diabetic rats (STZ) was investigated. Mineral content, phenolic and flavonoid contents of *Caralluma Flava* leaves were investigated with the antioxidant activity. Results indicated that the *Caralluma Flava* dried leaves samples showed to be rich source of many macro and microelements. The obtained results show that the concentration of total phenol contents in the extract was 8.56 mg/g. The total flavonoid content was 2.87 mg/g. Serum glucose levels were significantly decreased in the *Caralluma Flava* treated rats in a dose dependent manner in comparison to the STZ diabetic control. Liver superoxide dismutase catalase and glutathione peroxidase levels were apparently increased in the *Caralluma Flava* treated rats at 0.2 and 0.5 g/kg compared to STZ diabetic control. In conclusion, it is illustrated that the *Caralluma Flava* extract showed hypoglycemic effect and antioxidant effect on the streptozotocin-induced diabetic rats.

Keywords: *Caralluma Flava* N.E.Br; aqueous extract; antioxidant; mineral; diabetes

1. Introduction
The use of herbal medicines for the treatment of diabetes mellitus has gained importance globally. The World Health Organization has also recommended and encouraged this practice, especially in countries where access to the conventional treatment of diabetes is not adequate. There is an increased demand for using natural products with antioxidant activity to treat and prevent diabetes. Oxygen free radicals was reported to be a contributory factor in complications of diabetes mellitus [1] which seems to be an oxidative stress related disorder and the antioxidants may be useful in preventing it [2]. Therefore, supplementation of therapeutics with antioxidants may have a chemoprotective role in diabetes [3]. Many studies showed that the herbal medicine possesses significant efficacy, low incidence of side effects, low cost and relative safety [4], while synthetic anti-diabetic agents can produce serious side effects, as hypoglycemic coma and disturbances of the liver and kidneys [5].
Many species of genus Caralluma are found in tropical Asia and Mediterranean regions especially in Yemen. Some species have been used as traditional medicine for the treatment of many diseases [6]. *Caralluma Flava* N.E.Br (CAF) is a flowering plant, which belongs to family Apocynaceae. It is a leafless, succulent and angular plant, which grows wildly in many regions of Yemen. In addition, it is consumed freshly by many people in Yemen in the treatment of rheumatism, diabetes, leprosy, gastric ulcer and as antipyretic [7]. In traditional Yemeni folk medicine, CAF is used in the case of diabetes [8], but no scientific evidence to support the use of the CAF leaves in treating diabetes. The present study was undertaken with the aim to evaluate the antihyperglycemic activity of aqueous extract of leaves of CAF in streptozotocin induced diabetic rats. Apart from that, the study was performed to determine phenolic and flavonoid contents of CAF leaves and the antioxidant activity of the CAF aqueous extracts.
The role of some inorganic elements like zinc, sodium, potassium, calcium, copper, manganese, and traces of chromium in the improvement of impaired glucose tolerance and their indirect role in the management of diabetes mellitus are being increasingly recognized. In traditional methods, medicinal plants are being used, which contain both organic and inorganic constituents. Even trace elements play an important role in the formation of active constituents. Analysis of mineral content in CAF leaves was performed to investigate the

Mineral composition, which was not reported in the literature, and it may contribute to the hypoglycaemic role of CAF in experimental rats.

2. Materials and methods

2.1 Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma-Aldrich (St. Louis, MO). Ascorbic acid was purchased from Fisher Scientific (Loughborough, UK). Methanol and ethanol were purchased from Fisher Scientific (Fisher Scientific Co Ltd., Ottawa, ON). Omeprazole was purchased from Ibn HyanPharmcy in local market in Sana`a. STZ, sodium citrate, glucose and glibenclamide, were purchased from Sigma.

2.2. Plant collection

Caralluma flava plant leaves were collected from Rada, Albyda and from Bani Matr, Sana`a Yemen, the plant was identified and authenticated by a plant taxonomist at Department of Botany, Faculty of Agriculture, Sana`a University, Yemen. The plant was dried and protected from the light; the dried plant was stored at 4ºC and protected from light prior to further use.

2.3. Mineral Analysis

Mineral content of *CAF* dried leaves was determined by Association of Official Analytical Chemists methods (AOAC, 1997) using the flame system of the Atomic Absorption Spectrophotometry (AAS), (GBC 908AA, USA). Dried CAF leaves plants were ashed at 550°C overnight and the ash was dissolved in concentrated hydrochloric acid and filtered, diluted to 100 mL with distilled water and the absorbance of the samples was read directly on the AAS.

2.4. Determination of phenolic and flavonoid contents

The total phenolic content of *CAF* dried leaves was determined using the Folin–Ciocalteu method with gallic acid as standard according to [9]. Different concentrations of gallic acid solution (5 mg/100 mL) were used to plot the calibration curve. The total flavonoid content in the dried leaves of CAF plant was determined and was expressed in micrograms of gallic acid equivalent (GAE) as described by [10].

2.5. Preparation of the aqueous extract

The extract was obtained as follows; 100 g of dried leaves of *CAF* was added in 1 liter of boiling water and boiled for 15 min followed by filtration with filter paper (Milipore filter 0.45 µm Ref HAWP04700) and lyophilization by freeze dryer (Labconco, USA) into powder form. The powder was dissolved in water daily just before analysis.

2.6. Scavenging Activity of DPPH Radical

The antioxidant activity of the CAF aqueous extract extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical, were determined by the method described by Benzie and Strain [11]. L ascorbic acid was used as standard antioxidants and methanol was used as the control. An aliquot of 0.5 ml of a methanolic solution of DPPH (50 mg DPPH/100 mL MeOH) was added into the different concentration (1, 0.5, 0.25 mg/ml) of the aqueous extract and ascorbic acid as long as control samples (both extract and ascorbic acid were dissolved in methanol). All samples were incubated in the dark at room temperature for 30 min before absorbance values were read at 517 nm (Amersham 2100Pro, UV-vis spectrophotometer, UK).The radical scavenging effect was calculated as follows: Radical scavenging effect (%) = Ac – As/Ac × 100

Ac = absorbance of control and As = absorbance of test sample.

Where control is the absorbance of the DPPH radical+ methanol.

2.7 Animal Study

Male Albino rats aged 8-10 weeks (180-250 g) were sued in this study. Animals had free access to water and a standard laboratory diet. Experiment was carried out according to the guidelines for the use of animals and approved by the Animal Care and Use Committee (ACUC) of Faculty of Agriculture, University of Sana`a Yemen.

2.8 Induction of experimental diabetes

After an overnight fast, diabetes was induced by intraperitoenal (i.p.) injection of a freshly prepared solution of streptozotocin (STZ) (Sigma, St. Louis, Mo) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight. Animals were then allowed to drink 5% glucose solution overnight to overcome the drug-induced hyperglycaemia. Control rats were injected with citrate buffer alone. After 1 week, rats with fasting blood glucose levels of greater than 14 mmol/l were considered as diabetic and used in the present study (Ravi *et al*., 2004). Rats were divided into six different groups each group contain 6 rats; normal control rats were not induced with STZ and served as reference group. STZ diabetic control rats were given normal saline. two STZ diabetic groups were treated with CF leaves aqueous extract at 0.2 and 0.5 g/kg, daily continuously for 28 days. The fifth group received the reference drug, glibenclamide (6 mg/kg) in aqueous solution orally for 28 days. The doses of the extract were chosen based on dose-response studies that were conducted earlier in our laboratory. Body weight, food and water consumption were measured weekly. After 28 dyes, blood was collected by cardiac puncture for biochemistry tests. At the end of this period the rats were anaesthetized with diethyl ether and killed by cervical dislocation.

The liver tissues were rapidly excised, washed in ice-cold. About 10% (w/v) homogenate of liver tissue was mixed with cold distilled water. The suspended mixture was centrifuged at 3000 g for 10 minutes. The resulting supernatant was used for the assay of activities of antioxidant enzymes including SOD, CAT and GPX.

2.9 Parameters

Plasma glucose levels, Liver antioxidant enzymes including SOD, CAT and GPX levels, Liver enzymes including alanine aminotransferase (ALT), Gamma-glutamyltransferase (GGT), creatinine and urea were determined using their serum blood. All the blood analysis were carried out in Alulaqi Specials Med. Lab in Sana`a Yemen.

2.10 Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using the analysis of variance (ANOVA). Differences were considered to be significant when $P < 0.05$.

3. Results

3.1 Minerals Content

Mineral composition of the *CAF* dried leaves samples are shown in Table 1. Ten different elements (macro and micro) were determined. Results indicate that *CAF* dried leaves is

rich source of macro minerals such as calcium, potassium and magnesium. In addition, *CAF* dried leaves is rich source of micro minerals such as iron, zinc and selenium.

Table 1: Minerals content of *CAF* dried leaves

Elements	Content (µg/g)
Calcium	22516.53 ±292.74
Copper	3.43 ±0.11
Iron	195.2133 ±5.15
Potassium	15026.45 ±260.49
Magnesium	5325.78 ±265.76
Manganese	29.75667 ±0.99
Sodium	10056.31 ±121.29
Phosphorus	1092.973 ±53.32
Zinc	13.86 ±0.76
Selenium	125±4.76

Method of AOAC (1996) was used to analyze the hydrochloric acid solution of the ash samples using the flame system of the Atomic Absorption Spectrophotometry (AAS), (GBC, 908AA, USA). Each value represents the mean of three replications± SD.

3. 2 Total phenol and flavonoid contents

The obtained results show that the concentration of total phenol contents (TPC) in the *CAF* dried leaves extract was 8.56 mg/g. The total flavonoid content was 2.87 mg/g in the plant extract. The total phenolic and flavonoid contents of the extract of *CAF* leaves were significantly higher when compared to the gallic acid standard.

3.3. Antioxidant activity

Table.2 shows DPPH scavenging activity of *CAF* aqueous extracts and L- ascorbic acid. Result from this assay clearly showed that the extract exhibited high antiradical activity towards DPPH radical and the activity was approximately similar to the pure antioxidant standard, ascorbic acid. After 30 minutes of the reaction, the *extract,* at concentration of 0.1 mg/ml scavenged more than 18% of the total radicals in the reaction system. Subsequently, the scavenging activity of *CF extract* were gradually increased to more than 81% of the total radicals, at higher concentration (1mg/ml).

Table 2: The DPPH scavenging activity of the *CAF* aqueous extract

Samples	Radical scavenging effect (%)			
	Concentration (mg/ml)			
	1.00	0.5	0.25	0.1
L- ascorbic acid	90±40	85±53	79±61	47±52
CAF aqueous extract	81.9±54	52.9±37	32.3±35	18.8±20

The values represent means ± SD for three different experiments.

3.4 Serum glucose levels

Serum glucose levels of the experimental period is shown in Table 3. The diabetic control rats and normal control rats had comparable levels of blood glucose. The STZ control group had higher of serum glucose level compared to normal group. After four weeks of treatment, the diabetic rats that received *CAF* leaves extract at doses of 0.2 and 0.5g/kg and glibenclamide had reduced blood glucose concentrations compared with STZ control group. The reduction of blood glucose in rats treated with *CAF* aqueous extract was dose dependent. STZ treated rats with glybenclamide showed more reduction in blood glucose level and the level was nearly from the normal rats at the end of the experiment period.

Table 3: Plasma glucose level in experimental rats

Group	Glucose level at W0	Glucose level at W4	Glucose level at W8
Normal rats	5.68 ±0.4	6.78 ±0.6	6.95 ±0.4
STZ diabetic control	5.12 ±0.6	13.67±1.23	14.23 ± 1.67
STZ diabetic+0.2 g/kg of *CAF*	6.0 8 ±0.4	12.89±1.14	10.56 ± 32
STZ diabetic+0.5 g/kg of *CAF*	6.32 ±0.7	13.15±0.98	8.45 ±68
STZ diabetic+glybenclamide	5.75 ±0.2	12.67±0.87	7.85±34

All values are expressed as Mean ± SEM, (n=6)

3.5 Antioxidant Enzymes

Superoxide dismutase (SOD) activity was significantly decreased ($p < 0.05$) in the diabetic control group compared to normal control group. The treated diabetic rats with *CAF* extract at 0.2 and 0.5 g/kg, showed significantly ($p < 0.05$) increased SOD activity compared to control diabetic rats and the SOD levels were similar to those found in normal control group. (Table 4).

Catalase (CAT) activity was significantly decreased ($p < 0.05$) in the diabetic control group compared with normal control group. The treated diabetic rats either with *CAF* extract at different doses or glibenclamide showed significantly ($p < 0.05$) increased CAT activity compared to control diabetic rats and (Table 4). The diabetic control group exhibited significant decreased in the activity of GPx compared to normal control group. Administration of *CAF* extract at different doses to diabetic rats significantly increased GPx activity achieving values similar to those found in normal group (Table 4).

Table 4: Effect of *CAF* aqueous extract on activity of Sod, GPX and catalase in liver of Experimental rats

Group	SOD activity (U/ml)	GPX activity (nmol/min/ml	CAT activity nmol/min/ml
Normal rats	9.05 ±0.48	3.91 ±0.55	58.23±4.47
STZ diabetic control	6.56 ±0.62	0.66±0.18	25.86± 5.67
STZ diabetic+0.2 g/kg of *CAF*	8.0 8 ±0.58	2.24±0.45	35.76 ± 3.61
STZ diabetic+0.5 g/kg of *CAF*	9.49 ±0.99	4.39±0.98	59.80 ±2.11
STZ diabetic+glybenclamide	8.17 ±0.62	1.77 ±0.6	44.40±4.52

All values are expressed as Mean ± SEM, (n=6),

4. Discussion

The aim of the present study was to investigate the hypoglycaemic and antioxidantive effect of *CAF* aqueous extract on streptozotocin (STZ)-induced diabetic. Apart from that, mineral content and antioxidant activities of *CFA* aqueous extract was evaluated.

Data of minerals analysis of *CAF* dried leaves revealed that the seeds contain an abundant amount of calcium, magnesium, potassium, phosphorus and iron. From the results, it becomes evident that CAF dried leaves provide an abundance of many minerals and could be considered as a good source of many elements. Current results showed that the CAF dried leaves also provide an abundance of zinc and Selenium. The purpose of determination of the mineral content of the dried leaves to know the mineral composition which might be involved in the hypoglycemic effect that was shown in this study since the mechanism of hypoglycaemic effect of *CAF* dried leaves was not investigated in this work. Many herbs have been shown to have hypoglycemic action in animals and humans [7]. They might be used in various forms like food and medicines, which contain both organic and inorganic constituents. Even trace elements play an important role in the formation of active constituents in medicinal plants [8].

Antioxidants are important in maintaining good health and there is a growing interest in the investigation of antioxidant activity from medicinal plants with higher potency and lower toxicities than the synthetic ones currently available. Our attention has been focused, in particular, on the CAF grown in Yemen which used by many Yemenis as traditional medicine for diabetes prevention. The concentration of total phenol contents (TPC) in the CAF aqueous extract was. 56 mg/g. The total flavonoid content was 2.87 mg/g. The TPC of ethanolic extract of the CAF leaves was reported to be 10.14 mg/g. and the total flavonoid content was 4.13 mg [11].

Phenolic compounds and total flavonoid content directly contribute to the anti-oxidative action. The antioxidant activity using DPPH showed that the *CAF* extract reduced the DPPH radical. It was observed that activity increased with increasing concentration of the extract in the assay and the radical scavenging capacity reach 81% at highest concentration. In accordance to the result of this effort, CAF can be considered as a natural source with antioxidant activity.

The radical scavenging capacity may be attributed to phenolic com-pounds and flavonoids content in the aqueous extract of the CAF leaves aqueous extract. The results of a recent study by [11]. revealed that CAF leaves ethanol extract has good antioxidant power and thereby it is concluded that the intake of this plant, as an antioxidant-rich food, may reduce diabetes risks and cellular oxidative stress.

The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves over-production and decreased utilization of glucose by the tissues. In our study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation, revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals, at the end of the 28 days experimental period. When aqueous extract CAF leaves at two different doses were administered to rats, a decrease in plasma glucose level was observed after 2 weeks and 4 weeks of the treatment and the reduction was dose dependent.

In diabetes, changes in the antioxidant parameters status have been reported in various tissues [12] and there are contradictory results in the literature regarding the effect of diabetes-induced hyperglycaemia on antioxidant enzymes activities [13, 14] The present study revealed that SOD1, CAT and GPX2 activities decreased in STZ animals, which may be due to altered antioxidant status. This is in accordance with results that indicated a decreased SOD and GPX in STZ animals may be due to the utilization of antioxidant enzymes in the removal of released H_2O_2 released [15]. These findings are supported by previous studies [16].

Our study examines whether the activity of the antioxidant enzymes, such as SOD1, GPx2, and CAT are differentially affected in the liver of the diabetic rats receiving either aqueous extract CAF leaves or glibenclamide treatment. In this present study, we showed that SOD1, CAT and GPX2 activities were increased in CAF leaves treated diabetic rats, suggesting that CAF might decrease the superoxide radicals under diabetic conditions. SOD and CAT are the major scavenging enzymes that remove radicals *in vivo*. A decrease in the activity of these antioxidant enzymes can lead to an excess availability of superoxide radicals, such as superoxide anion and hydrogen peroxide [17]. The observed increase in SOD activity in liver of CAF treated rats can lead to an important elimination of superoxide ions, which can then inhibit the formation of hydroxyl radical in this tissue. The H_2O_2 produced by SOD is excreted as H_2O based on the activity of glutathione peroxidase and catalase, therefore protecting the body from oxygen toxicity. The present investigation shows that the CAF treatment initiated 4 weeks after induction of diabetes is effective in controlling the antioxidant system in the liver by enhancement of antioxidant enzyme level.

5. Conclusion

We conclude that *aqueous extract CAF leaves* may provide a useful option in the reversal of oxidative stress induced by diabetes in liver of diabetic rats possibly due to the presence of many potent antioxidants. However, further investigations to identify the biologically active ingredients and to define the precise mechanism(s) through of hypoglcymic and antioxidative effects are required.

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