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Hypoglycemic effect and *in vitro* antioxidant activity of methanolic extract from *Argel* (*Solenostemma Argel*) plant

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ABSTRACT
The present study was undertaken to examine the hypoglycemic effect of methanolic extract of *Argel* leaves in normal and in Methylprednisolone induced- hyperglycemia rats. Hyperglycemia was induced in the by intraperitoneal injections of Methylprednisolone (55 mg/kg twice). Then induced rats were treated with either *Argel* extract at 1g/kg orally daily for 10 days or treated with glibenclamide at 6 mg/kg. Antioxidant activity using DPPH was found to increase in a concentration dependent manner. Results showed that oral administration of *Argel* leaves extract, significantly lowered the plasma glucose level compared to the untreated rats. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were significantly lower in *Argel* group compared to untreated group. In conclusions: the methanolic extract of *Argel* leaves showed hypoglycemic and antioxidant potency. The present investigation suggests that *Argel* leaves extract may be a potential source of natural antioxidant with good hypoglycemic effect.

Keywords: *Argel*; hypoglycemic effect; antioxidant; radical scavenging; ALT; AST.

1. Introduction

The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Many investigations are carried out to identify new alternative natural product agents for the treatment of human diseases such as diabetes and cardiovascular disease [1]. In developing countries and particularly in Yemen, a large segment of the population still relies on folk medicine to treat serious diseases. Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas.

Argel (*Solenostemma argel*) is a desert plant of traditional medical used in folk medicine in different places in the world especially in African country. *Argel* belongs to the Asclepiadaceae family [2]. *Argel* is considered to be medicinally important in Sudan, Libya and Chad [3]. *Argel* leaves are used in herbal medicine for the treatment of some liver and kidney diseases and some allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica [4]. Also, it is used as incense in the treatment of measles and sometimes crushed and used as remedy for supporting wounds. The leaves are infused to treat gastrointestinal cramps, stomach ache, colic, cold and urinary tract infections and are effective as anti-syphilitic if used for prolonged period of 40-80 days [5]. Phyto-chemicals of medicinal properties from *Argel* shoots had been reported by many workers [6, 7, 8] Sulieman *et al.*, [9] reported that the aqueous extracts of *Argel* have antifungal and antibacterial properties.

Argel leaves nowadays are used as traditional medicine in Yemen for prevention of diabetes and the leaves are consumed as tea. Since a literature search indicated the absence of information regarding biological and investigations of the effect of the leaves of *Argel* as hypoglycemic agent, the main aim of the study was is to carry out biological investigation on *Argel* as a hypoglycemic agent.

The selection of these plants was based firstly, on traditional use for the leaves of this plant in the treatment of diabetes and secondly on the results of our screening assay for the antioxidant activity from this plant to have potent antioxidant capacity. As a potent antioxidant capacity, this plant should be explored to establish new pharmacological possibilities as anti-diabetic agent.

2. Materials and Method

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 DMSO were purchased from Fisher Scientific (Fisher Scientific Co Ltd., Ottawa, ON). Methylprednisolone and glibenclamide were purchase from Ibn HyanPharmcy in local market in Sana`a.

2.2 Plant collections

Argel, was purchase from local market in Sana`a. The plant was identified and authenticated by a plant taxonomist at Department of Biology, Faculty of Sciences, Sana`a University Yemen. The dried leaves were ground, powdered, stored at 4 ºC and protected from light prior to further use. The analysis has been carried out in 2013 at the Laboratory of Food science and Technology Faculty of Agriculture Sana`a University Yemen and Department of Biology, Faculty of Science, University of Sana`a, Yemen.

2.3 Plant extraction

Argel leaves finely ground using an electrical grinder (Waring Blender, Tokyo, Japan) at speed 6 for 2 min and ground samples (100g) leaves were soaked for two days in methanol at 1:5 ratio at room temperature. The mixture of samples and solvent was filtered through a filter paper (Whattman No. 2). The solvent was dried. The extract was transferred into glass sealed amber dark bottles and then stored in at 4 °C for subsequent analyses. The *Argel* leaves methanolic extract showed yield of 10%.

2.4. Determination of Antioxidant Activity

The antioxidant activity of methanolic leaves of *Argel*, on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Benzie and Strain (1996). 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 leaves extract and ascorbic acid as long as control samples (both extract and ascorbic acid were dissolved in methanol) .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 decrease in absorbance was calculated as an IC50 and expressed as µg/ml, which is the concentration of sample required for 50% scavenging of DPPH radicals in the specified time period.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.

2.5. Animal study

Rats were divided into four different groups (n=6); normal control rats were not induced with Methylprednisolone and served as reference group. The three groups were received a single injection of Methylprednisolone at 55mg/kg body weight intraperitoneal. 5 days later, Blood was collected and analyze for glucose level to be sure that the level of glucose is elevated. Then induced rats with methylprednisolone were divided as following: control rats were given normal saline only. *Argel* group was treated with *Argel* methanolic extract at 1g/kg orally daily continuously for 14 days. The reference drug group received glibenclamide at 6 mg/kg in aqueous solution orally for 14 days. Body weight, food and water consumption were measured weekly. Blood was collected at baseline and at the end of the treatment period by cardiac puncture. At the end of this period the rats were anaesthetized with diethyl ether and killed by cervical dislocation. Liver, kidney, heart weight were measured. Serum glucose, alanine transaminase (ALT), aspartate aminotransferase (AST) urea and creatinine levels were measured using reagent kits (Instrumentation Laboratory,USA) by ILab Chemistry Analyzer 300 PLUS (Instrumentation Laboratory, USA).

2.6 Statistical analysis

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

3. Results

3.1 Antioxidant activity

The results of the free radical scavenging activity of *Argel* extract and the authentic antioxidant L-ascorbic acid was summarized in Table (1). Result from this assay clearly showed that all extract exhibited high antiradical activity towards DPPH radical. The scavenging activity of the pure antioxidant standard, ascorbic acid was 46% at lower concentration of 250µg/ml. The radical scavenging activity was concentration dependent and it increased by concentration to 63 and 77% at 500 and 1000µg/ml respectively. After 30 minutes of the reaction *Argel* extract, at lower concentration (250 µg/ml) scavenged 32%, of the total radicals in the reaction system. Subsequently, the scavenging activity *Argel* extract was gradually increased by 57 and 84% of the total radicals respectively, at higher concentration (1000 µg/ml).

Table 1: The DPPH free radical scavenging activity of the methanolic extract of *Argel* and L-ascorbic acid.

Samples	Radical scavenging effect (%)		
	Concentration (mg/ml)		
	1.00	0.5	0.25
L- ascorbic acid	77 ±6.21	63 ±3.12	46±2.51
Argel	84± 5.32	57± 3.21	32 ±6.13

Values are mean ±STD.

3.2. Serum glucose levels

Serum glucose levels of the experimental period is shown in Table 2. Methylprednisolone injection at 55 mg/kg resulted in significant higher of serum glucose level when compared to reference group after 5 days of the injection. The induced control group had significantly ($P<0.01$) higher of serum glucose level compared to reference group. After two weeks of treatment, the diabetic rats that received *Argel* and glibenclamide had significantly ($P<0.05$, reduced blood glucose concentrations compared with induced not treated control group.

Table 2: The effect of 10 days treatment of *Argel*methanolic extract on blood glucose of Methylprednisolone induced diabetic rats

Group	Serum glucose level at baseline	Serum glucose level after 5 days induction with Methylprednisolone	Serum glucose level at the end of the experiment
Reference group	73±2.12[a]	74±3.15[a]	70±2.30[a]
Control untreated	72±3.20[a]	114±6.24[b]	88±4.27[b]
Treated with *Argel*	76±4.12[a]	143±3.52[c]	43±3.12[c]
Treated with glibenclamide	78±3.45[a]	141±4.25[c]	37±6.02[d]

Values are mean ±STD. Within column, different letters indicated a significant difference

3.3. Body weight

The body weights of experimental are shown in Table 3. There was significant difference in the final body weights in different groups treated rats compared to entail weight at the beginning of the experiment. There was significant difference in the final body weights in treated groups either with *Argel* or glibenclamidgroups compared to control induced and normal group.

Table 3: Body weight of the experimental rats

Group	Body weight at baseline	Body weight after 5 days induction with Methylprednisolone	Body weight at the end of the experiment
Reference group	88±3.43[a]	120±7.13[b]	129±6.43[c]
Control untreated	125±7.34[a]	102±8.44[b]	128±9.21[a]
Treated with *Argel*	147±8.16[a]	111±6.42[b]	94±5.52[c]
Treated with glibenclamide	106±9.35[a]	93±8.85[b]	93±7.37[b]

Values are mean ±STD, within column, different letters indicated a significant difference.

3.4. Serum ALT, GGT, creatinine, and urea levels

Table 4 shows the ALT, GGT, creatinine, and urea levels in the serum of the experimental rats. The results show that inducting rats with Methylprednisolone at 55 mg/ml resulted in a significant elevation of ALT, GGT, in untreated group compared to the reference group. Whereas, no significant different in urea and creatinine levels in the untreated group compared to the reference group. ALT, GGT, creatinine and urea levels were significantly higher in the glibenclamide treated group compared to another groups. Whereas, administration of *Argel* extract at diabetic induced rats caused reversal of the elevations in ALT and AST were elicited by Methylprednisolone to the levels shown by normal non diabetic control group.

Table 4: ALT, GGT, urea, and creatinine levels in serum of experimental rats

Group	ALT (U/L)	AST (U/L)	Creatinine mg/dI	Urea mg/dI
Reference group	189±5.23[a]	62±4.16[a]	0.65±0.01[a]	34±1.14[a]
Control	225±12.54[b]	50±4.62 [b]	0.55±0.09.[a]	30±2.23 [a]
Treated with *Argel*	196±16.43[c]	59±8.62[b]	0.60±0.05. [a]	31±4.74[a]
Treated with glibenclamide	1170±42.35[d]	211±4.57[d]	1.70±0.067. [b]	108±7.87[b]

Values are mean ±STD. within column, different letters indicated a significant difference.

5. Discussion

Diabetes mellitus (DM) is growing concern and represents one of the main threats to human health. Numerous studies have reported the potential hypoglycemic effect of plant aqueous extract in reducing the glucose level in blood in diabetic rats [10, 11, 12]. But up to now, there is no a viable study about the hypoglycemic effect of methanolic extract from *Argel*. Therefore, the present study was undertaken to investigate the hypoglycemic effect of methanolic extract of *Argel* in Methylprednisolone induced diabetic rats. Glibenclamide was used as reference drug to compare the antidiabetic properties of *Argel* extract. Methylprednisolone which commercially known as a DEPOPRED 80 was used to induce hyperglycemia. Methylprednisolone produced significant increases in fasting glucose in most patients without diabetes [13]. Further, the Methylprednisolone diabetic animals may exhibit most of the diabetic complications. In our study, 10 days treatment with *Argel* extract at 1g/kg daily at significantly reduced fasting serum glucose in Methylprednisolone induced diabetic rat compared to untreated. The result showed that the serum glucose levels in treated group with 1g/kg were steadily lowered during the whole experiment. Administration of glibenclamide, resulted in significant reduction of blood glucose level in comparison to the control untreated group.

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color.

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. From the results, it may be postulated that the plant extracts have hydrogen donors thus scavenging the free radical DPPH

The levels of Plasma levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as creatinine and urea in the plasma are key indicators of in vivo hepatocyte damage and renal abnormality, respectively. In this study, administration of Methylprednisolone induced hepatotoxicity in the experimental rats (control group), with a elevation in plasma ALT and AST. Surprisingly, no physical signs of toxicity could be observed in the *Argel* treated rats. Plasma ALT and AST levels in the *Argel*treated group were significantly lower than in the control untreated group, indicating a possible hepatoprotective effect by *Argel* extract. On the other hand, administration of glibenclamide, resulted in significant elevated of serum of ALT and AST levels in comparison to the all different groups. That is indicating the side effect of the glibenclamide treatment which lead to search and develop a natural product agent for treatment or prevention of diabetes.

Plasma levels of creatinine was not detectable in the control animals nor animals receiving extract, indicating normal renal function. The results show that *Argel* extract is nontoxic and possess hypoglycemic and antioxidant activity.

6. Conclusion

Thus from the above findings it is illustrated that *Argel* methanolic extract exhibited antioxidant and hypoglycemic effect without showing any toxic effect. The non-toxic effect of *Argel* extract provide support to the widespread use of the plant and it can be recommended for use as a nutritional supplement, health food and adjuvant in the management of diabetes and patho-oxidative disorders. However, the mechanism of action by which *Argel* extract exert It`s action needs to be established by a thorough phytochemical investigation to identify the constituents responsible for the antioxidant and hypoglycemic activity

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8. References

1. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003; (66):022-1037.
2. Elkamali HH, Khalid SA. The most common herbal remidies in Dongola province, Northern Sudan. Fitoterapia 1996; (69):118-121.
3. Ahmed MA. The efficacy of four systemic insecticides using two methods of application against the green date palm pit scale insect (*Asterolicanium phoenicis* Rao) (Homoptera: Asterolicaniidae) in northern Sudan. ActaHorticulturae 2007; (736):369-389.
4. Tharib SM, El-Miggirab, Veitch GBA. A preliminary investigation of the potential antimicrobial activity of *Solenostemma argel*. Pharm Biol 1986; (24):101-104.
5. Boulos L. Medicinal plants of North Africa. Publications Inc Algonac Michigan USA ISBN 1983; (10):48001- 286.
6. Roos SA, Medgalla SE, Dishay DW, Awad AH. Studies for determining antibiotic substances in some Egyptian plants: Screening for antimicrobial activities. Fitoterapia 1980; (5):303-308.
7. Hamed AI. New steroids from *Solenostemma argel* leaves. Fitoterapia 2001; (72):747-755.
8. Kamel MS, Ohtani K, Hasanain HA, Mohamed H, Kasai R, Yamasaki K. Monoterpene and pregnaneglucosides from S*olenostemma argel*. Phytochemistry 2000; (53): 937-940.
9. Sulieman AE, Elzobair WM, Abdelrahim AM. Antimicrobial activity of the extract of *Solenostemma argel* plant. J Sci & Technol 2009; (10):104-115.
10. Eddouks M, Lemhadri A, Zeggwagh NA, Michel JB. Potent hypoglycaemic activity of the aqueous extract of *Chamaemelum nobile* in normal and streptozotocin-induced diabetic rats. Diabetes Res ClinPr 2005; (67): 189-195.
11. Shirwaikar A, Rajendran K, Kumar DC, Ramgopal Bodla R. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. J Ethnopharmaco 2004; (91):171-175.
12. El Hilaly J, Lyoussi B. Hypoglycaemic effect of the lyophilised aqueous extract of Ajugaiva in normal and streptozotocin diabetic rats. J Ethnopharmaco 2002; 800:109-113.
13. Tamez Perez HE1, Gómez de Ossio MD, Quintanilla Flores DL, Hernández Coria MI, Tamez Peña AL, Cuz Pérez GJ *et al.* Glucose disturbances in non-diabetic patients receiving acute treatment with methylprednisolone pulses. Rev Assoc Med Bras. 2012; (58):125-8.