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ANTIDIABETIC EFFECT OF SOME MEDICINAL PLANTS EXTRACTS

Hassan, R. A. ; S. T. AboTaleb ; M. I. Sanad and Sahar E. Hamed
Agric. Chem. Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt

ABSTRACT

Medicinal plants play an important role in cure diseases such as diabetes mellitus. From these plants are Vinca rosea (*Catharanthus roseus)* and Sage (*Salvia officinalis*) which belong to Apocynaceae and Lamiaceae, respectively. The purpose of the present study is to examine the anti diabetic effect of streptozotocin (STZ) in deuced diabetic rats. Vinca rosea and Sage leaves were extracted and applied at 250 and 500 mg /kg b.w.

Results revealed that all plant extracts caused a reduction in serum glucose levels. The highest decrease was observed with Vinca rosea extract (500 mg/kg b.w.) from 445.00 to 128.61 (71.0%) after 30 days.

It was also clear that, investigated plant extracts improved the triglyceride, the levels of total lipid, HDL, LDL ,vLDL and cholesterol .

Further studies are necessary to prove the antidiabetic extracts and to evaluate their active components.

Keywords : Vinca rosea, *Catharanthus roseus,* Sage, *Salvia officinalis*, diabetic, hyperglycaemia, Rats.

INTRODUCTION

Vinca rosa had been used to cure some diseases as diabetes mellitus, high blood pressure and infection. This plant is the source of over 70 different indole alkaloids. Two of the common anti-cancer drugs which are derived form this plant are vincristine and vinblastine. (Shams *et al*., 2009) , Sage is used as traditional medicine it has many biological activates such as a antiseptic, a tonic, digestive, astringent, anti oxidant, anti inflammatory, anti cancer and antispasmodic. Sage is listed by the Council of Europe as a natural source of food flavouring. (Dweck and Kintzios, 2000).

The aim of this work is to evaluate the extracts of two plants under study i.e. Vinca rosea and Sage as antidiabetic agents in experimental animal through serum glucose levels. Liver function (AST and ALT), serum triglyceride, total cholesterol, HDL, LDL and vLDL were also estimated.

MATERIALS AND METHODS

The aerial parts of Vinca rosea and Sage leaves plants were collected from agricultural collage garden of Mansoura University and were bought from local market, respectively.

Extraction of plants:

Vinca rosa plant was extracted according to *(Singh et al.,* 2001*)*. Dried leaves, twigs and flowers were made into powder in a grinder and were

extracted by soaking in 10 volumes of dichloro methane: methanol (1:1, v/v) at the rate of 1:1 (w/v) for 48 h.

The method of (Lis-Balchin *et al*. 1998) was applied for Sage plant as follows: air dried plant sample was extracted by soaking in methanol at the rate of 1:1 (w/v) for 48 h.

Animals :

Sixty male albino rats (100-120 g) were obtained from the animal house of Pharmacy Collaged, Mansoura Univ.

Sixty rats were kept two weeks for adaptation under laboratory conditions. Five rats were housed as negative control. The other 55 rats were STZ injection to induce diabetes as described by (Ghasemi *et al*., 2007). The diabetic rats were then randomly divided to 5 groups (11 diabetic rats in each) as follows:

Group 1: negative control and received a basal diet for 30 days, group2 : positive diabetic rats and received a normal diet for 30 days, group 3: as group 2 with vinca rosea extract at 250 mg/kg b.w, group 4: as group 3 but vincea rosea dose was 500 mg/kg b.w, group 5: as group 4 but with sage methanolic extract (250 mg/kg b.w), group 6: as group 5 with sage methanolic extract (500 mg/kg b.w).

Blood chemical constituents: .

Blood samples were collected from the eye canthus by heparinized tubes every 7 days after the beginning of extracts administration. Then, each blood sample was centrifuged to obtain clear serum where serum glucose levels for fasting animals were determined immediately. Serum blood samples were kept in refrigerator under freezing conditions for the determination of the lipid profile (triglycerides, total cholesterol, HDL, LDL and vLDL).Serum glucose was colorimetrically determined using glucose oxidase (GOD) by commercial kits of Human GmbH (Germany). As described by (Ashwell., 1957).

Serum triglycerides were determined by colorimetric enzymatic method described in a commercial kits by Human GmbH (Germany) according to *(*Trinder, 1969).

Serum total cholesterol was determined by enzymatic colorimetric method of (Richmond, 1973) described in a commercial kits by Human GmbH (Germany).

High density lipoprotein (HDL) was determined according to (Richmond, 1973) as described in commercial kits of Human GmbH (Germany).

Low density lipoprotein (LDL) was estimated by the following calculation according to (Friedewald *et al*., 1972)*,* using the following equation:-

$$\text{LDL-cholesterol mg/100 ml serum} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL-cholesterol}$$

f) Very low density lipoprotein (vLDL):

Very low density lipoprotein (vLDL) was calculated by the following equation according to (Friedewald *et al*., 1972), using the following equation:

$$\text{vLDL mg / 100ml serum} = \frac{\text{Triglycerides}}{5}$$

Statistical analysis:

Statistical analysis of all experimental data was done using the statistical software package (CoStat, 2005). Ayanalysis of variance (ANOVA) were determined used with Duncan's multiple range test at $p<0.05$ as the level of the significance (Duncan, 1955).

RESULTS AND DISCUSSION

a) Effect of plant extracts on serum glucose level:

Streptozocin (STZ) injection at dose of 4.5mg/100g. b.w caused a highly significant increase in serum glucose level. Treated rats showed an increase of serum glucose level from 117.80 to 415.80mg/dl for non diabetic and diabetic rats at zero time ,respectively. Gradual increase was observed during the experimental periods (7, 14, 21 and 30 days) until reached the maximum level of 470.00mg/dl at the end of experiment. This increase may be due to the destructive effect of streptozocin on β-cells of Langerhans islets which lead to insulin deficiency. In addition to the absence of available insulin in blood circulation, these may be the main causes of hyperglycemia which observed in the treated rats with streptozocin as reported by (Vessal *et al*., 2003).

From Table (1), it could be seen that the concentration of 500mg/kg b.w of Vinca rosea extract was effective for reducing serum glucose level for all plants extracts. The initial antidiabetic activity was observed after 7 days then gradually decreased for all groups to reach the maximum decrease at the end of the experiment (30 days).The obtained results revealed that the most effective treatment was that of the Vinca rosea extract which cause a decrease value of 71.09% at a dose of 500 mg/kg b.w.

Crude methanol extract of Sage (500 mg/100g b.w) acheved the second level with the average decreasing value of 67.30. However, Crude methanol extract of Sage (250 mg/100g b.w) had the lowest effective value, It cause a decrease value of 43.77% for serum blood glucose after 30 days.

However , Vinca rosea extract cause a percentage decrease value of 48.31 at a dose of 250mg/kg b.w.

(Singh *et al*., 2001), reported that serum glucose levels in rats were significantly decreased after treatment of dichloro methane : methanol (1:1,v,v) of Vinca rosea extract . Dose of 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively

While, (Perry *et al*., 2003), observed a significant reduction in serum glucose level after treatment of sage methanol extract at concentration of 150, 250 and 500 mg /b.w. These decrease reached 15.20, 35.2 and 42.80% after 20 days, respectively.

Finally, it could be concluded that lowering of serum glucose levels which was observed in the diabetic animals may be due to the stimulation of β-cells of pancreatic islets. This reduction come through stimulation of insulin release

resembling the oral hypoglycemic drugs or peripheral glucose utilization (Esmaeili and Yazdanparast, 2004).

Table (1): Effect of plants extract on blood glucose level (mg/dl) .

Groups	Zero time	7days	14days	21days	30days
Group 1	117.80	117. 40	117. 90	116.44	117.40
Group 2	415.80	416.00	416.40	414.60	445.00
Group 3	417.43	350.00	307.20	279.00	230.00
Group 4	399.24	289.00	222.80	166.40	128.61
Group 5	367.20	333.00	282.42	262.34	250.21
Group 6	402.48	351.40	252.80	169.62	145.50

2. Effect of plant extracts on serum triglycerides:

Data recorded in table 2 revealed that serum triglyceride increased from 167.48 to 270.54 mg/dl by injection of STZ. This increase reached about 38.09% of that obtained for non diabetic rats at zero time. It could be seen also that the injection of albino rats with Vinca rosea extract at dose of 500 mg/kg b.w. daily for 30 days caused a decrease in serum triglyceride by 46.88 comparing with the positive control .

The present results agreed with those mentioned by (Sivakumar *et al*., 2010), who found that the high concentration of Vinca rosea decreased the levels of triglycerides and total lipids than the control group.

On the other hand sage methanolic extracts decreased serum triglycerides levels in average percentage values of 33.97 and 29.00 at dose of 250 and 500 mg/kg b.w in comparison with the control, respectively.

Table (2): Effect of plants extract on Serum triglycerides (mg/dl) .

Groups	Zero time	7days	14days	21days	30days
Group 1	167.48	169.94	171.28	171.54	173.16
Group 2	270.54	276.72	275.70	300.92	320.22
Group 3	280.68	302.84	179.02	177.32	179.10
Group 4	283.00	289.00	184.70	172.32	170.10
Group 5	284.52	268.52	244.00	235.56	226.92
Group 6	281.03	273.60	234.34	225.74	211.41

3. Effect of plant extracts on serum total cholesterol level :

Table 3 showed that STZ induced rats treatment caused an increase in serum total cholesterol from 192.68 in non diabetic rats to 376.42 mg/dl in diabetic rats at zero time. Aserum total cholesterol gave a decreasing percentage values 32.15 and 51.50 at dose of 250 and 500 mg/kg b.w, respectively . Also, Sage methanolic extracts decrease total cholesterol and the average percentage values of decreasing reached about 36.65 and 48.82 % at dose of 250 and 500 mg/kg b.w, respectively.

These findings are in agreement with those mentioned by *(*Singh *et al*., 2001).They reported that plasma cholesterol was significantly decreased by Vinca rosea extract .

On the same trend, (Baricevic and Bartol, 2000),found that Sage extract reduced cholesterol, blood pressure and symptoms associated with diabetes mellitus .

Table (3): Effect of plants extract on Serum total cholesterol (mg/dl) :

Groups	Zero time	7days	14days	21days	30days
Group 1	192.68	189.72	191.68	193.28	196.88
Group 2	376.42	400.40	409.62	412.76	413.10
Group 3	399.24	405.84	389.42	349.35	280.26
Group 4	398.04	376.98	289.52	208.90	200.33
Group 5	396.40	391.20	374.22	266.00	261.67
Group 6	388.41	393.60	353.72	245.65	211.41

4. Effect of plant extracts on blood high density lipoprotein (HDL):

Data in table (4) revealed that the oral administration with plants extracts of all samples led to an increase in serum HDL which raised from 20.96 to 41.34 and 45.03 at dose 250 and 500 mg/kg b.w of Vinca rosea respectively. On the other hand the crude methanolic extract of sage showed a gradual increase in serum HDL cholesterol from 20.96 to 38.41 and 41.03 at dose 250 and 500 mg/kg b.w ,respectively.

The obtained results agreed with those of many authors proved that treatment with STZ caused a decrease in HDL- cholesterol. For instance Tilvis *et al*., 1988 noticed a decrease in HDL in alloxan diabetic rats which lowerd to 10 comparing with 22 mg/dl in non diabetic rats at zero time respectively. Fernandez *et al*., 2001demonstrated that a decreasing in serum HDL- cholesterol level by injection of streptozocin may be due to decrease of lecithin cholesterol acetyl transferase, which is responsible for estrification of cholesterol in HDL. Hessien, 2003 found that alloxan induction at 8 mg/100g.b.w decrease HDL- cholesterol level from 2.0g/l in normal rats to 0.9 g/l in diabetic one . Zarzuelo *et al* 1990 reported a significant decrease in plasma triglycerides and an increase in HDL levels in sage extract which taken orally, compared to the control group. In another study, Sage oil administration to rats significantly decreased serum total cholesterol, LDL and triglycerides and increased HDL.

Chatopadhyay *et al*. 1991, mentioned that administration of alkaloid of V.rosea decreased significantly the serum cholesterol, triglyceride, and LDL and increased significantly HDL .

Table (4): Effect of plants extract on Serum HDL cholesterol (mg/dl):

Groups	Zero time	7days	14days	21days	30days
Group 1	44.14	44.92	45.32	46.60	47.00
Group 2	24.52	23.18	21.90	23.60	20.96
Group 3	26.60	29.46	30.02	35.44	41.34
Group 4	25.32	32.80	37.90	43.68	45.03
Group 5	26.32	25.32	35.50	36.62	38.41
Group 6	28.22	33.60	33.66	38.31	41.03

5 . Effect of plant extracts on Serum low and very low density lipoprotein (LDL and vLDL):

Tables (5 and 6) and figures (5 and 6) showed that both LDL and vLDL-cholesterol increased by injection of STZ which reached 322.62 after 30 days . This result are similar to those obtained by several researchers. For instance **Tilvis *et al*., 1988** reported that many compositional abnormalities of the lipoproteins have been found in diabetic patients and the major cause of hyper triglyceridemia patients appears to be the over production of vLDL which is attributed to hyperglycemia and/or increased influx of free fatty acids into the liver.

Fernandez *et al*., 2001 demonstrated that the increasing in LDL level may be attributed to some reasons such as an increase of intestinal absorption of lipid, an increase of cholesterol synthesis and increase of liver lipid synthesis or liver disfunction. On the same trend Hessien ,2003, mentioned that LDL- cholesterol increased from 100 to 909 mg/dl, while vLDL cholesterol increased from 33.3 to 56.0 mg/dl by injection of alloxan to experimental rats at zero time respectively. Also, Soltani *et al*., (2007) observed that STZ diabetics induction increased LDL and vLDL- cholesterol.

From the same tables (5 and 6), it could be seen that all plants extracts during experimental periods showed a decrease in serum LDL - cholesterol which became 108.22 and 199.06 mg/dl for dose 250 and 500 mg/kg b.w of V. rosea respectively. The results showed also that methanolic sage extract after 30 days were 172.67 and 129.36 using 250 and 500 mg/kg b.w respectively

The obtained results showed also that vLDL decreased from 68.50 mg/dl in STZ diabetic rats to 36.90 and 32.74 mg/dl after treatment with V.rosea extracts after 30 days by using 250 and 500 mg/100g b.w respectively. On the other hand vLDL values decreased which became 41.20 and 35.70 mg/dl after treatment with sage extracts after 30 days by using 250 and 500 mg/100g b.w, respectively.

Table (5): Effect of plants extract on Serum LDL cholesterol (mg/dl) :

Groups	Zero time	7days	14days	21days	30days
Group 1	105.02	107.96	107.67	108.22	109.01
Group 2	288.78	293.82	296.04	301.62	322.62
Group 3	282.70	288.30	254.66	236.82	199.06
Group 4	284.26	288.06	222.20	215.66	108.22
Group 5	284.20	275.00	253.33	238.00	172.67
Group 6	288.46	258.60	249.40	236.02	129.36

Table (6): Effect of plants extract on Serum vLDL cholesterol (mg/dl) :

Groups	Zero time	7days	14days	21days	30days
Group 1	32.78	33.06	32.34	34.22	33.12
Group 2	60.94	62.68	62.02	68.82	68.50
Group 3	60.78	65.02	55.30	38.30	36.90
Group 4	61.44	62.50	56.34	36.80	32.74
Group 5	61.02	63.74	53.18	44.72	41.20
Group 6	64.08	61.42	51.56	40.08	35.70

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رمضان أحمد حسن, سامي طلعت أبو طالب , مصطفى ابراهيم سند وسحر السيد حامد*
قسم الكيمياء الزراعية , كلية الزراعة , جامعة المنصورة , المنصورة , مصر
*** كلية الزراعة - جامعة دمياط**

تلعب النباتات الطبية دور مهم فى تنظيم مرض السكرى خاصة فى الدول النامية وتقدم هذه الدراسة بعض النباتات الطبية التى لها تأثير لهذا المرض من هذه النباتات نبات الونكا ونبات المريمية حيث ان هذان النباتان يتمتعان بالكثير من التاثيرات الحيوية المهمة مثل تعمل كمضاد للاكسدة ومضاد لمرض السرطان ومرض لوكيميا الدم ومرض الزهيمر ومضادة لنشاط الكائنات الحيوية الدقيقة . تتناول هذة الدراسة تأثير بعض المستخلصات من هذين النباتين على بعض فئران التجارب المصابة بمرض السكرى . حيث تم دراسة مستخلص نبات الونكا (dicholoromethane:methanol.1:1v/v) بتركيز ٢٥٠ - ٥٠٠مجم /كجم من وزن الجسم ومستخلص الميثانول لنبات المريمية بتركيز ٢٥٠ - ٥٠٠مجم /كجم من وزن الجسم. وقد اوضحت النتائج ان مستوى السكر فى الدم قد انخفض بدرجة ملحوظة ولكن كانت أعلى نسبة للانخفاض سجلت لمستخص نبات الونكا بتركيز ٥٠٠مجم / كجم من وزن الجسم حيث انخفضت من ٤٤٥.٠٠ الى ١٢٨.٦١مجم /ديسلتر من الدم كما اوضحت النتائج ان هناك تحسن فى وظائف الكبد ، الكلى ، الكوليسترول الكلى وكذلك الكوليسترول المنخفض الكثافة .على سبيل المثال قد انخفضت نسبة الجلسريدات الثلاثية triglyceride من ٣٢٠.٢٢ الى ١٧٠.١٠مجم /ديسلترمن الدم بينما اعطى الكرياتينين على نسبة انخفاض بالمقارنة بالمستخلصات الاخرى حيث وصل الى ١.٢٥ مجم / ملليتر .

قام بتحكيم البحث

أ.د / حسان بركات حامد داود	**كلية الزراعة – جامعة المنصورة**
أ.د / سيد سليمان السيد	**خارجى**