Anti Diabetic Effect of *Artemisia.sieberi* In Rabbits that Induced Diabetic by Alloxan

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Abstract — The purpose of this study is to demonstrate the effectiveness of some of the material extracted from the plant Artemisia . sieberia and their impact on the reduction of diabetes in rabbits that have been injected with alloxan induced diabetic rabbits , Where was the study on rabbits, which were divided into three groups, each group of three rabbits group 1 normal rabbits received 1ml day -1 of dimethyl sulphoxide [control]; group 2 diabetic rabbits received asingle dose [80 mg kg -1 b.wt.] of essential oil aqueous extract of Artemisia .sibberi ; group 3 diabetic rabbits received single dose [80 mg kg -1 b.wt.] of essential oil coholex extract of *Artemisia.sieberi* .all treatments were orally administered once aday for three weeks .changes in blood glucose concentration ,body weight and food and water intake were measured and the data obtained were compared with that of control .the essential oil extract significantly [p0.05] lowered blood glucose level as well as food and water intake in diabetic rabbits accompanied by an increase in body weight again with no apparent side effect when compared with untreated diabetic rabbits .these effects were found to be closely similar to that of aqueous extract and coholix extract and a metaphormin ,acommon anti diabetic drug .on other hand ,no apparent improvement on body weight again in diabetic rabbits treated with metaphormin . in addition , for all parameters measured , the oil extract showed no effect in normal rabbits .in conclusion ,the essential oil of Artemisia . sieberi exhibited anti diabetic activity in alloxan –induced diabetic rabbits . In recent times it has become dependent on medicinal plants as a treatment for diabetes in humans and various types of diseases and published research on the plant Artemisia .sibberi proved that

Index Terms — ***Artemisia .sibberi*** **, alloxan , Diabetes mellitus , blood glucose, pancreas, Anti-diabetic**

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1. Introduction

Diabetes mellitus is achronic condition which affects ±10% of the general population, characterized by ↑ serumglucose and a relative or absolute ↓ in pancreatic insulin production, or ↓ tissue responsiveness to insulin; if not properly controlled, theexcess glucose damages blood vessels of the eyes, kidneys, nerves, heart Types Insulin dependent–type I and non-insulin dependent–typeII diabetes Symptoms type 1 DM is associated with ↑ urine output, thirst, fatigue,+

and weight loss ; type 2 DM isassociated with, in + addition, non healing ulcers , oral and bladder infec- and feet, and itchingCardiovascular MI, stoke Eyes Retinal damage, blindness Legs/feet Nonhealing ulcers, cuts leading to gangrene and amputation Kidney sHTN, renal failure Neurology Paresthesias, neuropathy Diagnosis Serum glucose above cutoff points after meals or when fasting; oncetherapy is begun, serum levels of glycosylated Hb are measured periodically to assess adequacy of glucose control Management Therapyre-
flects type of DM; metformin andtriglitazone have equal and additive effects on glycemic control Prognosis A function of stringency ofglucose control and presence of complications.

Oral medications are available to lower blood glucose in Type II diabetics Konuklagil , B,Deniz, G,Yildiz , O,Senoz,s and saygi, s, 1997 . In 1990
, 23.4 outpatient prescriptions for oral antidiabeticagents were dispensed. By 2001, the number had increased to 91.8 million prescriptions. Oral antidiabetic agents accounted for more than$5 billion dollars in worldwide retail sales per year in the early twenty-
first century and were the fastest-
growing segment of diabetes drugs.The drugs first prescribed for Type II diabetes are in a class of compounds called sulfonylureas and include tolbutamide, tolazamide,acetohexamide, and chlorpropa-
mide. Newer drugs in the same class are now available and include glyburide, glimeperide, and glipizide.How these drugs work is not well understood, however, they seem to stimulate cells of the pancreas to produ-
ce more insulin. Newmedications that are available to treat diabetes include metformin, acarbose , and troglitizone . The choice of medication
depends in part onthe Individual patient profile . All drugs have side effects that may make them inappropri-
ate for particular patients. Some for example, maystimulate weight gain or cause stomach irritation, so they may not be the best treatment for someone who is already overweight or whohas stomach ulcers. Others, like metformin, have been shown to have positive effects such as reduced cardiovascular mortality, but butincreased risk in other situations. While these medications are an important aspect of treatment for Type II diabetes, they are not asubstitute for a well planned diet and moderate exercise. Oral medications
have not been shown effective for Type I diabetes, in which thepatient produces little or no insulin.

Since olden days, plants are used to treat many ailments. India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties (Grover JK, et al. J Ethnopharmacol. 2002;81:81). Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethnobotanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycemic and antihyperglycemic activity. (Grover JK, et al. J Ethnopharmacol. 2002;81:81) The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents.(Fabricant DS, Farnsworth NR. Environ Health Perspect. 2001;109:69) Several species of medicinal plants are used in the treatment of diabetes mellitus, a disease affecting large number of people world-wide. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (Nammi S, et al. BMC Complement Altern Med. 2003;3:4)

Essetial oils are commonly used in traditional medicine as antiseptic, antimicrobial, virucidal, fungicidal, analgesic, seedactive, anti-inflammatory, spasmolytic and anesthesia as well by Arabs. Biochemical analyses showed that essential oils are mixture of a variety of lipid soluble and volatile compounds such as terpenes and terpenoids , phenol-derived aromatic and aliphatic components that are characterized by their strong odor(Zareba et al., 2005; Burt,2004;Bakkali et al., 2008; Sabu and Kuttan, 2002).These analyses also characterize most of them as antioxidants. Essential oils extracts have also been known to contain at least 100 alkaloid compounds as well as other pharmacologically active compounds. Moreover,their quality and quantity were (shown King , H R,E. Auburt and W. H .Herman, 1998 ; Global burden of diabetes 1995-2005) to vary according to method of extraction, climate, soil composition, plant organ, age and vegetative cycle stage.

Furthermore, ethanobotanical studies reported that at least 1,200 species of plants with anti hyperglycemia or hypoglycemia activity have been identified as remedies for DM around the world (Marrif et al., 1995; Khalil, 1995; Afif and Irmaileh, 2000; Irshaid and Mansi, 2009a,b).*Artemisia.sieberi* (A. sieberi)is one of them which grows wild in Jordan and it is commonly known as sheeh (Khalil et al., 1995; Afif and is becoming very popular in Jordan for the treatment of DM and other aliments (Afif and Irmaileh, 2000, Amr, 1995). According to a recent study by Hudaib and Aburijai (2006), forty different compounds have been identified in the essential oils of this plant by GC/MS analyses. These include phenol, alcohol, ketone and monotepenes compounds. Recently , its hypoglycemic activity in aqueous extract has been reported in diabetic animals (Mansi and Lahham, 2008). However,prior to our current study, the effects of essen-

as in food industry as preservative (Zareba et al., 2005; Burt, 2004; Bakkali et al., 2008; Sabu and Kuttan, 2002). Essential oils were firstextracted from aromatic plants by steam or hydro-distillation

tial oils extract obtained from A. sieberi have not yet been evaluated in Jordan. Furthermore,there is no report on the antid iabetic activity of essential oil derived from *A.sieberi* in the available literature. Therefore, the present research work was oil extract from *A.sieberi* and metaformin on blood glucose concentration, water intake, food intakeand body weight in alloxan induced diabetic rabbits.

1.1. Artemisia .sieberi plant

For thousands of years ago, plants have been extensively used to treat numerous ailments. In Jordan, about 2500plant species belonging to seven hundred genera are recorded (Al-Eisawi, 1982) of which around 485 species from 99 plant families are categorized as medicinal plants (Affix and Abu Iramileh, 2000). Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community. They contain valuable medicinal properties. A number of plants have shown varying degrees of hypoglycemic and anti-hyperglycemic activities (Grover et al., 2002). The active principles of many plant species were isolated fordirect use as drugs or pharmacological agents (Fabricant et al., 2001). Several species of medicinal plants are being. One of anti diabetic phytotherapies is Artemisia sieberi which is a well-known medicinal plant that has been used in the Middle East traditional medicine for treating various diseases including diabetes mellitus. It is used as an anthelminthic by the local population. The plant is also used as antimicrobial, poison antidote, and emmenagogue (Ziyyat et al., 1987). Others (Suleiman et al., 1988; Gharaibeh etal., 1988; Tanira et al., 1996; Konuklugil et al., 1997)

reported that *Artemisia sieberi* to possess antidiabetic effects and have been used in many countries of Middle East and Turkey as an herbal medicine for treatment of diabetes, high blood pressure and gastrointestinal ailments. Administration of aqueous extract of *Artemisia sieberi* normalized serum lipids, secondary to the diabetic state. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishna Kumar et al., 2000).

2 Material and Methods

2.1 material

- plant of *Artemisia .Siberia*
- Distilled water
- Ethanol 70%
- Detol

2.2 Instruments and Equipments

- Containers
- Oven in (45%)
- Refugerg . for freezing
- Sense balance
- Refugerg . for freezing
- Gloves
- Sense balance
- Syringes
- Knife
- ACCU CHEK Active(diabetic appearterus)

Fig. 1 CCU CHECK ACTIVE Diabetic APPERTUS

2.3 Plant collection

Aerial parts of Artemisia sieberi were collected in the spring 7fromAl-Mafraq (68 KM North-East Amman). The plant was identified by Prof. Jamil Lahham (Plant Taxonomist, Department of Biological Sciences; Yarmouk University). Voucher specimens were deposited in the Department of Biological Science, Faculty of

Science, Al-aLBayt University (NO.AHE-2-007).(Kameswara Rao, B, Kesavulu, MM, Giri,R.and Appa Rao, CH. 1999) The whole plant was air-dried, ground mechanically, and preserved in a deep freezer until use.

2.4. Blood collection and glucose determination

For blood glucose level determination, blood samples were collected from fasted rabbits of the three groups prior to the treatment with above schedule and three times per weeks after oral administration of treatments up to 3 weeks. Blood samples were collected by snipping ear with sharp razor and blood glucose level was then measured immediately by Haemo-Glukotest (20_800Rglucose strips supplied by M/S Boehringer Mannheim India Ltd .

2.5 Extraction preparation

We take (300 gm) from Artemisia . Siberia in powdered from after we and put (150 gm) in all flask and preparation of a ethanol cohol in (70 %) of concentration by add (30 %) of distiller water water to it to become the concentration (100%) and we add it to the plant in the flask and we covered it and leave it in the dark room for 2 days then we put it in oven in (45 C) for 9 hours and we out it and put it in sub-freezing for (24 hours) then we get it to extraction it by (ethanol) and purification in other flask and we in putted in Pe-

tri dish and we put it in incubator in (45C) for (2days) we get it on(4gm)from extract plant then we out it to collection by removing it from petridish by knaive and put it in container then we put it in freezing or cold place to use it . extract by distiller waters: we take (150 gm) from *Artemisia . siberia* in powdered from then add distiller water warm (500ml) and began to extraction it and purification that we kept it in glass flask with good cover closed to use it later .

To preparation the dose extraction of alcohol . we take(1gm) and add it (10ml) from distiller water and then take (10ml) to anther container add to it (10ml) from distiller water and it ready to use it for injection rabbits orally

The mount dose is (80mg kg-1 b.wt.) for three weeks and we recorded the result .

To preparation of dose from water extraction we take (10 ml)and add (10 ml)from distilled water in then we take (10 ml) from the mix and (10ml) distilled water to it then it become ready to use it .

2.6 Induction of diabetes in rabbits

Male Wister rabbits weighing 1.5g to 2g were used for this study .all rabbits were obtained from the animal house of al – muthanna university of science in biology department . the rabbits were harbored in stainless steel cages under standard laboratory condition of 12 h light/dark cycle throughout the experimental periods .they had access to normal food .the animals were carefully checked and monitored every day for any sign of toxicity or changes during entire period of experimental .alloxan monohydrate was purchased from market ltd [Poole ,England]and was dissolved in sterile normal saline [8.5 nacl].to induce diabetes , the rabbits were injected intraperitonially with freshly prepared aqueous solution of alloxan following a24 h fast in adose of 150 mg kg bwt. after measuring fasting blood sugar , diabetic status was determined ,rabbits with blood glucose of 250 mg dl-1 or more were classified as diabetic rabbits and were used for the subsequent experimental non- diabetic control rabbits were injected with normal saline [0.5 ml kg -1 b.wt.] instead of alloxan .

2.7 Experimental design

Rabbits were divided in to three experimental groups of rabbits each group .group 1 consisted of normal rabbits as contrall group .group 2 consisted of alloxan –induced diabetic rabbits that received A. seiberia oil ethanol or alcohol extraction group III consisted of alloxon – induced diabetic rabbits that received A. Siberia oil water extraction . Rabbits were minted in these treatment regimens dose of (80mg kg-1 b.wt.) gave to the Rabbits daily for 3 weeks with free access to food and water ad libitum . these experiments complied with guidelines of our animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care . Dialy measurement of body weight and food and water intake were .

3 RESULTS

The extract had no effect on the weight of the studied animals. In Table I , the extract produced significant decrease ($p<0.05$) in blood glucose level in diabetic rabbits after 10 days of treatment by significantly increasing ($p<0.05$) the secretion of insulin and decreasing the serum level of glucagons(Table II). The hypoglycemic effects of *Artemisia sieberi* on the diabetic rabbits were observed within 2 h, continued for about 8 h, and lasted to the end of the experiment (10 days). The administration of Artemisia sieberi indicates significant decrease ($p<0.05$) of blood glucose concentration and increase of serum insulin was found to be anti-diabetic. None of the animals treated with Artemisia sieberi showed any visible

serious symptoms of toxicity; however, there were mild signs of respiratory distress, diarrhea, and convulsions, as shown in these tables:

Table 1 : effect of oral administration of *Atrmisia sieberi* essntal oil cholics extract (80 mg kg 1- b. wt)for third week an body week gain in alloxan diabetic rabbits

Serum glucose concentration (mg/dl-1) :

No	First Week	Second week	Third week	Control by Induced alloxan
1	198 mg/dl	105 mg/dl	80 mg/dl	300 mg/dl
2	155 mg/dl	100 mg/dl	78 mg/dl	280 mg/dl
3	157 mg/dl	135 mg/dl	100 mg/dl	250 mg/dl

Table 2 : effect of oral administration of *Atrmisia sieberi* essntal aqueous extract (80 mg kg 1- b. wt)for third week an body week gain in alloxan diabetic rabbits

No	First week	Second week	Third week	Control by Induced alloxan
1	170 mg/dl	150 mg/dl	120 mg/dl	300 mg/dl
2	175 mg/dl	160 mg/dl	140 mg/dl	280 mg/dl
3	160 mg/dl	143 mg/dl	123 mg/dl	250 mg/dl

4 DISCUSSION

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Szuldelski, 2001) . Our observations in this study correlates with the previous research findings, in that the
blood glucose levels significantly increased in alloxan untreated diabetic rabbits .

Alloxan induces damage and death of pancreatic islet-cells in several experimental animal models, thus causing diabetes mellitus and decreasing the secretion of insulin. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species, Alloxan and the product of its reduction, dial uric acid; establish a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide.

Therefore, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of ß-cells. The blood glucose data obtained clearly indicate that aqueous extract from Artemisia sieberi produced significant hypoglycemic effects in alloxan-induced diabetic rabbits . The obtained results were similar to those obtained by (Marrif et al., 1995) and (Twaij et al., 1988). It is possible that the plant may reverse the catabolic features of insulin deficiency, decrease the release of glucagons or increase that of insulin, stimulate directly glycol sis in peripheral tissues,
increase glucose removal from blood or reduce glucose absorption from the gastrointestinal tract (Mari et al., 1995). Hypoglycemic effects of Artemisia sieberi could,
possibly, be due to increased peripheral glucose utilization. Inhibition of the proximal tubular reabsorption mechanism for glucose in the kidneys, if any, can also contribute towards blood lowering effect (Sharma et al.,1983). Body weight in all diabetic rabbits was increased. This is the normal effect of diabetes mellitus. After the treatment of the diabetic rabbits , their body weight increased again. Similar effects were also observed by other researchers (Boriky et al., 1996; Twaij et al., 1988; Sharma et al., 1983). The synthetic oral hypoglycemic agents can produce a series of side effects. As can be seen from this study, rabbits treated

with *Artemisia sieber* ,

showed only mild visible undesirable clinical symptoms**.** We have noticed a significant reduction in food and water intake in alloxan diabetic rabbits . This could be the result of improved glycemic control produced by aqueous extract of *Artemisia sieberi*. The present study indicated that *Artemisia sieberi* treatment might ameliorate some disturbed hematological parameters of diabetic rabbits It has been suggested that anemia occurrence in DM is due to the increased nonenzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (Kennedy and Baynes, 1984). Oxidation of these glycosylated membrane proteins and hyperglycemia in DM cause an increase in the production of lipid peroxides causing a hemolysis of RBC. In this study, the RBC membrane lipid peroxide levels in diabetic rabbits were not measured. However, (Meral et al., 2001) demonstrated

that serum lipid peroxide level increased in diabetic rabbits. Thus, increased RBC count of *Artemisia sieberi* treatment rabbits could be due to the lowered lipid peroxide level in RBC membrane leading to a decrease susceptibility of RBC to hemolysis. Since nonenzymatic glycol solutions of membrane proteins correlate with hyperglycemia (Kennedy and Baynes, 1984), it might be suggested that *Artemisia sieberi* produced its effect by decreasing the elevated glucose However, more studies by measuring the RBC fragility, and serum folic acid, iron, cobalt, vitamin B12 and calcium levels are needed to demonstrate the exact mechanism of action of *Artemisia sieberi* on increased RBC count of diabetic rabbits

Fig.2 The flowers of *arteimisea .sieberi*

Fig.3 The appearance of *Arteimisea.sieberi* plant with stem and leaves

5 CONCLUSIONS

Results obtained suggest that taking the plant extract of *Arteimisea.sieberi* by mouth showed positive effects of reducing diabetes in rabbits which was introduced by diabetes through Alaoxan compared to metaphormin which is used to lower diabetes in the blood which is the most common, has been observed rabbits in response to the impact of the pancreas and its physiological as well as the Note toxicity by giving Abstract did not show any signs indicating that a very safe extract, and found other experiments conducted on mice showed the results of an approach which shows the effectiveness of their impact on diabetes

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