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


**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* sibirii; group 3 diabetic rabbits received single dose [80 mg kg⁻¹ b.wt.] of essential oil coholdex 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 ,a common 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* sibirii proved that

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

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1. **Introduction** — Diabetes mellitus is a chronic condition which affects ±10% of the general population, characterized by ↑ serum glucose and a relative or absolute ↓ in pancreatic insulin production, or ↓ tissue responsiveness to insulin; if not properly controlled, the excess glucose damages blood vessels of the eyes, kidneys, nerves, heart Types Insulin dependent–type I and non-insulin dependent– typell diabetes Symptoms type 1 DM is associated with ↑ urine output, thirst, fatigue,
and weight loss; type 2 DM is associated with, in addition, non-healing ulcers, oral and bladder infections, and itching. Cardiovascular MI, stroke, Eye 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. Therapy reflects type of DM; metformin and trilaglitzone have equal and additive effects on glycemic control. Prognosis. A function of stringency of glucose 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 antidiabetic agents 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 chlorpropamide. 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 produce more insulin. New medications that are available to treat diabetes include metformin, acarbose, and troglitizone. The choice of medication depends in part on the individual patient profile. All drugs have side effects that may make them inappropriate for particular patients. Some for example, may stimulate weight gain or cause stomach irritation, so they may not be the best treatment for someone who is already overweight or who has stomach ulcers. Others, like metformin, have been shown to have positive effects such as reduced cardiovascular mortality, but increased risk in other situations. While these medications are an important aspect of treatment for Type II diabetes, they are not a substitute for a well planned diet and moderate exercise. Oral medications have not been shown effective for Type I diabetes, in which the patient 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 ethnomedical 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)
Essential oils are commonly used in traditional medicine as antiseptic, antimicrobial, virucidal, fungicidal, analgesic, seedactive, anti-inflammatory, spasmyloytic and anesthesia as well as in food industry as preservative (Zareba et al., 2005; Burt, 2004; Bakkali et al., 2008; Sabu and Kuttan, 2002). Essential oils were first extracted from aromatic plants by steam or hydro-distillation 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 antihyperglycemia 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 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 essential oils extract obtained from *A. sieberi* have not yet been evaluated in Jordan. Furthermore, there is no report on the antidiabetic activity of essential oil derived from *A. sieberi* in the available literature. Therefore, the present research work was oil extract from *A. sieberi* and metformin on blood glucose concentration, water intake, food intake and 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 2500 plant 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 Irmaileh, 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 for direct 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 anthelmintic 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 et al., 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 apperturus )

Fig. 1 CCU CHECK ACTIVE Diabetic APPERTUS

2.3 Plant collection
Aerial parts of *Artemisia sieberi* were collected in the spring from Al-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 (2 days) we get it on (4 gm) from extract plant. then we out it to collection by removing it from petridish by knife 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 (500 ml) and began to extraction 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 (1 gm) and add it (10 ml) from distiller water and then take (10 ml) to another container add to it (10 ml) from distiller water and it ready to use it for injection rabbits orally. The mount dose is (80 mg 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 then we take (10 ml) from the mix and (10 ml) distilled water to it then it become ready to use it.

2.6 Induction of diabetes in rabbits

Male Wister rabbits weighing 1.5 g to 2 g 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 the 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 intraperitoneally with freshly prepared aqueous solution of alloxan following a 24 h fast in a dose 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 into three experimental groups of rabbits each group. Group 1 consisted of normal rabbits as control group. Group 2 consisted of alloxan-induced diabetic rabbits that received A. siberia oil ethanol or alcohol extraction. Group III consisted of alloxan-induced diabetic rabbits that received A. Siberia oil water extraction. Rabbits were minted in these treatment regimens dose of (80 mg 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 glucagon (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* essential oil cholics extract (80 mg kg⁻¹ b. wt) for third week and body weight gain in alloxan diabetic rabbits

<table>
<thead>
<tr>
<th>No</th>
<th>First week</th>
<th>Second week</th>
<th>Third week</th>
<th>Control by Induced alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>198 mg/dl</td>
<td>105 mg/dl</td>
<td>80 mg/dl</td>
<td>300 mg/dl</td>
</tr>
<tr>
<td>2</td>
<td>155 mg/dl</td>
<td>100 mg/dl</td>
<td>78 mg/dl</td>
<td>280 mg/dl</td>
</tr>
<tr>
<td>3</td>
<td>157 mg/dl</td>
<td>135 mg/dl</td>
<td>100 mg/dl</td>
<td>250 mg/dl</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>No</th>
<th>First week</th>
<th>Second week</th>
<th>Third week</th>
<th>Control by Induced alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170 mg/dl</td>
<td>150 mg/dl</td>
<td>120 mg/dl</td>
<td>300 mg/dl</td>
</tr>
<tr>
<td>2</td>
<td>175 mg/dl</td>
<td>160 mg/dl</td>
<td>140 mg/dl</td>
<td>280 mg/dl</td>
</tr>
<tr>
<td>3</td>
<td>160 mg/dl</td>
<td>143 mg/dl</td>
<td>123 mg/dl</td>
<td>250 mg/dl</td>
</tr>
</tbody>
</table>

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 β-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 glycolysis 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 \textit{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 \textit{Artemisia sieberi}. The present study indicated that \textit{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 non-enzymatic 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 \textit{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 \textit{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 \textit{Artemisia sieberi} on increased RBC count of diabetic rabbits.

5 CONCLUSIONS

Results obtained suggest that taking the plant extract of \textit{Artemisia sieberi} by mouth showed positive effects of reducing diabetes in rabbits which was introduced by diabetes through Alloxan compared to metformin 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.

References

[4] Zareba et al., 2005; Burt, 2004; Bakkali et al., 2008; Sabu and Kuttan, 2002
[5] Marrif et al., 1995; Khalil, 1995; Afif and Irmaileh, 2000; Irshaid and Mansi, 2009a,b
[8] Fabricant et al., 2001
[12] Marrif et al., 1995