International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (7): 782-789, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.782.789

Antidiabetic, Antihyperlipedemic and Antioxidant Effects of Aqueous Extract of the Roots of *Cynara cornigera* in Alloxan-induced Experimental Diabetes Mellitus

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Abstract: Diabetes mellitus is one of the most common diseases due to which world's population suffers from it. Nowadays, the medical management of diabetes is not adequate due to the serious side effects of synthetic hypoglycemic drugs. The aim of this study was to investigate the antidiabetic, antihyperlipidemic and antioxidant activities of *Cynara cornigera* as an herbal drug free from side effects. Hyperglycemia was induced by a single intraperitoneal injection of alloxan (150 mg kg^{-1}). Administration of the *Cynara cornigera* extract (1.5 g kg^{-1}) and glibenclamide (10 mg kg^{-1}) orally in diabetic rats resulted in a significant reduction ($p<0.05$) on blood glucose level (from 330.80±10.11 mg dL^{-1} to 229.70±7.94 and 195.50±6.53 mg dL^{-1} for the extract and for glibenclamide, respectively). In addition, it significantly recovered serum insulin levels (from 18.00±1.23 μL mL^{-1} to 27.00±1.19 and 32.00±1.73 μL mL^{-1} for the extract and for glibenclamide, respectively), liver glycogen content (from 14.80±1.10 mg g^{-1} to 33.65±1.96 and 39.10±2.47 mg g^{-1} for the extract and for glibenclamide, respectively) and prevented the decrease in body weight (from -12.5 to +4.2% and +6.2% for the extract and for glibenclamide, respectively). Hyperlipidemia, marked increase in lipid peroxide levels and concomitant decrease in glutathione content and catalase activity from homogenized liver sample were observed in untreated diabetic rats. Treatment with *Cynara cornigera* extract significantly reversed these conditions to near normal levels. The results of the present study show that administration of aqueous extract of the root of *Cynara cornigera* may be a good alternative antidiabetic agent.

Key words: Hyperglycemia, *Cynara cornigera*, insulin, hyperlipidemia, oxidative stress

INTRODUCTION

Diabetes Mellitus is considered as one of the most common metabolic disorder that widely spread all over the world. Diabetes is caused by many causative factors; these factors either cause defects in secretion, insulin action, or both. It is characterized by chronic increase in the blood sugar as a result of disturbance in carbohydrates, protein and fat metabolism (Karim *et al*., 2011). There are two main causes of type 2 Diabetes, either by cells resistance to insulin with deficiency in insulin secretion or highly deficiency in insulin secretion with or without insulin resistance (WHO, 1999). The incidence of diabetes is greatly increased in all countries within the last few years and many studies have been conducted in this field. It was found that diabetes in 1995 was increased by about 4% in adults less than 20 years of age and this rate is expected to increase in 2025 by 5.4% (Moller and Flier, 1991). Serious side effects were detected on synthetic hypoglycemic medications in treatment of diabetes, therefore these medications are contraindicated to use in pregnancy. Examples of these side effects are hepatorenal defects and hypoglycemic coma. (Oubre *et al*., 1970; Holman and Turner, 1991; Suba *et al*., 2004; Rahman and Zaman, 1989). The limitations for use the synthetic hypoglycemic medications are depend on their pharmacokinetic properties; increase their failure rates and also their serious side effects (Hussain, 2002). Many studies have been done to overcome the restrictions of the synthetic hypoglycemic medications and search for safer and potentially effective medications. Hypoglycemic agents that derived from plants and other natural sources can replace the synthetic hypoglycemic medication in treatment of diabetes and have fewer side effects (WHO, 1980; Bailey and Day, 1989).

Cynara cornigera L. (Asteraceae) commonly called as Gaamool in Libya. It is a plant that is widely grown in Mediterranean countries. Asteraceae is a plant family considered to be one of the important families of plants with potent hypoglycemic effects (Nazni *et al*., 2006). It is rich in natural antioxidants mainly polyunsaturated fatty acids (Vardavas *et al*., 2006a), vitamins C, k, α-Tocopherol and β-Carotene (Vardavas *et al*., 2006b). Recently, Pandino *et al*. (2011) also reported that they are rich in

polyphenols, mainly Caffeoylquinic acids and Luteolin. The present study was undertaken to evaluate the antidiabetic, antihyperlipedemic and antioxidant activities of aqueous extract of the root of *Cynara cornigera* on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Chemicals: Alloxan monohydrate was purchased from Sigma chemical company. All other chemicals and biochemicals were of analytical grade obtained from local firms.

Plant material: Fresh roots of *Cynara cornigera* were purchased from the local market in Benghazi, during the month of November 2010 and it's identifying in the Botany department of the college of Science, University of Garyounis.

Preparation of the extract: Roots of *Cynara cornigera* were cleaned and dried at room temperature under shade. Thereafter, 150 g of dried roots were grounded and the obtained powder was mixed with 1000 mL of distilled boiling water for a period of 15 min under continuous stirring. The obtained mixture was filtered in a filter funnel and the obtained liquid was vacuum dried until a concentrated residue (25%, w/w) was obtained. This stock extract was maintained at -20°C until being used. Lower concentrations of the extract were prepared by dilution of the stock with cold and sterile 0.9% saline solution.

Animals: Male Wistar rats weighing 180-200 gram were used for all experiments. Animals were obtained from the central animal house of Garyounis University, Benghazi, Libya. Rats were fed a standard laboratory chow and had free access to water. They were kept in wire bottomed stainless steel cages. All experimental procedures involving animals were conducted in accordance with the guidelines of National Institutes of Health (NIH guidelines). The study protocols were approved by Ethical committee of Garyounis University.

Induction of diabetes: The animals were fasted for 12 h with free access to water prior to the induction of diabetes. Alloxan monohydrate freshly prepared in 0.9% v/v cold normal saline solution was administered intraperitoneally (i.p.) at single dose of 150 mg kg^{-1} body weight after base line glucose estimation was done (Tanko *et al*., 2011). Since alloxan is capable of producing fatal hypoglycemia, as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani *et al*., 2002). Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of alloxan. Rats with blood glucose level of above 200 mg dL^{-1} were considered to be diabetic and used for the studies.

Experimental design: The rats were randomized into four groups comprising of eight animals in each group as given below. Normal saline/*Cynara cornigera* (1.5 g/kg/day)/Glibenclamide were administered orally using an intra-gastric tube once daily for 30 days:

Group 1: Normal control rats, received cold normal saline solution
Group 2: Diabetic control rats received alloxan in single dose (150 mg kg^{-1})
Group 3: Diabetic rats received *Cynara cornigera* (1.5 g/kg/day), 5 days after alloxan treatment
Group 4: Diabetic rats received Glibenclamide (10 mg/kg/day), 5 days after alloxan treatment

At the end of the experimental period, all rats were sacrificed the next day after an overnight fast by decapitation. Blood was collected and sera were separated by centrifugation at 3000 rpm for 10 min. The serum was stored at -80°C after separation until it was assayed as described below.

Biochemical measurements: The serum glucose and serum insulin levels were estimated using kits from bio-Merieux, France. The concentrations of cholesterol, triglycerides, High-Density Lipoprotein (HDL) cholesterol, Low-Density Lipoprotein (LDL) cholesterol and Very Low-Density Lipoprotein (VLDL) cholesterol in serum were estimated using standard enzymatic kits (ERBA diagnostic Mannheim GMBH, Germany) spectrometrically. The glycogen content in the liver was determined by the anthrone method (Carroll *et al*., 1956). Lipid peroxidation in the liver was estimated by measuring the formation of the Thiobarbituric Acid Reactive Substances (TBARS) (Ohkawa *et al*., 1979). The glutathione (GSH) content was determined spectrophotometrically at 412 nm using 5,5-dithiobis-2-nitobenzoic acid (Ellman, 1959). The activity of Catalase (CAT) was assayed as suggested by Bock *et al*. (1980), based on the rate of H_2O_2 degradation by the action of catalase contained in the examined samples.

Statistical analysis: The experimental results were expressed as Mean±MSE, Statistical comparison was done using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) when more than two groups were involved. The p-values<0.05 were considered significant.

RESULTS

Body weight changes: The results obtained in the present study showed a drastic decrease in body weight in diabetic rats (Group 2) by -12.5%, $p<0.05$, compared to normal control rats (Group 1). This loss in body weight was compensated after *Cynara cornigera* extract administration (Group 3) (+4.2%, $p<0.05$) and with glibenclamide treatment (Group 4) (+6.2%, $p<0.05$), compared to diabetic rats (Table 1).

Antidiabetic effect of cynara cornigera

Blood glucose level: The results of blood glucose levels in normal, alloxan induced diabetic rats, extract treated diabetic rats and glibenclamide treated diabetic rats were shown in Fig. 1. There was a significant ($p<0.05$) increase in blood glucose levels in alloxan induced diabetic rats (Group 2) when compared with normal rats (Group 1). Administration of aqueous extract of roots *Cynara cornigera* (Group 3) and glibenclamide (Group 4) to diabetic rats resulted in a significant decrease in blood glucose level. The *Cynara cornigera* extract and glibenclamide treated groups remained to have higher serum glucose level throughout the experimental period when compared with normal rats.

Serum insulin: Serum insulin levels decreased significantly in diabetic groups when compared to normal control groups. After treatment with aqueous extract of roots of *Cynara cornigera* and glibenclamide the insulin levels increased to near normal (Fig. 2).

Liver glycogen: Figure 3 shows the content of liver glycogen in liver tissues of normal and experimental animals. There was a significant ($p< 0.05$) low content of glycogen during diabetes when compared to the normal control group. Administration of aqueous extract of roots of *Cynara cornigera* and glibenclamide tend to bring the level to near normal which has been indicated by the higher levels of hepatic glycogen in the drug treated diabetic animals.

***In vivo* antioxidant activities of *Cynara cornigera*:** Table 2 shows the effect of administration of aqueous extract of roots of *Cynara cornigera* on TBARS, GSH and CAT in liver tissue of different groups of rats. There

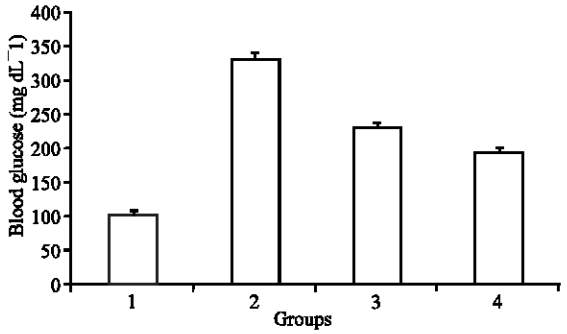

Fig. 1: Effects of *Cynara cornigera* and glibenclamide treatments on blood glucose level in alloxan-induced diabetic rats. All the values are Means±SEM of eight individual observations. *Significance difference from Group I at $p<0.05$ and **Significance difference from Group II at $p<0.05$. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic +*Cynara cornigera* treated rats and Group 4, diabetic+ Glibenclamide treated rats

Table 1: Effects of *Cynara cornigera* and glibenclamide treatment on body weight changes alloxan-induced diabetic rats

	Body weight (mg) (% increase or decrease from the initial weight)	
Groups	0th day	30th day
Group 1	192.32±3.65	209.36±4.90 (+8.9%)
Group 2	193.20±7.90	169.06±5.40 (-12.5%)*
Group 3	191.82±9.30	199.85±8.31 (+4.2%)**
Group 4	190.47±4.21	202.30±6.49 (+6.2%)**

All the values are Means±SEM of eight individual observations. *Significance difference from Group I at $p<0.05$, **Significance difference from Group II at $p<0.05$. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic+ *Cynara cornigera* treated rats and Group 4, diabetic +Glibenclamide treated rats

Table 2: Changes in the levels of GSH and TBARS and the activity of CAT in the liver extracts in alloxan-induced diabetic rats treated with *Cynara cornigera* and glibenclamide

Groups	TBARS (μmol g^{-1} tissue)	GSH (μmol g tissue)	CAT (μmol of H2O2 utilized/min/mg protein)
Group 1	43.22±1.35	4.58±0.21	58.61±2.91
Group 2	90.00±3.21*	1.86±0.25*	26.29±3.48*
Group 3	59.98±2.09**	3.43±0.22**	42.16±3.82**
Group 4	48.51±1.43**	3.74±0.16**	48.50±2.24**

All the values are Means±SEM of eight individual observations. *Significance difference from Group I at $p<0.05$, **Significance difference from Group II at $p<0.05$. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic+ *Cynara cornigera* treated rats and Group 4, diabetic +Glibenclamide treated rats. TBARS: Thiobarbituric acid reactive substance; GSH: Reduced glutathione; CAT: Catalase

Table 3: Serum lipid and lipoprotein profiles in alloxan-induced diabetic rats treated with *Cynara cornigera* and glibenclamide

Groups	TC (mg dL^{-1})	TG (mg dL^{-1})	VLDL-c (mg dL^{-1})	LDL-c (mg dL^{-1})	HDL-c (mg dL^{-1})
Group 1	71.05±1.23	62.15±1.41	12.31±0.84	20.78±0.97	48.57±0.85
Group 2	209.38±2.67*	186.32±2.16*	36.58±0.92*	103.94±1.68*	20.12±0.97*
Group 3	99.50±1.75**	93.46±1.11**	20.74±0.89**	40.69±0.96**	41.25±0.90**
Group 4	87.30±1.62**	70.12±1.39**	14.26±0.92**	24.72±0.96**	43.47±0.89**

All the values are Means±SEM of eight individual observations. *Significance difference from Group I at p<0.05, **Significance difference from Group II at p<0.05. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic+ *Cynara cornigera* treated rats and Group 4, diabetic+ Glibenclamide treated rats. TC: Total cholesterol; TG: Triglycerides; VLDL-c: Very low density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol

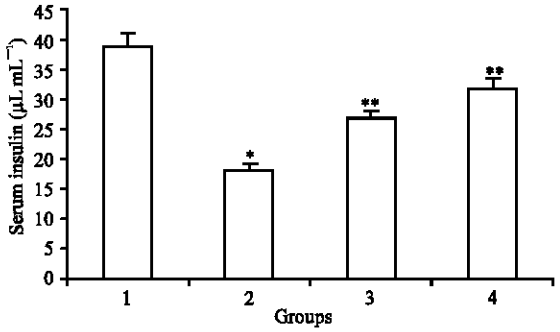

Fig. 2: Effects of *Cynara cornigera* and glibenclamide treatments on serum insulin level in alloxan-induced diabetic rats. All the values are Means±SEM of eight individual observations. *Significance difference from Group I at p<0.05 and **Significance difference from Group II at p<0.05. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic+ *Cynara Cornigera* treated rats and Group 4, diabetic+ Glibenclamide treated rats

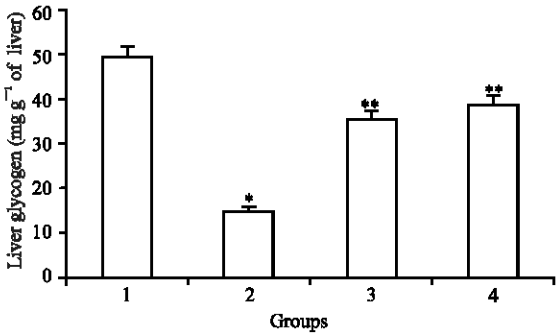

Fig. 3: Effects of *Cynara cornigera* and glibenclamide treatments on liver glycogen content in alloxan-induced diabetic rats. All the values are Means±SEM of eight individual observations. *Significance difference from Group I at p<0.05 and **Significance difference from Group II at p<0.05. Group 1, normal control rats, Group 2, diabetic control rats, Group 3, diabetic+ *Cynara Cornigera* treated rats and Group 4, diabetic+ Glibenclamide treated rats

was a significant (p< 0.05) elevation in tissue TBARS in diabetic rats (Group 2) as compared to normal rats (Group 1). Treatment with *Cynara cornigera* extract (Group 3) for 30 days resulted in a significant decrease in liver tissue TBARS as compared to diabetic rats. GSH content and CAT activity in diabetic control rats were significantly (p<0.05) decreased in liver tissue when compared with normal rats. *Cynara cornigera* extract treatment significantly restored GSH content and CAT activity as compared with diabetic control.

Antihyperlipidemic effect of *Cynara cornigera*: Alloxan treatment (Group 2) resulted in a significant (p<0.05) elevation of TG, TC, VLDL-C, LDL-C and reduction of HDL-C levels as compared to the normal control rats (Group 1). The administration of *Cynara cornigera* extract (Group 3) and glibenclamide (Group 4) to diabetic rats resulted in a significant reduction in elevated TG, TC, VLDL-c, LDL-c and HDL-c level was restored respectively when compared to diabetic control (Table 3).

DISCUSSION

The antidiabetic, antihyperlipidemic and antioxidant activities of the aqueous extract of roots of *Cynara cornigera* were examined in this study. In agreement with the obtained results in this study, Shirwaikar *et al.* (2004), stated that induction of diabetes in rats resulted in characteristic loss of body weight. They attributed that to increased muscle wasting and loss of tissue proteins. The extract treated diabetic rats showed significant recovery in body weight gain. This may be due to controlling muscle wasting and improvement in insulin secretion as well as glycaemic control by the extracts. The increased content of hepatic glycogen in extract treated experimental animals could be contributed to the decreased endogenous glucose output from the liver. These changes were accompanied by a significant decrease in lipid peroxidation in the liver. These results are in accordance with those of other investigations using different *Cynara* species (Nazni *et al.*, 2006). The active component of the hypoglycemic medications that derived

from plant sources can act by many different mechanisms. Some groups of this kind of hypoglycemic medications act by increase activity of pancreatic β cells which include cells regeneration, insulin synthesis and release (Hsu *et al*., 2009). Other groups can act by increase cells sensitivity to insulin or by increase the inhibitory effects against insulinase (Lee *et al.,* 2009; Alonso-Castro *et al*., 2010). Others have suggested that the mechanisms may involve improved glucose homeostasis (Ahmad *et al*., 2000), increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen (Yuan *et al*., 1998) and or decrease of glycogenolysis acting on enzymes (El-Missiry and El-Gendy, 2000). Nicola *et al.* (1996) and Munari *et al.* (1998) disenibed the hypoglycemic effect of phytochemicals to inhibition of intestinal glucose absorption and reduction of glycaemic index of carbohydrates, respectively. Another possible mechanism of action is reduction of the effect of glutathione (Raza *et al*., 1996). The data obtained from this study was in agreement with these previous studies as the presence of the active components from the aqueous extract of roots of *Cynara cornigera* may be responsible for their antidiabetic effect due to one of the suggested mechanisms reported above.

Baynes (1991), reported that hyperglycaemia develops after alloxan administration and stimulates the production of advanced glycosylated end-products, enhances the polyol pathway and activates protein kinase C. These conditions might lead to increased generation of Reactive Oxygen Species (ROS), such as superoxide anion (O^-) (Nishikawa *et al*., 2000) which rapidly reacts with NO leading to the formation of ^-ONOO which is highly oxidant and capable of damaging several biological molecules (Chiueh, 1999). The peroxidation of polyunsaturated fatty acids with the concomitant formation of aldehydic products is an indicator that ^-OH has been formed (Bromme *et al*., 2000). The present study was in agreement with these previous studies as alloxan administration elevates TBARS, indicating an increased oxidative stress due to over production of ROS. Also a significant decline of endogenous antioxidants, including GSH content, SOD and CAT activities in liver (Cheeseman, 1994; Maritim *et al*., 2003). It is accepted that membrane peroxidation alters the membrane fluidity and permeability and leads to a loss of membrane integrity (El-Missiry *et al*., 2004). This in turn might contribute to the disruption of the intracellular and membrane redox state of hepatocytes, hence disrupting glucose and lipid metabolism. The improvement recorded by aqueous extract of roots of *Cynara cornigera* administered after alloxan treatment might suggest a potential protective effect against alloxan action.

Diabetes is associated with hyperlipidemia (Maiti *et al*., 2005). It is well documented that there is elevation of serum lipid concentration in diabetics (Chase and Glasgow, 1976) and such an elevation represents the risk of Coronary Heart Disease (CHD) (Prince *et al*., 1999; Maiti *et al*., 2005). Lowering of serum lipids concentration through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease (Rhoads *et al*., 1976; Sharma *et al*., 2003). The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipemia that characterised the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony *et al*., 1994; Pari and Saravanan, 2002).

In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides were decreased significantly by the treatment of *Cynara cornigera* extract as well as glibenclamide in alloxan diabetic rats showing their beneficial effects. *Cynara cornigera* extract also has therapeutic effect on hypertriglyceridemia and hypercholesterolemia that associated with diabetes. This effect can be explained by the ability of *Cynara cornigera* to act as insulin secretagogue and may also due to presence of hypocholesterolemic compounds. These compounds act as inhibitors to some enzymes such as hydroxy methylglutaryl CoA reductase which has a role in cholesterol synthesis. The decreased concentration of cholesterol could be due to reduction of its absorption from intestine by phytochemicals. These phytochemicals may also have a role in regulation of blood lipid by increasing the activity of lecithin cholesterol acyl transferase (Sharma *et al*., 2003).

Our data were in line with notion as the alloxan treated diabetic rats exhibited a significant elevation of serum LDL-c, VLDL-c and significant reduction of HDL-c levels. Treatment with *Cynara cornigera* extract for 30 days was sufficient to produce a significant reduction in the LDL-c, VLDL-c and significant increase in HDL-c levels in diabetic rats. In IDDM patients, the HDL-c levels correlate with lipoprotein lipase (LPL) levels (Nikkila *et al*., 1977). Also increased LDL-c may arise from glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the LDL receptor and hence, decreased metabolism (Golay *et al*., 1986). Hence it is

evident that the plant extract may be helpful in controlling the metabolism of certain lipoproteins which results in significant attenuation of serum HDL-c and LDL-c towards normal levels thereby supporting the hypolipidimic effect of the extracts. The deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. Bruan and Severson (1992) and Lopes-Virella *et al.* (1983) reported that treatment of diabetes with insulin served to lower plasma triglyceride levels by returning lipoprotein lipase levels to normal. Administration of plant extract to alloxan-induced diabetic rats improved the serum triglyceride levels suggesting its insulin like activity.

The synthetic oral hypoglycaemic agents can produce a series of side effects including haematological, gastro-intestinal reactions, hypoglycaemic coma and disturbances of liver and kidney. In addition, they are not suitable for use during pregnancy (Larner, 1985).

CONCLUSION

The data obtained from the present study can support that *Cynara cornigera* is a herbal drug that has strong antidiabetic, hypolipidaemic effects and antioxidant activity. This could be due to different types of active principles, each with a single or a diverse range of biological activities. Hence, *Cynara cornigera* proves to be a safer antidiabetic agent and might help in preventing diabetic complications. It can serve as a good adjuvant in the present armamentarium of antidiabetic drugs. Further biochemical and pharmacological investigations are still needed for the evaluation of the active principle(s) of the *Cynara cornigera* extract.

ACKNOWLEDGMENTS

The author particularly wishes to thank the traditional practitioner who helped to collect the plant material. Author also thank the botanists of the Botany department of the college of Science, University of Garyounis for the botanical determination of the plant.

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