Evaluation of hypoglycaemic and hypolipidemic activities of Globularin isolated from *Globularia alypum* L. in normal and streptozotocin-induced diabetic rats

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ABSTRACT

The present study investigates the effect of intraperitoneal administration of Globularin on blood glucose levels in normal and streptozotocin diabetic rats. Globularin was an iridoid glucoside which was isolated from the leaves of *Globularia alypum* (3.4 %). This compound   was identified by means of physical constants and spectral data UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMBC. The acute toxicity test demonstrated that Globularin is not lethal up to dose of 1000 mg/kg body weight after intraperitoneal injection. In normal and streptozotocin diabetic rats, single intraperitoneal administration of Globularin at a dose 100 mg/kg body weight produced significant decrease of blood glucose levels. However, in prolonged treatment study, the repeated intraperitoneal administration of Globularin (2 x 100 mg/kg body weight) decreased significantly the blood glucose levels when compared to   the diabetic control rats. In addition, daily injection of Globularin (2 x 100 mg/kg body weight) reduced significantly serum levels of total cholesterol (varied from 0.53 to 0.41 g/L) and triglycerides (varied from 1.71 to 0.79 g/L) in the diabetic rats.

INTRODUCTION

Diabetes mellitus is the root cause of several chronic and progressive diseases that adversely affect a number of organs including the nervous and vascular systems. Diabetes is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 2000 of 150 million people, and 220 million people in 2010 (Zimmet *et al.,* 2001).The diabetes population is likely to increase to 300 million or more by the year of 2025 (British Medical Association, 2004; Tielmans *et al*., 2007). The classes of drugs currently available include insulin and insulin analogues, sulfonyl ureas, glinides, biguanides, thiazolidinediones and α-glucosidase inhibitors. However most of drugs can cause problems including compliance, hypoglycaemia and obesity (Seltzer, 1989; O' Moore-Sullivam and Prins, 2002).

Many papers indicated that natural products play a highly significant role in drug discovery (Newman, 2003). *Globularia alypum* L. is a medicinal plant with approved hypoglycaemic effect (Skim *et al.,* 1999).

Globularia alypum is a wild plant belonging to *Globulariaceae* family. It is a perennial shrub which is found throughout the Mediterranean area. The plant is known for a variety of purposes (Sezik *et al.,* 1991; Jouad *et al.,* 2001).

In the Algerian traditional pharmacopoeia, *Globularia alypum* locally named "Ain Larnab" is one of the most traditional plant remedies. Its leaves are traditionally used as hypoglycaemic agent, laxative, cholagogue, stomachic, purgative and sudorific (Allali *et al.,* 2008; Baba Aissa, 1999). It also used in the treatment of cardiovascular and renal diseases as demonstrated by ethnobotanical surveys, which showed that *Globularia alypum* is one of the most used medicinal plant in Morocco (Jouad *et al.,* 2001; Bellakhdar *et al.,* 1991).

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The infusion of *Globularia alypum* exhibiting no toxicological effects was thus shown to produce a significant hypoglycaemic activity in rats both by oral and intraperitoneal administration (Skim *et al.,* 1998; Skim *et al.,* 1999).

A significant antileukemic activity of an aqueous extract of leaves of *Globularia alypum* was also reported (Caldes *et al.,* 1975). Methanol and dichloromethane extracts of *Globularia alypum* were also shown to reduce histamine and serotonin contraction in *vitro* (Bello *et al.,* 2002*)*. The methanolic extract of *Globularia alypum* decreases hyperglycaemia in streptozotocin – induced diabetic rats (Zennaki *et al.,* 2009). However, different extracts of *Globularia alypum* were significant source of compounds with antioxidant, antigenotoxic and anti-tuberculosis activities (Khlifi *et al.,* 2005; Harzallah *et al.,* 2010; Khlifi *et al.,* 2011).

In addition, *Globularia alypum* L. was shown to exert an anti-ulcer activity against the gastric mucosal damages caused by indomethacin that the mechanism of action may result from an inhibition of intraepithelial lymphocytes migration (Fehri and Aiache, 2010). Recently, the *Globularia alypum* aqueous extract has a beneficial effect on plasma triglycerides and gives a promising perspective for hypertriglyceridemia treatment. Moreover, in rats (muscle and kidney) fed a high – fructose diet, *Globularia alypum* is effective by lowering lipid peroxidation and improves antioxidant enzymes (Taleb – Dida *et al.,* 2011).

The wide use of this plant for the treatment of many diseases in addition to the fact that only few studies are reported on the Algerian *Globularia alypum* strain (Boutiti *et al.,* 2008; Zennaki *et al.,* 2009, Taleb – Dida *et al.,* 2011), prompted us to study the hypoglycaemic activity of its major compound. So far, the most important chemical investigations of *Globularia alypum* are those of Chaudhuri and Sticher (1981), Ben Hassine et al. (1982), Es Safi et al. (2005; 2006; 2007) and Boutiti et al. (2008), where the presence of some glycosidic iridoids, phenolic acids, flavonoids and a lignan diglucoside was reported.

In the present work, we report the isolation, the structure elucidation of Globularin, which was the major compound of the leaves of *Globularia alypum.* Because there is no report on hypoglycaemic and hypolipidemic effects of Globularin, we were interested to the evaluation of these effects of this compound.

MATERIALS AND METHODS

Plant material

Globularia alypum wa**s** collected from Achba – Tlemcen, Algeria, during the fructification period of the specie, in summer (July). Botanical identification of plant was conducted by Prof. Noury BENABADJI and a voucher specimen of the plant was deposited in the Herbarium of the Laboratory of Botany, Department of Biology, University of Tlemcen, Tlemcen, Algeria.

Extraction and isolation

The fresh leaves of plant were air – dried in shade at room temperature and powdered. From the obtained powder, 100 g were extracted with 3 x 400 mL of mixture of distilled water – acetone (40%–60%) under reflux for 3 x 9 h.

The residue was filtered and then the acetone   was evaporated under vacuum. The aqueous phase was successively extracted with hexane (3 x 400 mL), diethyl ether (3 x 400 mL) and ethyl acetate (3 x 400 mL). The solvents were evaporated to dryness in vacuum.

Ethyl acetate extract (5.70 g) was applied to a column of silica gel 60 and eluted with a gradient of ethyl acetate – methanol with increasing polarity to give several fractions. One of these fractions (3.4 g) corresponds to Globularin (Yield (%) of isolation of Globularin with respect to the dry matter: 3.4 %).

General experimental procedures

Silica gel was used for column chromatography. Thin layer chromatography (TLC) was conducted on pre-coated commercial silica gel plates (Merck, 60 F_{254}) with ethyl acetate – methanol (90:10) as a developing solvent system. Globularin was detected by UV fluorescence and spraying with vanillin H_2SO_4, followed by heating at 100 °C for 10 min.

A melting point was determined on apparatus: Wagner Emunz Nr 6666, Heizbank, System Kofler Type WME. Optical rotation was measured on a Schmdt Haensch 22955 Polarimeter. A UV spectrum was recorded using a Perkin Elmer Lambda 5 Spectrophotometer. An IR spectrum was measured on Genesis M Ftir–Mattson, FT–IR Spectrometer, in KBr pellet. A MS spectrum was recorded with an AEI MS-50 spectrometer.
NMR measurements (^{1}H, ^{13}C, DEPT, COSY and HMBC) in CD_3OD at room temperature were performed on a Bruker AC 400 Spectrometer operating at 400 and 100.61 MHz for ^{1}H and ^{13}C respectively.   Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard.

Hypoglycaemic activity

Animals used

The present tests were achieved on female and male adult wistar rats "*Rattus norvegicus*" (150–320 g). All the animals were housed in an air – conditioned animal room at 25°C and fed with standard pellet diet and water ad libitum.
They were divided into eleven groups as follows:

- Group I (n = 10 rats) : for the evaluation of the toxicity dose of Globularin;
- Group II, III, IV, V, VI, VII (n= 30 rats): for the evaluation of the effect of Globularin in short term treatment of normal and diabetic rats (8 h);
- Group VIII, IX, X, XI (n= 32 rats): for the evaluation of the effect of Globularin in prolonged treatment of diabetic rats (7 days).

Toxicity evaluation in rats

Globularin was tested for its acute toxicity in overnight fasted female rats (group I). It was injected intraperitoneally at different doses: 300, 600, 800 and 1000 mg/kg body weight, to the rats (two female were used for each dose), control rats received

saline solution. Mortality and general behaviour of animals were observed periodically for 48 h. Animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration. The observation of rats was continued until the 14th days (Twaij *et al.,* 1983).

Streptozotocin – induced diabetic rats

Diabetes was induced in overnight fasted male rats by single intraperitoneal injection of streptozotocin (60 mg/kg body weight), which was dissolved in citrate buffer (5mL/kg body weight; pH 4.5) immediately before administration. Vehicle injected animals acted as control. The rats with fasting blood glucose concentration of over 3 g/L at 72 h after streptozotocin injection were considered to be diabetic and were used for the experiment.

Collection of blood samples

Blood samples were collected from the tip of the tail for glycaemia measuring and from the retro orbital sinus for biochemical parameters measuring. In all study, blood glucose concentrations were determined at the defined time patterns, by means of Accu-Chek glucometer and blood glucose test strips based on the glucose oxydase method (Teixeira *et al.,* 1990).

Experimental bioassays
Evaluation of the effect of Globularin in short term treatment of normal and diabetic rats

Male rats were fasted for 16 h, but allowed free access to water, the body weight was determined before the start of the experiment. The rats were divided randomly into six groups (five rats per group) including three normoglycaemic groups (II, III and IV) and three diabetics groups (V, VI and VII), and treated by one intraperitoneal injection in the following manner : groups II and V served as controls, receiving isotonic saline solution ISS (5mL/kg body weight), groups III and VI, received glibenclamide (0.6 mg/kg body weight) and groups IV and VII, received Globularin (100 mg/kg body weight).

Blood samples were collected from the tip of tail 0 h (just before drug administration) and 1, 2, 3, 4, 6, 8 h (after drug administration). The percentage change in glycaemia was calculated by applying the following formula (Gharaibech *et al.,* 1988):

Percentage of blood glucose level reduction = $[(G_0 - G_t)/G_0] \times 100$
G_0 : glucose concentration at $t_0 = 0$.
G_t : glucose concentration at t.

Evaluation of the effect of Globularin in prolonged treatment of diabetic rats

The effect of Globularin was also tested for a prolonged treatment. The rats were divided into four groups of eight rats each. The rats of group VIII (normal rats) and group IX (diabetic rats) were used as controls, receiving ISS (5 mL/kg, twice a day). The diabetic rats of group X received Glibenclamide (0.6 mg/kg

body weight; twice a day). The rats of group XI (diabetic rats) received Globularin which was injected at dose of 100 mg/kg body weight, twice a day. The administration of Globularin was continued for 7 days. Blood samples were collected from the tip of the tail of non-fasted animals at days 1, 3, 5 and 7 just after the first injection. The body weight of animals was also determined at the same days. On the day eight, after overnight starvation, serum glucose, total cholesterol and triglycerides were measured.

Estimation of biochemical parameters

Serum glucose was estimated spectrophotometrically by Trinder method (1969) using a commercial assay kit (Quimica Clinica Aplicada S.A). Serum cholesterol was estimated by the method of Fasce and Vanderlinde (1972). Triglycerides in the serum was estimated by Fossati and Prencipe method (1982) using a kit (Quimica Clinica Aplicada S.A).

Statistical analysis

The results are expressed as mean ± standard error of mean (S.E.M.), the significant of various treatments was calculated using student's t – test and were considered statistically significant when $P < 0.05$ (Snedecor, 1967).

RESULTS

Isolation and characterization of Globularin

The ethyl acetate extract prepared from leaves of *Globularia alypum* was subjected to column chromatography to give Globularin in pure form.
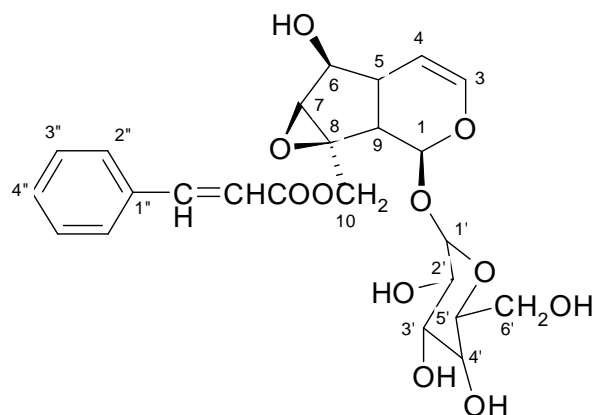

Fig. Isolated iridoid glucoside: Globularin.

Globularin was identified by means of physical constants, spectral data (UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMBC) and comparison with spectral data of literature (Di Maio and Panizzi, 1966; Chaudhuri and Sicher, 1981; Faure *et al.,* 1987).

Globularin ($C_{24}H_{28}O_{11}$) was obtained as an amorphous powder: R_f value (Ethyl acetate – MeOH : 90-10) = 0.44; m.p.= 115 -117°C; $[\alpha]_D^{25}$ (c= 0.8, MeOH) = - 63.5°. The UV spectrum (MeOH) exhibited maxima (λ_{max}) at 279.55 nm. The FTIR

spectrum showed absorption bands at 3401.14 (br OH), 2888.12 - 2919.90 (C-H), 1701.41 (C=O ester), 1636.44 (C=C–O) and 1508 cm^{-1} (aromatic ring). Its molecular weight was concluded to be 492 in agreement with a $C_{24}H_{28}O_{11}$, as confirmed by EIMS analysis, which showed different fragmentations at m/z: 131(100.00 %), 28 (40.06 %), 103(35.80 %), 148 (28.02 %),182 (7.38 %), 330 (4.48 %), 492 (5.80 %). The remaining resonances in the NMR spectra were consistent with of Globularin. The complete assignment of the proton and carbon spectra of Globularin is presented in Table 1. ^{13}C multiplicities were determined by DEPT method; the interpretation of the spectra was achieved comparison of heteronuclear ^{13}C - ^{1}H chemical shift correlation (HMBC) and ^{1}H homonuclear correlation (COSY).

Hypoglycaemic activity of Globularin
Toxicity evaluation in rats

In the acute toxicity study, Globularin did not show any mortality up to a dose of 1000 mg/kg body weight in rats. Even at this high dose there was no gross behavioural change. After drugs administration, the parameters observed specially in the first hours were grooming, sedation and sometimes contractions. During the rest of observation, no sign was mentioned, the rats were in good health until the fourteen day. The acute toxicity test demonstrated that Globularin is not lethal up to dose of 1000 mg/kg body weight after intraperitoneal injection. After determination of the lethal dose, we have tested the hypoglycaemic activity of Globularin at short (8 hours) and prolonged (7 days) times.

Effect of streptozotocin on body weight and blood levels on the third day after diabetes induction

The administration of streptozotocin at 60 mg/kg body weight to the fasted rats markedly decreased the body weight and increased blood glucose levels as compared with control rats on the third day after diabetes induction (Table 2).

Evaluation of the effect of Globularin in short term treatment of normal and diabetic rats

The changes of blood glucose levels in normal and streptozotocin diabetic rats at various time intervals after single intraperitoneal administration of Globularin (100 mg/kg) are shown in Table 3.

In normal rats, Globularin at a dose 100 mg/kg produced a significant decrease of glycaemia at 2 h when compared with the basal value ($p < 0.05$), this effect was persisted for one hour. However, blood glucose remained at very low level at 8 hours ($P < 0.01$). Glibenclamide at a dose 0.6 mg/kg also produced a significant decrease in the blood levels from 2 h ($P < 0.05$, $P < 0.01$). In streptozotocin – diabetic rats, the blood glucose levels of untreated rats increased significantly 4 h after single intraperitoneal administration, due to the aggravation of diabetic state in absence of any hypoglycaemic treatment. Globularin reduced significantly the blood glucose levels at 3 h ($P < 0.05$), 6 h ($P < 0.05$) and 8 h ($P < 0.05$), while Glibenclamide at a dose 0.6 mg/kg did not show any effects on hyperglycaemia.

Table. 1: ^{1}H and ^{13}C NMR Spectroscopic Data and HMBC Correlations for Globularin in CD$_3$OD[a].

C/H	δ$_C$ (ppm)	DEPT	δ$_H$ (ppm)	Correlation ^{1}H-^{1}H (COSY)	^{1}H-^{1}H coupling constant J (Hz)	^{13}C-^{1}H correlation (HMBC)
1	94.08	CH	5.01(d)	H-9	9.8	H-1', H-3, H-9
3	140.32	CH	6.31(m)	H-4, H-5	6.0, 1.9	H-1, H-5
4	102.20	CH	5.05(dd)	H-3, H-5	6.0, 4.4	H-3, H-5, H-6
5	37.51	CH	2.29(m)	H-3, H-4, H-6, H-9	1.9, 4.4, 7.9, 7.7	H-3, H-4, H-6, H-7
6	77.95	CH	3.89(d)	H-5, H-7	7.9, 1.2	H-5, H-7, H-9
7	61.51	CH	3.46(s)	H-4	1.2	H-9, H$_2$-10
8	62.03	C				H-7, H-9, H$_2$-10
9	42.08	CH	2.66(dd)	H-1, H-5	9.8, 7.7	H-1, H-1', H-4, H-5, H-6, H$_2$-10
10	63.02	CH$_2$	5.00(d)	H-10b	12.7	H-9
			4.24(d)	H-10a	12.7	
1'	98.76	CH	4.71(d)	H-2'	7.7	H-1, H-2'
2'	73.29	CH	3.17(dd)	H-1', H-3'	7.7, 8.8	H-3'
3'	76.31	CH	3.27(t)	H-2'	8.8	H-2'
4'	69.95	CH	3.24[b]			H-2', H-3', H-5', H$_2$-6'
5'	76.96	CH	3.26[b]	H-6'a, H-6'b	1.7, 5.9	H-1', H-2', H-3', H-4'
6'	61.28	CH$_2$	3.89(d)	H-5', H-6'b	1.7, 11.9	
			3.65(dd)	H-5', H-6'a	5.9, 11.9	
1"	134.22	C				
2"	128.50	CH	7.35(m)			H-α, H-3", H-4"
3"	127.85	CH	7.55 (m)			H-β
4"	130.06	CH	7.37 (m)			H-2", H-6"
5"	127.85	CH	7.55(m)			H-β
6"	128.50	CH	7.35(m)			H-α, H-4", H-5"
α	117.12	CH	6.55(d)	H-β	16.0	H-β
β	145.16	CH	7.71(d)	H-α	16.0	H-α, H-2", H-6"
C=O	166.89	C				H-α, H-β, H$_2$-10

[a] All proton and carbon assignments are based on 2D NMR (COSY, HMBC). [b] Signal patterns are unclear due to overlapping.

Table. 2: Effect of streptozotocin on body weight and blood glucose levels on the third day after diabetes induction.

Treatment		Body weight (g)	Blood glucose (g/L)
Control	t = 0	301.17±10.13	0.74±0.01
(citrate buffer 5mL/kg; n = 11)	t = 72 h	308.57±11.12	0.92±0.10
Streptozotocin (60 mg/kg; n = 11)	t = 72 h	289.25±7.27**	3.59±0.10***

Values are expressed as mean ± SEM.
Statistical significance: ** $P < 0.01$; *** $P < 0.001$ (compared to control rats on the third day after diabetes induction).

Table. 3: Effect of Globularin in short term treatment on blood glucose levels (g/L) after intraperitoneal administration to normoglycaemic and streptozotocin - diabetic rats.

Group	Treatment	Time (h)						
		0	1	2	3	4	6	8
II (n=5)	Normal control (ISS)	0.96±0.06	1.15±0.06	1.04±0.05	0.93±0.07	0.86±0.05	0.85±0.05	0.75±0.08
III (n=5)	Normal+ Glibenclamide (0.6 mg/kg)	0.92±0.02	0.91±0.03	0.79±0.06* (14.13 %)[a]	0.75±0.04** (18.48 %)[a]	0.67±0.02** (27.17 %)[a]	0.70±0.05** (23.91 %)[a]	0.70±0.02** (23.91 %)[a]
IV (n=5)	Normal + Globularin (100 mg/kg)	0.96±0.06	1.01±0.05	0.86±0.06* (10.42 %)[a]	0.86±0.05* (10.42 %)[a]	0.90±0.06	0.76±0.04** (20.83 %)[a]	0.71±0.05** (26.04 %)[a]
V (n=5)	Diabetic control (ISS)	4.19±0.27	4.21±0.28	4.08±0.14	3.92±0.03	4.24±0.15	3.53±0.15	3.71±0.16
VI (n=5)	Diabetic +Glibenclamide (0.6 mg /kg)	3.21±0.20	3.65±0.14	3.81±0.19	3.58±0.17	3.49±0.20	3.52±0.19	3.39±0.10
VII (n=5)	Diabetic + Globularin (100 mg/kg)	3.81±0.12	4.01±0.12	3.63±0.13	3.43±0.11* (9.97 %)[a]	3.54±0.08	3.38±0.16* (11.29 %)[a]	3.44±0.16* (9.71 %)[a]

Values are expressed as mean ± SEM.
Statistical significance: ** $p < 0.01$;* $p < 0.05$, when compared with t=0.
[a] percentage of blood glucose level reduction.

Table. 4: Effect of Globularin in prolonged treatment on blood glucose levels (g/L) in streptozotocin - diabetic rats.

Group	Treatment	Time (days)			
		1	3	5	7
VIII (n = 8)	Normal control (ISS)	1.36±0.03	1.30±0.06	1.30±0.02	1.38±0.05
IX (n = 8)	Diabetic control (ISS)	5.04±0.25	5.53±0.20	5.52±0.22	5.84±0.09
X (n = 8)	Diabetics + Glibenclamide (2 x 0.6 mg/kg)	5.23±0.27	5.73±0.12	5.30±0.28**	5.01±0.19**
XI (n = 8)	Diabetic +Globularin (2 x 100 mg/kg)	4.76±0.31**	5.57±0.20	5.11±0.34**	5.17±0.20**

Values are expressed as mean ± SEM.
Statistical significance: ** $p < 0.01$, when compared to diabetic control

Table. 5: Effect of Globularin on serum glucose, total cholesterol and triglycerides levels in streptozotocin – induced diabetic rats.

Group	Treatment	Biochemical parameters (g/L)		
		Serum glucose	Total cholesterol	Triglycerides
VIII (n = 8)	Normal control (ISS)	1.03±0.07	0.39±0.02	0.70±0.12
IX (n = 8)	Diabetic control (ISS)	4.23±0.27	0.53±0.04**[a]	1.71±0.20**[a]
X (n = 8)	Diabetics + Glibenclamide (2 x 0.6 mg/kg)	4.79±0.11	0.51±0.03*[b]	0.95±0.23*[b]
XI (n = 8)	Diabetic + Globularin (2 x 100 mg/kg)	4.22±0.36	0.41±0.02**[b]	0.79±0.07**[b]

Values are expressed as mean ± SEM.
Statistical significance: ** $p < 0.01$; * $p < 0.05$, [a] when compared to normal control, [b] when compared to diabetic control.

Evaluation of the effect of Globularin in prolonged treatment of diabetic rats

The effect of Globularin was also tested for a prolonged treatment (7 days) on streptozotocin – induced diabetic rats. The results of repeated intraperitoneal administration of Globularin and Glibenclamide in diabetic rats are shown in Table 4.

In untreated diabetic rats, the blood glucose levels increased from 5.04 to 5.84 g/L. In treated diabetic rat, Globularin at a dose 2 x 100 mg/kg decreased significantly the blood glucose levels at the first, fifth and seventh days (P < 0.01) when compared to the diabetic control rats (untreated). In addition, for the Glibenclamide at a dose 2 x 0.6 mg/kg, the decrease in blood glucose levels was noted from the fifth day of treatment (P < 0.01). In normal rats, it was observed no significant variation of the glycaemia (1.30 - 1.38 g/L), while significant differences in blood glucose levels were observed between the untreated normal and diabetic Globularin treated groups.

The effect of Glibenclamide is comparable to that of Globularin. After 7 days of treatment, diabetes mellitus persists. Globularin possess an effect on decrease of blood glucose level of diabetic rats in comparison with the diabetic control rats, but it did not exhibit significant anti- hyperglycaemic activity in streptozotocin – induced hyperglycaemic rats (glycaemia: 4.76 – 5.57 g/L) when compared to the normal rats.

In addition body weight was progressively reducing, and amount of water and food intake were significantly increased (data not shown). However, in the untreated diabetic rats, serum levels of total cholesterol and triglycerides were significantly increased when compared to the normal rats. These complications of diabetes were attenuated with the administration of Globularin. The effects of the standard drug (Glibenclamide) on serum levels of total cholesterol and triglycerides in the diabetic rats were less significant than those of the Globularin (Table 5).

Discussion

Previous investigations of patients suffering from diabetes, have demonstrated that *Globularia alypum* represented the second highest percentage of medicinal plant utilization (9.9 %) after *Trigonella foenum graecum* (13.3 %) (Ziyyat et al, 1997). The extract of the leaves of *Globularia alypum* possess a significant hypoglycaemic effect in normoglycaemic and hyperglycaemic rats after both oral and intraperitoneal administration (Skim *et al.,* 1999; Jouad *et al.,* 2002). The methanolic extract of *Globularia alypum* decreases hyperglycaemia in streptozotocin – induced diabetic rats (Zennaki *et al.,* 2009). The *Globularia alypum* aqueous extract has a beneficial effect on plasma triglycerides and gives a promising perspective for hypertriglyceridemia treatment (Taleb-Dida *et al.,*

2011). In the present study, a natural product has been isolated from the leaves of *Globularia alypum*. Globularin was obtained as an amorphous powder. The molecular formula $C_{24}H_{28}O_{11}$, was deduced by a combination of different methods such as UV, IR, MS and NMR. The low and moderate doses (0 -1000 mg/kg body weight) of the compounds did not cause any deaths or significant changes in general behaviour in rats.

In our studies, we attempt to detect the hypoglycaemic activity of Globularin which is the major compound of *Globularia alypum*, in the experimental model of streptozotocin diabetic rats. According to our results, we can speculate that Globularin at a dose 100 mg/kg showed a hypoglycaemic effect in normalglycaemic and hyperglycaemic rats after single intraperitoneal administration. In normal rats, this effect was comparable to that of well known hypoglycaemic compounds such as Glibenclamide used at 0.6 mg/kg.

The evaluation of the hypoglycaemic effect of Globularin in prolonged treatment was performed in non-fasted animals. In these conditions, a slight hyperglycaemia was caused. This effect can be observed in normal rats which have a blood glucose concentration (1.30 - 1.38 g/L) higher than 1 g/L.

However, daily injection of Globularin (2 x 100 mg/kg) and Glibenclamide (2 x 0.6 mg/kg) did not show significant effect on hyperglycaemia in streptozotocin diabetic rats in comparison with the normal control rats. This ineffectiveness of both compounds: Globularin and Glibenclamide, suggest that severe hyperglycaemia was induced by streptozotocin (specific cytotoxic agent) in rats, due to the massive destruction of pancreatic β-cells. Finally Globularin could be more effective in mild diabetes mellitus. Further studies will be necessary. Therefore, we can not exclude the possibility that Globularin which is soluble in methanol and water was responsible for the hypoglycaemic effect of methanolic and aqueous extracts of *Globularia alypum* (Skim *et al.,* 1999; Jouad *et al.,* 2002; Zennaki *et al.,* 2009). This natural product could act separately or synergistically to cause the hypoglycaemic effect.

In prolonged treatment study, hypercholesterolemia and hypertriglyceridemia have been reported in streptozotocin diabetic rats and a significant increase was observed in our experiment which was in accordance to these studies. Repeated administration of Globularin had decreased the serum levels of total cholesterol and triglycerides significantly. According to the results obtained by Taleb – Dida et al. (2011), the beneficial effect on plasma triglycerides of *Globularia alypum* aqueous extract can be due to the Globularin.

CONCLUSION

Globularin is an iridoid glucoside, which was the major compound of the leaves of *Globularia alypum* (3.4 %). Our study indicates that Globularin possess a significant hypoglycaemic effect in normal and streptozotocin diabetic rats after single intraperitoneal administration. In addition, no remarkable toxic effect of Globularin was noted for the doses used in this study

(0 – 1000 mg/kg body weight). In prolonged treatment study, daily injection of Globularin (2 x 100 mg/kg) reduced significantly serum levels of total cholesterol and triglycerides in the diabetic rats. The obtained results demonstrated that the isolated compound play an important role for the anti-hypercholesterolemia and anti-hypertriglyceridemia properties of *Globularia alypum* and give a scientific basis to the use of this plant in traditional medicine. Globularin has this pharmacological potency which should be used in the future.

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