Natural Product Sciences
11(4) : 233-239 (2005)

Flavonoids of *Gomphocarpus sinaicus* and Evaluation of Some Pharmacological Activities

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Abstract – The aerial parts of *Gomphocarpus sinaicus Boiss.* yielded four flavonoids that were identified as isorhamnetin 3-O-rhamnoglucoside (**1**), luteolin-7-O-glucoside-3-O-rhamnoside (**2**), rutin (**3**) and rutin-7-O-rhamnoside (**4**). All of the isolated flavonoids were identified by spectroscopic methods (UV, FAB-MS, ^{1}H-NMR & ^{13}C-NMR) and in comparison with the literature data. The isolated flavonoids **1**, **2** and **4** are reported here for the first time from *Gomphocarpus sinaicus Boiss.* Three sets of experiments were carried out using the defatted alcoholic extract of *Gomphocarpus sinaicus* Boiss: the 1st experiment indicated that the LD_{50} was 49.82 mg/100 g b.wt. of intraperitoneally (i.p.) injected mice. The toxic signs were recorded within the first 24 hr post-injection. The 2nd experiment revealed that the extract of the plant exhibited significant anti-inflammatory effects in normal rats. The 3rd experiment was found that the tested doses of the extract in diabetic rats induced a significant decrease in serum glucose, AST, ALT, triglycerides, cholesterol and LDL, while HDL caused a significant increase.
Keywords – *Gomphocarpus sinaicus* Boiss., *Asclepiadaceae*, flavonoids, carrageenan, indomethacin, streptozotocin

Introduction

A great deal of attention has been recently given to the therapeutic use of herbal remedies for safety, efficacy, and economy. Plants of the genus *Asclepias* (of the milkweed family, Asclepidaceae) have found medicinal uses in the treatment of cancers, tumours and warts (Koike, *et al.*,). Indeed the genus name is derived from the Greek "God of Healing". *Gomphocarpus sinaicus* Boiss. (*Asclepiadaceae*) is known to grow in tropical and subtropical countries and various medicinal properties are attributed to these plant (Chitmel, *et al.*, 2004 and Zhang, *et al.*, 2003). *Gomphocarpus sinaicus* Boiss. (*Asclepias sinaica* Muschl.), Asclepidaceae is one of the plants growing in sandy mountanous regions in Sinai, Egypt and is known to be toxic to man and animals. It is known in arabic as Ghalquit el-deeb or Hargel and it is not found as a commercial article (Täckholm, 1974). A survey of literature revealed that a number of flavonoid, terpenoids ,cardirolides and their glycosides (Zhang, *et al.*, 2003), have been found to occur in various amount in most Asclepiadaceae family. In a previous work, the cardiac glycosides of the plant were studied (Abdel-Azim *et al.*, 1996 and Abdel-Azim, 1998) and in this paper we report on the flavonoidal compounds and also evaluation of some pharmacological activities in order to throw possible utilization in local pharmaceutical industry.

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Experimental

General – FAB-MS spectral analysis in negative or positive mode was performed on a VG70-SEQ Hyprid Mass Spectrometer. All NMR spectra were run on a Bruker DRX-400 instrument. The chemical shifts were reported in δ values (ppm) with TMS as the internal standard. Carbon multiplicities were determined in DEPT-135 and DEPT-90 experiments. ^{1}H-and ^{13}C-NMR spectra were recorded in DMSO. UV spectra were recorded on a UVIKON 931 double beam UV-VIS spectrophotometer in the region of 200-500 nm. Thin layer chromatography was performed on Merck precoated Silica gel 60 F_{254} plates while column chromatography was carried out using Merck silica gel 60 (200-250 mesh) as adsorbent. Solvent system for TLC was ethyl acetate: acetic acid: formic acid: water (30 : 0.8 : 1.2 : 8). Solvent systems for paper chromatography were: 15% acetic acid in water and butanol:acetic acid:water(4 : 1 : 5). The plates and papers were sprayed by 1% alcoholic aluminum chloride

(Markham, 1982).

Plant material – The plant was collected from the sandy mountainous regions in South Sinai, Egypt and identified by Prof. Dr. K. El-Batanouny, Dept. of Botany, Cairo University, Cairo, Egypt. A voucher specimen has been deposited at the Herbarium of Dept. of Botany, Faculty of Science, Cairo University.

Extraction and isolation – The air-dried and powdered aerial parts of *G. sinaicus* Boiss. (1 kg) was defatted with n-hexane in a soxhlet and then extracted subsequently with $CHCl_3$ and 70% aqueous methanol. The aqueous alcoholic extract was evaporated under reduced pressure till free from alcohol. The aqueous solution was partitioned first against $CHCl_3$, EtOAc and then against n-BuOH.

The EtOAc fraction was subjected to column chromatography (CC) on Sephadex LH-20 using methanol with increasing amounts (10%) of water; four fractions (Go1-Go4) were collected after monitoring with TLC. The spots were detected with UV before and after spraying with alcoholic $AlCl_3$. The fraction Go2 was further purified by CC (Silica gel 60), the separation was initiated with $CHCl_3$ and polarity was gradually increased with steps of 5% of MeOH. The flavonoid-containing fraction was eluted with $CHCl_3$/MeOH/H2O (8 : 2 : 0.5) and then subjected to PTLC using silica gel plates developed with $CHCl_3$/MeOH (8:2 +5 drops H_2O) to give compounds **1-3**. The fraction eluted with $CHCl_3$/MeOH (7 : 3) was also subjected to PC using chromatographic paper Whatmann 3MM and developed with 15% acetic acid in water followed by CC on Sephadex LH-20 which eluted with 90% aqueous MeOH to give compound **4**.

Test animals – Rats of both sex weighing 150-200 g and mice 18-20 g were used in the experiments. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication NO. 85-23, revised 1985).

Standard laboratory chow was provided and water was available *ad libitum*. Whole blood was obtained under light ether anesthesia from retro-orbital venous pleux by means of thin heparinized capillary tubes. The blood was immediately centrifuged and serum was transferred to test tubes. Blood was withdrown at 0, 15 and 30 days after treatment.

Toxicological study – The LD_{50} was determined using mice according to the methods described by (Karber, 1931). The MLD and LD_{100} were also determined. The symptoms of acute toxicity and post mortem finding were recorded.

Induction of Diabetes – Diabetes was induced by a single intraperitoneal injection of streptozotocin in a dose of 60 mg/kg b.wt. freshly dissolved in citrate buffer (0.05M, pH45) (Korthuis *et al.*, 1987). Four days later blood glucose was assayed and diabetes was verified. The animals included in this study were divided into three groups each of 6 rats.

Group I – control diabetic rats.

Group II – was treated with extract 1/20 LD_{50} (25 mg/kg/d,i.p) for 30 days.

Group III – received extract 1/10 LD_{50} (50 mg/kg/d, i.p.) for 30 days.

Anti-diabetic assay – Serum glucose was measured by a glucose oxidase Kit (Trinder, 1969). Serum triglycerides and serum total cholesterol were measured by Bio Merieux Kits (Fossatip,1982 and Richmond, 1973). Serum "ALT & AST (Reitman & Frankel, 1957). Serum high density lipoprotein (HDL-cholesterol) and serum low density lipoprotein (LDL-cholesterrol) were measured by kit from Quimica Clinica Aplicade S.A. 43870 (Amposta, Spain, P.O. Box 20).

Anti-inflammatory testing – Inflammation was produced using carrageenan according to the method described by Winter (Winter *et al.*, 1962). The extract was given one hour before carrageenan injection. Indomethacin was used as a standard anti-inflammatory drug. Paw volume was measured with a plethysmometer (UGO Basile, Italy), at zero time and 1,3, 4 hs after the carrageenan injection.

Drugs – Carrageenan, streptozotocin (STZ) and sodium citrate (sigma, USA) and indomethacin (Kahira Pharm & Chem. IND Co., Cairo, A.R.E.) were used.

Statistical analsis – Data are shown as means ± standard error. The statistical analysis was performed with the analysis of variance. The results obtained at the end of each time phase were compared with those obtained form zero time for the same group using student's-*t* test for paired observations. Biochemical data were analysed by Kapure & Saxena, (1972) and Armitage, (1971).

Results and Discussion

Isorhamnetin-3-*O*-rhamno-glucoside (1) – The UV absorption spectrum of the compound showed peak-I at 352 nm in methanol indicating the flavonol type structure of the compound (Markham, 1982). Also, the absence of the *o*-dihydroxy system was confirmed through the $AlCl_3$/HCl spectrum where there is no hypsochromic shift in peak-I. A bathochromic shift (62 nm) with high intensity was observed on the addition of NaOMe indicating the presence of a free OH group at C-4'. A bathochromic shift

(7 nm) in peak II on addition of NaOAc indicates the presence of a free OH group at C-7.

The negative ion mode FAB mass spectrum displayed a molecular ion peak at m/z 623 (M^+-1) corresponding to the molecular formula $C_{28}H_{23}O_{16}$. Another important peaks at m/z 609 (M^+-CH_3), 593 (M^+-OCH_3), 477 (M^+-146) (deoxy sugar moiety) and 315 (M^+-[146+162]).

The ^{1}H-NMR (DMSO) spectrum showed signal at δ in ppm 7.95 (d, 1H, H-2'), 7.63 (d, 1H, H-6'), 6.91 (d, 1H, H-5') 6.8 (d, 1H, H-8) and 6.39 (d, 1H, H-6). Two anomeric protons at 5.2 and 4.5 ppm attributed to glucose and rhamnose, respectively. These two sugar moities were rutinoside (Harborne & Day,1982). Another important peaks at 3.94 (s, 3H, OCH_3) and 1.1 (d, 3H, CH_3 of the rhamnose moiety). The other protons of the two sugar moities were appeared in the region between 3 to 3.8 ppm. The nature of the interglycoside linkage and the position of linkages of the sugars were confirmed by analysis of ^{13}C-NMR data as shown in table (1). The acid hydrolysis of the compound revealed the presence of an aglycone identified as isorhamnetin (by co-chromatography) (Marbry *et al.*, 1970) and two sugars identified as glucose and rhamnose. So, these data confirm the presence of the two sugars at position-3 in the parent compound and accordingly the compound is identified as Isorhamnetin-3-*O*-rhamnoglucoside.

Luteolin-7-*O*-glucoside-3'-*O*-rhamnoside (2) – The UV absorption spectrum in methanol displayed peak-I at 347 nm indicating the flavone type structure of the compound (Markham, 1982). A bathochromic shift (53 nm) with high intensity in peak-I was noticed upon the addition of NaOMe indicates the presence of free OH group at C-4'. The absence of an ortho dihydroxy system was confirmed through $AlCl_3$/HCl spectrum where there is no hypsochromic shift in peak-I. There is no bathochromic shift in peak-II indicating the absence of a free OH group at C-7.

The negative FAB mass spectrum showed a sharp intense molecular ion peak M^+ at m/z 593 corresponding to the molecular formula $C_{27}H_{20}O_{15}$ (M^+-1). Another important fragments at m/z 447 (M^+-deoxysugar, 146) and 285 (M^+-[deoxysugar+hexose], 146+162) indicate the presence of an aglycone of molecular weight 286 (may be luteolin) with two sugar moieties. The ^{1}H-NMR spectrum (DMSO) showed signals at δ in ppm 7.4 (d, 1H, H-2'), 7.3 (d., 1H, H-6'), 6.85 (1H, d, H-5'), 6.6 (1H, d, H-8), 6.35 (2H, dd, H-6, H-3) and two anomeric protons at 5.1 (1H, broad.) 4.45 (1H) attributed to glucose and rhamnose, respectively. The CH_3 group protons of rhamnose displayed at 0.9 ppm.

The partial hydrolysis **(Markham, 1982)** of compound **2** revealed the presence of both glucose and rhamnose with luteolin aglycone. While the enzymatic hydrolysis **(Markham, 1982)** of compound **2** with β-glucosidase revealed the presence of glucose and luteolin 3-O-rhamnoside This indicates that the glucose moiety was present at C-7 while the rhamnose moiety was present at C-3'.

The ^{13}C-NMR spectrum showed the most important signals like C-4 at 181.1 ppm charactareshic for flavones, the signal for C-3 appeared at higher field (103.2 ppm) and C-3' (145.5 ppm). The other data were shown in Table 1. Thus, the compound is identified as luteolin-7-*O*-glucoside-3'-0-rhamnoside.

Rutin (quercetin-3-*O*-rhamnoglucoside, 3) – The compound appeared as deep purple spot under UV light and changed to yellow when exposed to NH_3 vapour. The compound had R_f 0.6 in 15% acetic acid indicating the glycosidic nature of the compound. The UV absorption spectra in methanol showed peak-I at 360 nm indicating the flavonol type structure of the compound (Markham, 1982). The compound showed a bathochromic shift (50 nm) with high intensity in peak-I upon the addition of NaOMe indicate the presence of free OH at C-4'.

The presence of an *ortho dihydroxy* system on ring-B was confirmed through the $AlCl_3$/HCl spectrum where peak I showed a hypsochromic shift (31 nm) relative to the $AlCl_3$ spectrum. Peak-II was bathochromically shifted (12 nm) in the NaOAc spectrum indicating the presence of a free OH group at C-7.

The negative FAB mass spectrum showed a molecular ion peak (M^+) at m/z 609 corresponding to the molecular formula $C_{27}H_{21}O_{16}$. The presence of fragment at m/z 301 indicates the presence of an aglycone of $M^+ = 301$ with two sugar moieties (Deoxy, 146 + hexose, 162)

The partial hydrolysis of compound **3** revealed the presence of rhamnose in addition to a glycoside compound identified as quercetin-3-*O*-glucose. The ^{1}H-NMR spectrum showed signals at δ ppm 7.45 (1H,d, H-2'), 7.32 (1H, d, H-6'), 6.85 (1H, d, H-5') 6.35 (1H, d, H-8), 6.2 (1H, d, H-6). The anomeric protons of glucose at δ 5.85 (1H,d,H-1'') and the other anomeric protons of rhamnose at δ 4.3 (d,1H,H-1'''). The methyl group protons of the rhamnose moiety were appeared at 0.9 ppm. The ^{13}C-NMR (DMSO) were in accordance with that reported for rutin (quercetin-3-*O*-rhamnoglucoside) (Harborne & Marbry, 1982) and the data were shown in Table 1. So, the chromatographic and spectroscopic data confirmed the identification of compound **3** as rutin.

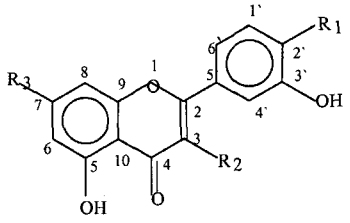
Rutin 7-*O*-rhamnoside (4) – The compound showed a

high R_f value (0.8) in 15% AcOH solvent system on paper chromatography indicating the high glycosidic nature. The UV absorption spectrum of the compound in methanol showed peak-I at 354 nm indicating the flavonol nature (Markham, 1982). A bathochromic shift was noticed in peak-I (50 nm) with high intensity in NaOMe spectrum indicate the presence of a free OH group at C-4'.

A hypsochromic shift (22 nm) was noticed in the $AlCl_3$/HCl spectrum in peak-I relative to the $AlCl_3$ spectrum indicating the presence of an ortho dihydroxy system in ring-B. There is no bathochromic shift in peak-II in NaOAc spectrum indicates the absence of free OH group at C-7.

The negative FAB mass spectrum of the compound showed a molecular ion peak M^+ at m/z 755 corresponding to the molecular formula $C_{33}H_{32}O_{21}$. Another important fragments at m/z 609 (M^+ -Deoxy hexose), 301 (M^+-(hexose+2 Deoxy sugars) confirms the presence of 3 sugar moieties.

The Acid hydrolysis revealed the presence of both rhamnose and glucose in addition to quercetin as an aglycone. The ^{1}Hnmr spectrum showed signal at δ in ppm 7.7 (1H, d, H-6'), 7.5 (1H, d, H-2'), 6.9 (1H, d, H-5), 6.75 (1H, d, H-8), 6.35 (1H, d, H-6), Three anomeric protons were appeared at 5.9 (1H, d, H-1", 3-glucose) 4.9 (1H, d, H-1"', 7-rhamnose) and 4.2 (1H, d, H-1"", 3-rhamnose). The methyl group protons of the two rhamnose moieties were appeared at 1.1 and 0.9 ppm, respectively. The ^{13}C-NMR spectrum (DMSO) confirms the presence of three anomeric carbons attributed to two rhamnoses and one glucose. Also, it confirms the presence of disubstituted quercetin at C-3 and C-7. The other data were shown in Table 1. So, the compound was identified as Rutin 7-*O*-rhamnoside.



1, $R_1 = OCH_3$, R_2 = *O*-rhamnosyl (1→6) glucose, R_3 =OH

2, R_1= *O*-rhamnosyl (1→6) glucose, R_2 = H, R_3 = O-glucose

3, R_1 =OH, R_2 = *O*-rhamnosyl (1→6) glucose, R_3 =OH

4, R_1= OH, R_2 = *O*-rhamnosyl (1→6) glucose, R_3 = *O*-glucose

Fig. 1. Structure of the flavonoids isolated from *G. siniacus*.

Table 1. ^{13}C-NMR spectral data of compounds **1-4**

Carbon no.	δ (ppm)			
	Compound (1)	Compound (2)	Compound (3)	Compound (4)
2	156.2	164.6	156.6	156.5
3	133.3	103.3	133.6	133.0
4	177.3	181.8	177.4	177.3
5	161.2	161.0	161.2	161.1
6	98.70	99.80	98.8	99.0
7	164.0	163.0	163.9	164.1
8	93.70	95.00	93.6	93.60
9	156.4	156.9	156.4	156.0
10	104.1	105.5	104.2	104.1
1'	121.2	121.6	121.6	121.0
2'	115.3	113.7	115.3	115.3
3'	149.9	145.7	144.6	144.5
4'	147.0	149.8	148.3	148.2
5'	113.9	116.0	116.5	116.2
6'	122.4	119.1	121.6	121.5
OCH_3	56.00	-	-	-
1"	101.4	100.4	101.5	101.5
2"	74.30	73.30	74.20	74.20
3"	76.70	77.30	76.80	76.70
4"	72.10	70.00	72.20	72.10
5"	76.00	76.60	76.10	76.00
6"	66.90	61.00	67.10	67.10
1"'	100.7	101.5	100.7	100.5
2"'	70.80	70.60	70.80	70.70
3"'	70.80	70.30	70.40	70.30
4"'	70.30	70.00	72.20	70.20
5"'	68.10	69.00	68.20	68.10
6"'	17.50	18.00	17.50	17.80
1""				100.6
2""				70.8
3""				70.6
4""				71.9
5""				69.8
6""				17.5

Compounds 1, 2 and 4 were isolated for the first time from this plant while compound 3 was previously isolated (Sarg *et al.*, 1993).

Acute toxicity studies – Symptoms of acute toxicity of *Gomphocarpus sinaicus boiss* extract in mice included increased respiratory rate and strong heart beats. After 2 hours post-injectionn, the animals suffered from general depression , shallow deep respiration and very weak heart beats that ended by death. The LD_{50} of the extract was found 49.82 mg/100g b.wt., while LD_{10} and LD_{100} were 30 mg and 96 mg/100g b.wt., respectively. Post mortem examination revealed general congestion of all intestinal organs particularly the lung and heart. The blood become dark brown in colour. The mucous membrane of he eyes was congested and changed to dark red colour. The heart was flabby and engorged with blood, which may indicate heart failure (Table 2).

Table 2. Determination of LD_{50} of defatted alcoholic extract of *Gomphocarpus sinaicus boiss* by Karber method using constant (1.4)

Dose used mg	M	Reaction	Z	D	Z.d
96.0	10	10	-		
68.6	10	8	9	27.4	246.6
49.0	10	6	7	19.6	137.2
35.0	10	3	4.5	14.0	63.0
25.0	10	-	1.5	10.0	15.0
					Σz.d = 461.8

Calculation

$$\cdot a \cdot M = DM - \frac{\Sigma Z \cdot d \cdot}{M}$$

Arithmatic means = 96–46.18
= 49.82

LD_{50} = 49.82 mg/100 g b.wt.

LD_{50} of defatted alcoholic extract of *Gomphocarpus sinaicus boiss* = 49.82 mg/100 g b.wt

Where,

a M: Arithmatic means.

DM: The dose by which all the animals reacted.

Z: Half the sum of the positive reacted animals from 2 successive doses.

d: the difference between the number of 2 successive doses.

M: the number of animals in each group.

Anti-inflammatory effect – The intraplantar injection of carrageenan into the rat hind paw elicited an inflammation (swelling and erythema) and a time dependent increase in paw oedema that was maximal at 4 h and remained elevated for more than 48 h following carrageenan. The acute paw oedema response induced by intraplantar carrageenan, was significantly reduced at 3 h by 64.14% in rats receiving indomethacin. the inflammatory response to carrageenan, i.e oedema, was significantly reduced at 3 h by two doses of *Gomphocarpus sinaicus boiss* extracts (2.5 & 5.0 mg/100 g b.wt) given I.P. by 31.87 and 45.22% vs control value respectively (Table 3).

Effect on glucose and liver function – Form Table (4) the administration of *Gomphocarpus sinaicus boiss* extract in two dose levels (2.5 mg and 5.0 mg/100 g b.wt) for 30 days to diabetic rats induced significant decrease in serum glucose, AST and ALT at high dose only. The low dose did not induce a significant decrease.

Effect on lipid component – Data presented in Table 5 revealed that administration of daily I.P doses of *Gomphocarpus sinaicus boiss* extract (2.5 g and 5 mg/100 g b.wt) for 30 days induced significant decrease in serum

Table 3. Effect of defatted ethanolic extract of *Gomphocarpus sinaicus Boiss* and Indomethacin on carrageenan induced paw oedema in rats (Values correspond to mean paw volume in ml ± S.E.)

Group	Dose mg/100 g b.wt	Volume of paw (ml) after carrageenan Administration				Total increase In paw volume (ml) after 3 hours	Percent inhibition
		0hour	2hours	3hours	4hours		
Control	-	0.85±0.04	1.42± 0.06	1.68± 0.06	1.72±0.07	o.84±0.08	-
Extract	2.5	0.85± 0.04	1.33± 0.09	1.42*± 0.06	1.42±0.05	0.57±0.08	31.87*
Extract	5.0	0.87± 0.08	1.21± 0.09	1.33**± 0.08	1.4± 0.06	0.46±0.07	45.22**
Indomethacin	5.0	0.73± 0.06	0.9 ± 0.09	1. 03***±0.05	1.03± 0.06	o.3±0.07	64.14***

*p < 0.05, **p < 0.01, ***p < 0.001 vs control at the same time

Table 4. Effect of defatted ethanolic extract of *G. sinaicus* on serum glucose, AST and ALT in diabetic rats

Groups	Time of sampling "days" after beginning of administration of extract mg/100 g b.wt. "I.P."								
	Zero			15			30		
	Glucose	AST	ALT	Glucose	AST	ALT	Glucose	AST	ALT
Control without treatment	263.37±18.4	62.43±5.57	55.25±4.14	250.34±22.66	56.74±4.18	52.95±3.71	228.32±20.77	53.9±5.18	48.8±4.25
2.5 mg	258.2±18.9	58.83±4.17	49.21±4.0	230.61±20.1	50.78±4.48	45.2±3.9	201.2±19.02	49.62±4.2	40.32±3.53
5.0 mg	229.33±20.56	64.17±1.58	52.17±3.32	179.17±10.03	55.82±5.02	44.17±3.0	159.17**±5.83	48.33**±3.07	39.83**±3.22

* Indicates a statistically significant difference of the value when compared with zero time in the same group.
(*p < 0.05, **p < 0.01, ***p < 0.001)

Table 5. Effect of defatted ethanolic extract of *G. sinaicus* on serum triglyceride cholesterol, HDL and LDL in diabetic rats

Groups	Time of sampling "days" after beginning of administration of extract mg/100 g b.wt. "I.P."											
	Zero				15				30			
	TG	Cholesterol	HDL	LDL	TG	Cholesterol	HDL	LDL	TG	Cholesterol	HDL	LDL
Control without treatment	150.26 ±13.0	160.0 ±14.3	26.42 ±2.42	40.6 ±3.32	143.14 ±10.3	150.26 ±13.02	25.38 ±1.9	37.0 ±3.27	138.78 ±8.9	154.3 ±12.2	29.42 ±2.91	35.4 ±2.91
2.5 mg	145.52 ±12.94	158.83 ±13.63	28.52 ±2.1	38.45 ±3.53	135.45 ±10.18	130.7 ±11.2	31.39 ±1.89	30.25 ±2.4	128.42 ±9.85	121.25 ±11.7	37.74** ±1.74	28.53* ±2.38
5.0 mg	154.17 ±12.81	150.0 ±11.55	32.17 ±0.95	36.0 ±1.53	130.0 ±7.75	124.17 ±9.87	34.33 ±1.33	29.5** ±1.18	105.0 ±13.35*	110.0 ±7.52*	38.0* *±1.24	24.83*** ±1.3

* Indicates a statistically significant difference of the value when compared with zero time in the same group.
(*p < 0.05, **p < 0.01, ***p < 0.001)

triglyceride, cholesterol and LDL in diabetic rats. While HDL levels showed significant increase.

Discussion – The present studies indicated clearly that *Gomphocarpus sinaicus* Boiss.extract is of therapeutic and economic values. The present work, which has evaluated the acute toxicity of the extract of defatted alcoholic extract of *G. sinaicus* revealed that the LD_{50} dose was 49.82 mg/100 g b.wt. The congestion of the intestinal organs and the dark brown color of the blood in post mortem findings, may be as a result of the effect of the one component of the tested extract (flavonoid or glycoside) in which it changed the oxyhaemoglobin to methaemglobin that gives the blood brown colour (Murray *et al.*, 1993).

The defatted ethanolic extract of *G. sinaicus* caused a significant reduction in the volume of the rat paw indicating considerable anti-inflammatory activity. The effect was in its maximum 31.87 and 45.22% at 3 hrs for two dose levels. Indomethacin showed a bigger reduction (64.14%).

The phytochemical analysis of the extract (Asclepiadaceae) revealed the prescence of sugar (Heinrich *et al.*, 1998), the flavonoids (Rahman &Wilcock, 1991), flavonol glycosides (Sen&Sahu, 1992), oxypregnance-oligoglycosides (Shibuya & Zhang, 1992), Terpenes, Terpene derivatives, pentacyclic triterpenoids and triterpenoids (Gupta &Ali, 2000). These constituents may mediate the anti-inflammatory property of the defatted alcoholic extract of, *G. sinaicus*.Furthermore, certain flavonoids can block early steps in the transduction of pro-inflammatory histamine e.g prostaglandins and leukotrienes (Middleton, 1998). Also, flavonols and flavones inhibit cyclooxygenase and lipoxygenase therepy ameliorating several eicosanoid-mediated pathophysiological disorders such as atherogenesis, inflammatory and immune-related disorders(Furst, 1995). They also markedly diminished symptoms of an atherosclerosis process in animals (Khushbaktova *et al.*, 1991).

On the other hand, obtained results revealed that administration of defatted alcoholic extract of *G.sinaicus* showed a significant decrease in serum glucose, lipid component and liver enzyme in diabetic rats are in harmony with (Furst, 1995 and Khushbktova *et al.*, 1991). It was noticed that plant favonoids are in acoordinate with reduction of cholesterol, triglyceride, LDL, AST and ALT serum levels while HDL exhibited significant increase. Available evidence suggests that flavonoids inhibit cAMP phosphodiesterase (Ferrel *et al.*, 1979). cAMP is a modulator of insulin secretion.

In conclusion, this study revealed that the defatted alcoholic extract of *G. sinaicus* at high dose improved glucose, liver enzyme and lipid component in diabetic rats. Also, these finding support the use of this extract as anti-inflammatory agent.

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(Accepted November 21, 2005)