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Formulation and evaluation of antihyperglycemic leaf extracts of *Zizyphus spina-christi* (L.) WILLD

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This study deals with the formulation of antihyperglycemic leaf extracts of *Zizyphus spina-christi* (L.) Willd. A bioactivity guided fractionation of different leaf extracts [defatted ethanol 70% (a), butanol (b), ethanol 70% (c), ethyl acetate (d) and petroleum ether (e) extracts] revealed that extract (c) possessed the highest antihyperglycemic activity followed by (b) and (a). HPLC was adopted for standardization of the extract (c) based on evaluation of the major saponin christinin-A which was used as marker. The detection limit was 9.45 mg/ml for Christinin-A. Extracts (a), (b) and (c) were separately formulated in soft (S) and hard (H) gelatin capsules. Two different formulations (F1 and F2) were tried using different excipients suitable for oral drug delivery. Formula 1, used for soft gelatin capsules [(F1) Sa, Sb, Sc] Formula 2, used for hard gelatin capsules [(F2) – Ha, Hb, Hc]. The recovery rates of the samples of saponin were in the range 99.43–101.86% at 200, 800 μg/ml and 1200 μg/ml. Saponin release rates from different formulae were carried out using dissolution tester USP XXIV. The highest release was obtained from formulation Sc. The release of the extracts followed diffusion mechanism. The selected formula Sc exhibited highest anti-diabetic activity ($P < 0.01$) on acute and long term administration and highest saponin release. This formula (Sc) contained poly-oxyethylene (20) cetyl ether (BC-20TX), PEG 400, PEG 6000, purified water, meglyol 810, ascorbic acid and 200 mg of extract (c).

1. Introduction

Zizyphus spina-christi has long been a subject for phytochemical and biological investigations (Michel 1993; Mahran et al. 1996, 2005). Results obtained suggested the leaves to be a promising antihyperglycemic agent for type II diabetes (Glombitza et al. 1994) through improvement of oral glucose tolerance and potentiation of glucose-induced insulin release in non-diabetic rats (Abdel-Zaher et al. 2005). Long-term treatment with the butanol extract of the leaves and its major saponin, christinin-A, decreased serum glucose level, liver phosphorylase and glucose-6-phosphorylase, while serum pyruvate level and liver glycogen were increased (Glombitza et al. 1994).

Attempts for the formulation of the different leaf extracts (a, b and c) were carried out in order to select the best oral antihyperglycemic preparation. Based on preliminary trials, the soft gelatin capsule has been used since it encapsulates a drug in a water-soluble non aqueous solution, oil solution, or suspension. Soft gelatin capsules can also improve both the bioavailability of a drug by accelerating disintegration, dispersion and release in the gastrointestinal tract, as well as drug stability due to poor permeability to oxygen (Amemiya et al. 1998a, 1999). Therefore, an oil-in-water emulsion type vehicle was developed for use in soft gelatin capsules (F1 – Sa, Sb, Sc) with a low content of surfactant and compared with another formula containing different excipients incorporated in a hard gelatin capsules (F2 – Ha, Hb, Hc). Plain extracts were also included in both soft (Spa, Spb, Spc) and hard gelatin capsules (Hpa, Hpb, Hpc) as listed in Table 1.

In the present study, standardization of the major saponin (christinin-A) using HPLC was performed. The saponin was chosen as marker based on its relatively high concentration, biological potential and phytotaxonomic significance. Formulation of the bioactive leaf extracts for oral drug delivery was achieved to select the formula showing the highest anti-diabetic activity and best release.

2. Investigations, results and discussion

A bioactivity guided fractionation of the leaves of *Zizyphus spina-christi* (L.) Willd extracted with different solvents revealed that the ethanol 70% extract (c) possessed the highest antihyperglycemic activity when compared to metformin ($P < 0.01$, Table 2). LD_{50} of the extract (c) was found to be 10 g/kg. b.wt. thus indicating the safety of the extract. Long term administration revealed that the ethanol 70% extract (c) had no toxic effect on cholesterol, triglycerides, creatinine, urea and liver enzymes (AST and ALT) serum level in rats after eight weeks, but that it showed a slight hypoglycemic effect within normal range of potency 5% ($P < 0.05$, Table 3). The antihyperglycemic activity of extract (c) showed the highest potency (83%)

Table 1: Formulation of different *Zizyphus spina-christi* extracts

Symbol	Name	Content
A	Plain extracts	Defatted ethanol 70% extract
B		Butanol extract
C		Ethanol 70% extract
Sa	(F1) – Formulated extracts	Formulated defatted ethanol 70% extract
Sb	in soft gelatin capsules	Formulated butanol extract
Sc		Formulated ethanol 70% extract
Ha	(F2) – Formulated extracts	Formulated defatted ethanol 70% extract
Hb	in hard gelatin capsules	Formulated butanol extract
Hc		Formulated ethanol 70% extract
Spa	Plain extracts filled	Defatted ethanol 70% extract
Spb	in soft gelatin capsules	Butanol extract
Spc		Ethanol 70% extract
Hpa	Plain extracts filled	Defatted ethanol 70% extract
Hpb	in hard gelatin capsules	Butanol extract
Hpc		Ethanol 70% extract

after 6 h at a dose of 200 mg/kg.b.wt. ($P < 0.01$) when compared to metformin (150 mg/kg.b.wt.). The defatted ethanol 70% extract (a) at the same dose level produced a potency of 48% after 6 h when compared with the same dose of standard as shown in Table 2. Selecting of the marker was achieved by pharmacologically screening the different extracts [butanol (b), ethyl acetate (d) and petroleum ether (e) extracts] at different dose levels (50, 100 mg/kg.b.wt.) for their antihyperglycemic activities. Extract (b) at a dose level of 50 mg/kg.b.wt. showed a potency of 44% after 6 h ($P < 0.01$). Extract (d) at the same dose level showed potency of (35%) after 6 h while the (e) extract showed no significant activity when compared to metformin (Table 2). Upon increasing the dose of both extracts (b) and (d) to 100 mg/kg.b.wt, the potencies were nearly similar after 6 h indicating that the available receptors were blocked. Therefore, extract (c) containing bioactive saponins, including the major saponin christinin-A (Michel 1993; Mahran et al. 2005; Zhu et al. 2004) was selected as marker. TLC investigation of the extract (c) using S1 as solvent system and spraying with P-anisaldehyde, located christinin-A spot at $R_f = 0.64$ (Michel 1993; Mahran et al. 1996).

Based on preliminary trials, determination and validation of the major saponin christinin-A in the ethanol 70% extract (c) was performed by HPLC (Table 4). Using an isocratic of acetonitrile : 10 mM potassium phosphate buffer pH 5.85 (60 : 40 v/v) as mobile phase and Diode Array Detector at 200 nm, retention time was 5.04 min, the detection limit was 9.45 mg/ml for christinin-A. The peak illustrated in the chromatogram (Fig. 1) was identified by direct comparison of retention time with authentic saponin (christinin-A), as well as, by cochromatography of the sample solution spiked with authentic christinin-A saponin (Mahran et al. 1996).

The calibration curve has a correlation coefficient close to 1. The resulting data and the mean regression equation was computed and found to be $y = 3300\,x + 12408$, where y is the absorbance, x is the concentration in μg/ml and correlation coefficient $R = 0.9984$.

Table 2: Effect of the different leaf extracts of *Zizyphus spina-christi* on blood glucose level in diabetic rats (n = 10)

Groups/doses	Zero time (mg/dl, mean ± S.E.)	4 h (mg/dl, mean ± S.E.)	6 h (mg/dl, mean ± S.E.
Diabetic untreated Control (10 ml saline)	258.6 ± 9.1	261.7 ± 12.3	265.1 ± 11.8
Diabetic treated with defatted ethanol 70% extract (a)			
(a) 100 mg/kg	257.2 ± 13.1	193.4 ± 8.2*	159.2 ± 7.4*
200 mg/kg	268.1 ± 14.3	181.2 ± 7.2*	139.4 ± 6.1*
Diabetic treated with butanol extract (b)			
(b) 50 mg/kg	264.3 ± 12.9	176.2 ± 8.4*	148.6 ± 5.3
100 mg/kg	251.5 ± 9.7	204.2 ± 8.1	136.1 ± 6.8*
200 mg/kg	255.1 ± 11.3	195.1 ± 7.6*	116.5 ± 5.9*
Diabetic treated with ethanol 70% extract (c)			
(c) 100 mg/kg	261.9 ± 12.3	171.6 ± 6.7*	128.2 ± 4.8*
200 mg/kg	268.3 ± 12.4	162.4 ± 7.2*	116.3 ± 3.9*
Diabetic treated with ethyl acetate extract (d)			
(d) 50 mg/kg	259.1 ± 11.2	198.5 ± 9.1*	169.3 ± 7.4*
Metformin 150 mg/kg (* $P < 0.01$)	265.2 ± 11.4	129.3 ± 5.6*	84.2 ± 2.1*

Table 3: Effect of long term administration of the ethanol 70% extract (c) of the leaves of *Zizyphus spina-christi* for eight weeks on cholesterol, triglycerides, glucose, creatinine, urea and liver enzymes (AST And ALT) serum level in rats (n = 10)

Group	Time in weeks	Biochemical changes of serum level						
		Cholesterol (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	AST	ALT
Control	Zero	77.8 ± 1.3	68.2 ± 1.5	84.5 ± 1.1	1.1 ± 0.02	24.7 ± 1.3	25.8 ± 1.2	19.3 ± 0.6
	4	76.4 ± 1.4	66.9 ± 1.4	83.2 ± 1.4	1.2 ± 0.01	22.5 ± 1.2	26.4 ± 1.1	18.9 ± 0.7
	8	77.6 ± 1.8	67.8 ± 1.5	86.5 ± 1.3	1.1 ± 0.02	22.1 ± 1.1	24.9 ± 0.9	19.1 ± 0.4
C_{50} mg/kg	Zero	79.2 ± 1.5	64.5 ± 1.6	83.6 ± 2.1	1.3 ± 0.04	24.6 ± 0.5	26.7 ± 0.8	21.7 ± 1.1
	4	78.7 ± 1.1	63.8 ± 2.1	82.7 ± 1.5	1.2 ± 0.01	25.1 ± 0.4	27.1 ± 0.5	22.2 ± 1.3
	8	76.4 ± 1.4	65.5 ± 1.3	80.1 ± 1.8	1.1 ± 0.02	23.6 ± 0.3	26.1 ± 0.7	21.9 ± 1.4
C_{100} mg/kg	Zero	77.1 ± 1.2	69.8 ± 1.4	85.1 ± 1.3	1.2 ± 0.02	25.1 ± 1.2	23.9 ± 1.1	26.4 ± 0.5
(*$P < 0.05$)	4	76.9 ± 1.3	69.1 ± 1.6	84.3 ± 1.1	1.3 ± 0.03	24.9 ± 1.3	22.8 ± 1.2	24.9 ± 1.1
	8	78.1 ± 1.5	70.1 ± 1.2	80.9 ± 1.2	1.1 ± 0.01	24.5 ± 1.2	23.4 ± 1.3	25.8 ± 0.9

Table 4: Results of HPLC assay validation for christinin-A

Parameters	HPLC
Accuracy	
Mean ± S.D.	101.86 ± 2.107
Precision	
Interday*	2.49
Intraday*	2.48
Linearity	
Slope	0.3300
S.E. of slope	0.00997
Intercept	12.408
S.E. of Intercept	9.6559
Correlation coefficient	0.9984
Range	100–1600 (μg ml^{-1})

*n = 3 (for concentrations of Precision: 200, 400, 1200 μg ml^{-1})

The percentage of saponin release from plain and formulated extracts packed in either soft and hard gelatin capsules were screened spectrophotometrically to select the formulae which allow the best saponin release. The rate of saponin release decreased in the following order: Sc > Sb > Sa > Hc > Hb > Ha) and Spc > Spb > Spa > Hpc > Hpb > Hpa. Therefore, dissolution test was performed with formulae Sc, Hc and Spc, determination followed the aforementioned HPLC conditions. Figure 2 displays the *in vitro* release of drug from these formulae as determined by HPLC. The release rate of saponin was found to decrease in the following order: Sc > Hc > Spc, and the highest saponin release rate (92.74 ± 0.99% after 45 min) was obtained from formula Sc in comparison to Hc and plain extract (c) which gave 78.77 ± 1.06% after 120 min. and 47.77 ± 2.45% after 90 min, respectively. The high release rate of saponin from soft gelatin capsules (Sc) may be attributed to the rapid release of the base in water. The greater solubility of saponin from soft gelatin capsules in the aqueous phase caused a faster saponin release than upon incorporation in hard gelatin capsules. The kinetic analysis of the extract release data from different formulae was calculated by linear regression according to zero, first order kinetics and simplified Higuchi model and followed diffusion model kinetics (Table 5).

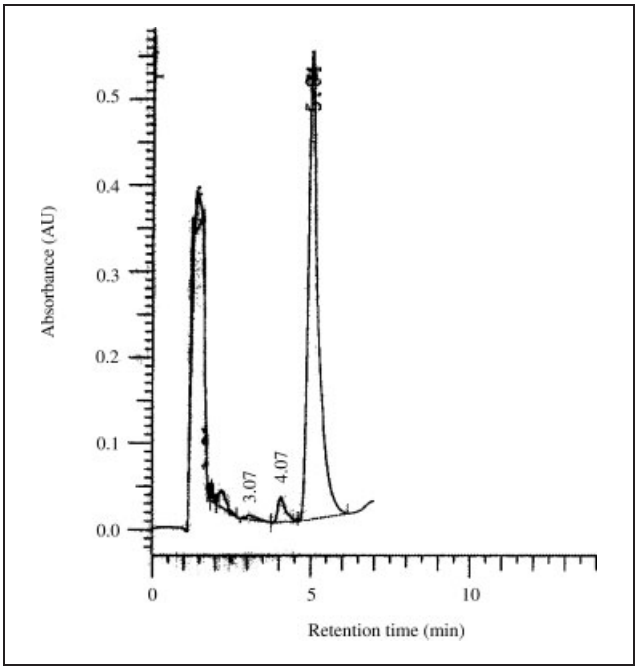

Fig. 1: HPLC chromatogram of 50 μl christinin-A (retention time 5.04 min, capacity factor 2.36, no. of theoretical plates 2540, tailing factor-1)

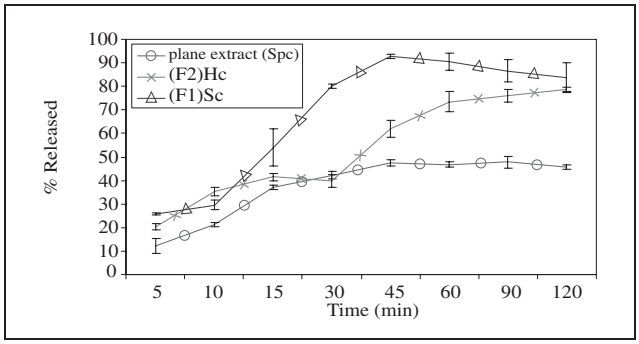

Fig. 2: Release profile of christinin-A determined by HPLC from soft (Sc) and hard (Hc) gelatin capsules in comparison with plain extract (Spc)

Based on the previous findings, formulations Sc, Hc, Spc formulations showing the highest release of saponin were tested for their antihyperglycemic activities. Sc exhibited a potency of 86%, Hc of 70% and Spc of 79% after 6 h when compared to metformin (150 mg/kg.b.wt., $P < 0.01$, Table 6). Long term administration of (Sc) led to potency of (95%) after eight weeks with same dose of the extract relative to the standard ($P > 0.01$, Table 7).

In conclusion, the ethanol 70% leaf extract (c) exhibited the highest antihyperglycemic activity at a dose of 200 mg/kg. b.wt. Long-term toxicity study of the extract (c) showed a wide range of safety. Selecting of Formula I

Table 5: Analysis of release data of saponin extract from different formulations (Sc, Hc, Spc) according to zero, first order kinetics and diffusion model

Formulation	Correlation coefficient			Mechanism of release
	Zero order	First order	Diffusion	
Formulated ethanol 70% extract in soft gelatin capsule (Sc)	0.591	0.570	0.793	Diffusion
Formulated ethanol 70% extract in hard gelatin capsule (Hc)	0.872	0.773	0.919	Diffusion
Ethanol 70% extract in soft gelatin capsule (Spc)	0.615	0.521	0.769	Diffusion

Table 6: Effect of the formulated leaf extracts of *Zizyphus spina-christi* (Sa, Sb, Sc) on blood glucose level in diabetic rats (n = 10)

Groups/doses	Zero time (mg/dl, mean ± S.E.)	4 h (mg/dl, mean ± S.E.)	8 h (mg/dl, mean ± S.E.)
Control (10 ml saline) Diabetic untreated	258.6 ± 9.1	261.7 ± 12.3	265.1 ± 11.8
(Formulated defatted ethanol 70% extract) Sa 100 mg/kg	258.4 ± 10.9	168.1 ± 7.2*	119.7 ± 5.3*
(Formulated butanol extract) Sb 100 mg/kg	258.5 ± 12.7	186.4 ± 7.6*	134.1 ± 6.2*
(Formulated ethanol 70% ext.) Sc 100 mg/kg	263.5 ± 12.3	151.2 ± 5.8*	109.4 ± 5.1*
Metformin 150 mg/kg (*P < 0.01)	265.2 ± 11.4	129.3 ± 5.6*	84.2 ± 2.1*

Table 7: Effect of long term administration of the formulated leaf extracts of *Zizyphus spina-christi* (Sa, Sb, Sc) on blood glucose level in diabetic rats (n = 10)

Groups/doses	Zero time (mg/dl, mean ± S.E.)	4 weeks (mg/dl, mean ± S.E.)	8 weeks (mg/dl, mean ± S.E.)
Control (10 ml saline) Diabetic untreated	251.2 ± 10.3	254.6 ± 9.5	261.4 ± 11.2
(Ethanol 70% extract) (c) 200 mg/kg	271.3 ± 9.6	189.3 ± 9.3*	120.2 ± 10.3*
(Formula F1) Sc 200 mg/kg	265.7 ± 11.4	149.3 ± 7.5*	98.9 ± 4.6*
(Formula F2) Hc 200 mg/kg	269.2 ± 12.8	178.4 ± 6.1*	106.5 ± 4.3*
Metformin 150 mg/kg (*P < 0.01)	259.3 ± 11.5	132.7 ± 6.4*	87.5 ± 4.1*

[poly-oxyethylene (20) cetyl ether (BC-20TX), PEG, 6000, purified water, Meglyol 810, and 200 mg of ethanol 70% leaf extract (Sc)] gave best saponin release determined by HPLC. Intraday and Interday precision showed that the sample was stable for at least one week at room temperature. The anti-diabetic activity of the selected formula (Sc) exhibited highest potency ($P < 0.01$) on acute and long term administration.

3. Experimental

3.1. Materials and apparatus

3.1.1. Plant material

Leaves of *Zizyphus spina-christi* (L.) Willd were collected during June 2006 from the cultivated plants in the Experimental Station of Medicinal Plants of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt. The identity was confirmed by Professor Dr. Nabil Hadidy, late Professor of Botany, Botany Department, Faculty of Science, Cairo University, Egypt. Voucher specimens are kept at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Air-dried leaves were coarsely powdered and packed in dark, coloured tightly-closed containers and kept for phytochemical and pharmacological studies.

3.1.2. Chromatographic study

Thin layer chromatography (TLC) was conducted on precoated silica gel 60 F_{254} plates (Macherey-Nagel, Germany). The solvent systems used were chloroform : methanol : water (65 : 40 : 10) [S1] and butanol : acetic acid : water (4 : 1; 5. upper phase) [S2]. Christinin-A was obtained from samples previously isolated and identified by Mahran et al. (1996). Reagents for HPLC analysis including acetonitrile, distilled water (HPLC grade), potassium dihydrogen phosphate and dipotassium hydrogen phosphate (analytical grade) were purchased from Merck chemicals (Germany).

3.1.3. Pharmacological studies

Albino mice weighing 20–25 g were used for LD_{50} determination. Adult albino rats of Sprague Dawley strain, obtained from the animal house colony, National Research Center, Cairo, Egypt, kept on standard laboratory diet and under the same hygienic conditions, were used for the antihyperglycemic study. Plant extract residues were given to animals at appropriate concentrations and the doses were expressed in terms of dried extract (mg/kg of body weight). The following drugs were used: Alloxan (Sigma Co., USA) for induction of diabetes and glimepiride (Amaryl®, Hoechst, Marion, Roussel, S.A.R.) and metformin (Amoun Co., Egypt) as standard antihyperglycemic agents.

3.1.4. Soft and hard gelatin capsules

Poly-oxyethylene (20) cetyl ether (BC-20TX), Croda, Bx 11143.U.K., Avicel FMC. (Brussels, Belgium), Meglyol 810 (Hüls AG, Witten, Ruhr, Germany), lactose, magnesium stearate, ascorbic acid and PEG 6000, PEG 400 were chosen, purchased from their respective sources. Soft gelatin capsules and hard gelatin capsules were gift samples supplied from Glaxo Co., Egypt.

3.1.5. Apparatus

UV-Spectrophotometer (Shimadzu, Kyoto, Japan), Electrical blender, Philips, Holland, Magnetic stirrer (Thermolyne Corporation, Dubuque, Iowa, U.S.A), Electrical balance (Sartorius GMBH, Göttingen, Germany), Thermostatically controlled electric water bath (Julabo Labortec Hink GMBH, Seelbach, Germany), USP release tester (Hanson SR6, Hanson Research Corporation, California, U.S.A), Sieves of mesh size 250 μm and 100 μm (VEB, Metal-Weberei, DDR) Italy were used. HPLC analysis was performed on a Merck Hitachi Lachrom Spectra system consisting of a L-7150 gradient pump and Diode Array Detector L-7455 at 200 nm. Data was processed with Chromatography software (D-7000 HSM). Standard addition with christinin-A was used. All injections were performed in triplicate unless otherwise stated. Column: Thermo BDS Hpypersil, C_{18}, 4.6×150 mm; Column Temperature: ambient temperature; flow rate: 1 ml/min; Detector: Scans 200 nm, Injection volume: 20 and 50 μl; Mobile phase: acetonitrile: phosphate buffer (pH 5.85; 40 : 60 v/v); Run time: 20 min.

3.2. Methods

3.2.1. Preparation of the standard solution and samples

A stock solution (1 mg/ml) of standard saponin (christinin-A) in methanol 80% was prepared. A standard calibration curve was established using serial dilutions of christinin-A viz. 100, 200, 800, 1200 and 1600 μg prepared from the stock solution. A typical calibration curve was prepared by plotting peak area (y) against injected amount (x, μg).
Ten grams powdered leaves were repeatedly refluxed with ethanol 70% until exhaustion (4 times, 200 ml). The ethanolic extract was concentrated to dryness under reduced pressure at 40 °C. The obtained residue (3.17 g) was extracted by ultrasonication with methanol 80% at room temperature for 20 min. The extract was filtered, clarified by centrifugation, transferred into a 50 ml volumetric flask and the volume adjusted with methanol 80%. An aliquot (1 ml) of the extract was transferred to a 5 ml volumetric flask and the volume adjusted with methanol 80%. Twenty μl of this solution were then injected into the HPLC system for analysis of the major saponin christinin-A.

3.2.2. Preparation of extracts for biological screening and formulation

Dried leaves (600 g) were defatted by refluxing with petroleum ether (60–80 °C) until exhaustion (5 l, 48 h). The petroleum ether extract was concentrated under reduced pressure at 40 °C to dryness to give petroleum ether extract (e). The mark was then refluxed with ethanol 70% until exhaustion (5 l, 48 h). The ethanolic extract was concentrated under reduced temperature and pressure to yield defatted alcoholic extract (a). Part of (a) was then suspended in water and partitioned with ethyl acetate (3 l, 48 h) followed by butanol saturated with water (3 l, 48 h) to afford butanol (b) and ethyl acetate (d) extracts respectively.
Another 100 g of the dried powdered leaves were similarly refluxed with ethanol 70% to give the total alcoholic extract (c). All the previous extracts (a, b, c, d and e) were screened for their antihyperglycemic activities. Results are listed in Table 2.

3.2.3. Toxicity study

To estimate acute toxicity, the median lethal dose LD_{50} of the ethanolic extract was determined according to Karber (1931). Chronic toxicity was studied after long-term administration of the most active extract (c) by determination of cholesterol (Allain et al. 1974), triglycerides (Trinder 1969), glucose (Eliasson and Samet 1969), creatinine (Henry 1979), urea (Evans 1968), and liver enzymes (AST and ALT) serum level in rats (Thefweld 1974). Results are listed in Table 3.

3.2.4. Acute and long-term antihyperglycemic activities

Diabetic rats were prepared according to the method described by Eliasson and Samet (1969). Results are recorded in Tables 6 and 7.

3.2.4. Preparation of soft gelatin capsules (F1: Sa, Sb and Sc)

The test formulation consisted of poly-oxyethylene (20) cetyl ether (BC-20TX) (3%), PEG 400 (74.69%), PEG 6000 (2.31%), purified water (10%), meglyol 810 (5%) and ascorbic acid (4.80%). The extracts (200 mg) of each (a, b, and c) were incorporated to yield 3.2 %. Materials were weighed in a stainless steel beaker, heated to 60 °C in a water bath, and then mixed (15 000 rev min^{-1} for 10 min) by a high-speed blender. The test formulation was prepared by encapsulating 450 mg of each preparation in a soft gelatin capsule (Amemiya et al. 1999; Uemastsu et al. 1996; Bateman and Uccellini 1984; Lesko et al. 1979; Ghirardi et al. 1977) prepared in our laboratory which was then sealed with gelatin solution. The control formulation was also prepared by separately encapsulating 200 mg of each plain extract (a, b, c) in a soft gelatin capsule (Amemiya et al. 1999) as presented in Table 1.

3.2.5. Preparation of hard gelatin capsules (F2: Ha, Hb and Hc)

This formula contained lactose (100 mg), magnesium stearate (8 mg), Avicel (42 mg) and 200 mg of each extract (a, b, c). The resultant mixture was pulverized so as to pass through a 250 μm sieve and to be retained on a 100 μm sieve, the required weight was then incorporated in hard gelatin capsules. Capsules containing 200 mg of each plain extract were similarly prepared and then kept in completely closed glass container.

3.2.6. Assay validation

A standard calibration curve was conducted by plotting the absorbance versus concentration of the marker christinin-A. Triplicate injections were performed to obtain the absorption plots ranging from 100–1600 μg/ml for christinin-A. The accuracy and precision of the assay were tested at concentrations of 200, 800 and 1200 μg/ml saponin (Table 4). Intra- and inter-day precisions were evaluated by triplicate injections of the marker christinin-A. Three injections were performed each day and on the first, second and the seventh day to determine reproducibility.

3.2.7. Release study

Determination of saponin release rates from different formulae were carried out using dissolution tester USP XXIV. The release studies of the individual plain extracts (Spa, Spb, Spc and Hpa, Hpb, Hpc), as well as, their incorporation in the various formulations either in soft (F1-Sa, Sb, Sc) or in hard gelatin capsules (F2-Ha, Hb, Hc) were performed. An accurately weighed amount of soft or hard gelatin capsules equivalent to 200 mg of each extract (a, b and c) was placed in an USP dissolution basket. The basket was rotated at 100 ± 5 rpm in 1000 ml distilled water at 37 ± 0.5 °C. At each time interval, 3 ml samples were withdrawn and replaced with 3 ml distilled water. Each release experiment lasted 2 h. Christinin-A in the different formulae was determined spectrophotometrically at each specified time interval at $\lambda = 200$ nm to select the formula explicating the highest release. Moreover, HPLC determination of the major saponin christinin-A was performed on the highest release formulae and compared with the plain extract.

References

Abdel-Zaher AD, Salim SY, Assaf MH, Abdel-Hady RH (2005) Antidiabetic activity and toxicity of Zizyphus spina-Christi leaves. J Ethnopharmacol 101: 129–138.

Amemiya T, Mizuno S, Hayashi Y (1998a) Development of emulsion type new vehicle for soft gelatin capsule. Selection of surfactant for development of new vehicle and its physicochemical properties. Chem Pharm Bull 46: 309–313.

Amemiya T, Mizuno S, Hayashi Y (1999) Absorption of vitamin K2 dogs after oral administration of a soft gelatin capsule formulation containing a new emulsion type vehicle. J Pharm Pharm 51: 1375–1380.

Allain CG, Poon LS, Chan CSG, Richmond WY, Fu PG (1974) Determination of blood cholesterol by enzymatic method. Clin Chem 20: 470.

Bateman NE, Uccellini DA (1984) Effect of formulation on the bioavailability of retinol, D-α-tocopherol and riboflavin. J Pharm Pharm 36: 461–464.

Eliasson SG, Samet JM (1969) Alloxan induced neuropathies lipid change in nerve and root fragments. Life Sci 81: 493–498.

Evans RJ (1968) Determination of blood urea. J Clin Path 21: 527.

Ghirardi P, Catenazzo G, Mantero O, Meroti GC (1977) Bioavailability of digoxin in a new soluble pharmaceutical formulation in capsules. J Pharm Sci 66: 267–269.

Glombitza KW, Mahran GH, Mirhom YM, Michel CG, Motawi TK (1994) Hypoglycemic and antihyperglycemic effects of Zizyphus spina-Christi in rats. Planta Med 60: 244–247.

Henry JB (1979) Estimation of blood urea and creatinine. Todd-Samford Clinical Diagnosis and Management by Laboratory Method"16th ed., W.B. Saunders Co. Philadelphia.

Karber G (1931) Determination of LD_{50}. Arch Exp Pathol Pharmacol 162: 480.

Lesko LJ, Canada AT, Eastwood G (1979) Pharmacokinetics and relative bioavailability of oral theophylline capsules J Pharm Sci 68: 1392–1494.

Mahran GH, Glombitza KW, Mirhom YW, Hartmann R, Michel CG (1996) Novel saponins of Zizyphus spina-christi (L.) Willd cultivated in Egypt. Plant Med 62: 163–165.

Mahran GH, Islam WT, Michel CG, Sleem AA (2005) Phytochemical investigation, pharmacological activity and nutritive value of Zizyphus spina-christi cultivated in Egypt. Bull Fac Pharm Cairo Univ 43: 107–117.
Michel CG (1993) Ph. D. Thesis, Faculty of Pharmacy, Cairo University, Cairo, Egypt,.
Trinder P (1969) Estimation of serum glucose and triglycerides by enzymatic method. Ann Clin Biochem 6: 24.
Thefweld W (1974) Enzymatic method for determination of serum AST and ALT. Dtsch Med 99: 343.
Uemastsu T, Nagashima S, Niwa M (1996) Effect of dietary fat content on oral bioavailability of menatetrenone in humans. J Pharm Sci 85: 1012–1016.
Zhu S, Zou K, Shaoqing C, Meselhy MR, Komatsu K (2004) Simultaneous determination of triterpene saponins in Ginseng drugs by high-performance liquid chromatography. Chem Pharm Bull 52: 995–998.