

## THE SEPARATION AND STUDY OF THE BIOLOGICAL ACTIVITY OF GLYCAN OF *SONCHUS OLERACEUS* L. HERB FAMILY ASTERACEAE

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د السكاكر المفصولة من النباتات مصدرا حيويا للمنتجات الطبيعية التي لها العديد من التأثيرات البيولوجية الهامة. وقد تم اختيار عشب نبات الجعضيض أو التلفاف التابع للعائلة المركبة لإجراء تلك الدراسة. حيث تم تحضير السكاكر المتعددة الخام وتعيين التركيب الأحادي لهذه السكاكر بعد حلماؤها جزئيا وكليا واستخدام كروماتوجرافيا الغاز المقترنة بتحليل طيف الكتلة وتبين أنه يتكون من مانوز وزيلوز وأربينوز وجلوكوز وجلالكتوز وحمض جلوكوبورنيك. تم تجزئة السكاكر المتعددة المفصولة بعد إزالة المعادن وإجراء عملية التصبن حيث تم الحصول على نوعين من السكاكر جزء مسحوق بني اللون لا يذوب في الماء والمحاليل المخففة للأحماض ولكن يذوب في محاليل القلويات وجزء مسحوق أبيض اللون يذوب في الماء والمحاليل المخففة للأحماض. وباستخدام كروماتوجرافيا العمود تم تجزئة السكاكر المتعددة المزالة المعادن على داى إيثيل أمينوليثيل سيليلولوز وأسفرت النتائج على أنه يتكون من بوليمر واحد. وأعيدت تجزئة جزء باستخدام خلات الصوديوم والسيتافلون (سيتيل ثلاثي ميثيل أمونيوم بروميد) وتعيين التركيب الأحادي لجميع السكاكر المفصولة بعد حلماؤها جزئيا باستخدام كروماتوجرافيا الغاز المقترنة بتحليل طيف الكتلة كيميا وكما. كما أسفرت نتائج المسح البيولوجي أن السكاكر الخام المتعددة المفصولة لها تأثير مضاد للالتهاب على فئران التجارب باستخدام جرعات مختلفة. كما أن جميع السكاكر المتعددة المفصولة من عشب نبات الجعضيض سواء الخام أو السكاكر المتعددة المزالة المعادن جزء وجزء لها تأثير كواقبات للكبد ضد سميتها برابع كلوريد الكربون في فئران التجارب وكانت أفضل النتائج لجزء والتي تعزى الى وجود حمض الجلوكوبورنيك حيث أنها تقلل مستوى بعض الإنزيمات الموجودة في الكبد وكذلك تحسن من بعض التغيرات الباثولوجية التي تطرأ على الكبد نتيجة سميتها برابع كلوريد الكربون.

*The wide biological and therapeutic applications of Asteraceae family motivated our attention to search on plant related to this family in our local flora. So, Sonchus oleraceus L. was the chosen plant.*

Received in 25/2/2006, Received in revised form in 31/3/2007 & Accepted in 2/4/2007

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The monomeric composition of the partially and completely hydrolysates of *S. oleraceus* L. herb crude polysaccharide complex was determined using GC/MS analysis. The obtained results revealed the presence of mannose, xylose, arabinose, glucose, galactose and glucuronic acid.

To determine the polymeric composition of the polysaccharide complex, different methods of fractionation were used. Demineralization was done first, then saponification. The demineralized glycan was chromatographed on DEAE-cellulose column. Elution being monitored spectrophotometrically using phenol-sulphuric acid reagent. The result revealed that the demineralized glycan composed of 1 major polymer. Both acidic and neutral monomers of the partially hydrolysed fractions were subjected to silylation and GC/MS analysis which provide qualitative and quantitative determination of the monomeric composition of each fraction. The polysaccharide complex was an acidic heterogenous polysaccharide.

The crude glycan of *S. oleraceus* L. herb showed significant anti-inflammatory effect and powerful hepatoprotective action against  $\text{CCl}_4$  induced hepatotoxicity in rats. Further investigation of hepatoprotective effect of demineralized, fraction I and fraction II glycans were done and the results were compared with that of the crude glycan. All of these glycans had powerful hepatoprotective action against  $\text{CCl}_4$  induced hepatotoxicity. Fraction I glycan (containing glucuronic acid) had the highest hepatoprotective activity.

## INTRODUCTION

Glycans are essential constituents of all living organisms and possess different vital functions. They constitute a structurally diverse class of biological macromolecules with a wide range of physicochemical properties, which are the basis for the different applications in the broad field of pharmacy and medicine<sup>1</sup>.

In the last decades, glycans of plant origin have emerged as an

important class of bioactive natural products. They showed antitumour<sup>2</sup>, immunological<sup>3&4</sup>, anticomplementary<sup>5</sup>, anti-inflammatory<sup>6</sup>, anticoagulant<sup>7</sup>, hypoglycemic<sup>8</sup>, antiviral<sup>9</sup> and hepatoprotective activities<sup>10&11</sup>.

The genus *Sonchus* (Asteraceae family) comprises more than 50 species. *Sonchus oleraceus* L. is one species of this genus<sup>12&13</sup>. It grows wild in different localities of Egypt. Many reports were found concerning the constituents of *Sonchus* species. These reports

include: isolation of sesquiterpene lactones, triterpenes, sterols, flavonoids, coumarins, vitamins, minerals, proteins and amino acids. In addition to the previously mentioned constituents, *S. oleraceus* L. contains traces of saponins and a novel disaccharide ( -D-erythrofuranosyl-(1→5)- -D-glucopyranoside) as well as volatile components<sup>14-18</sup>.

The plant has been extensively used in folk medicine. The infusion of the roots and leaves was used as tonic and febrifuge. The plant was also used to relieve many gastrointestinal disturbances. In addition, it has diuretic, mucolytic and expectorant effects. An infusion of the herb, when locally applied is efficient as anti-inflammatory and emollient in several skin conditions<sup>19</sup>.

Rutowitsch *et al.*<sup>20</sup> reported that *S. oleraceus* L. was successfully used for the treatment of two cases of vitiligo. The plant leaves are used as a source of provitamin A in Brazil<sup>21</sup>.

*S. oleraceus* L. is a component in the diet of New-Zealand Maori people. It had plausible colorectal cancer protective properties<sup>22</sup>. Nehir *et al.*<sup>23</sup> reported that the radical scavenging and iron-chelating activities were obtained from *S. oleraceus* L. which used as traditional dishes in Mediterranean diet.

The present work was planned to investigate the following items:

- 1- Separation of the crude glycan from *S. oleraceus* L. Purification and determination of the monomeric composition of the separated glycan.
- 2- Biological screening for anti-inflammatory and hepatoprotective effects for the isolated crude glycan.
- 3- Separation of individual glycans from the crude glycan. Study the hepatoprotective activity of some of the separated individual glycans.

## MATERIALS AND METHODS

### Plant materials

*S. oleraceus* L. herb was obtained from plants growing in Gharbia Governorate (Egypt) during the flowering stage from February to May 2001-2002. Identity was kindly verified by Prof. Dr. Abd El-Fatah Badr, Head of Botany Dept., Faculty of Science, Tanta University. The air dried collected plant organ was reduced to fine powder, particle size range was from (400-500 µm) then packed in tightly closed container.

### Chemicals

#### For glycans study

Sugars (authentic): glucose anhydrous, galactose anhydrous, fructose, xylose, rhamnose, arabinose, D-galacturonic acid and D-glucuronic acid were purchased from Sigma Chemical Co., St. Louis, Mo, U.S.A.

### **For biological studies**

Liver enzyme kits ALT and AST (Diamond Diagnostic Co., Egypt). Carrageenan sodium salt (BDH-England). Indomethacin (Sigma Chemical Co., St. Louis, Mo, U.S.A). All the remaining chemicals were of the highest analytical grade commercially available.

### **Experimental animals**

Male albino rats, 80-100 g., fed on normal diet were used for anti-inflammatory studies.

Male albino rats, 100-120 g., fed on normal diet were used for hepatoprotective studies.

### **Apparatus**

- 1- Spectrophotometer (UV/VIS) V-530. (Japan).
- 2- pH meter: membrane pH meter, HI 8314, HANNA instrument. (Portugal).
- 3- GC/MS: Varian equipped with Finnigan mat SSQ 7000 mass spectrometer, Fabricated by Thermo Inst. Sys. Inc., (U.S.A) Column: DB-5, (30 m x 0.25 mm), fabricated by J. & W. Sci., (U.S.A).

### **Experimental protocol**

#### **I- Phytochemical studies**

##### **A- Extraction and isolation of crude glycan**

Dried powdered (100 g) of *S. oleraceus* L. herb were extracted with boiling distilled water by placing on a boiling water bath with

occasional stirring for one hour, then filtered while hot and the process was repeated twice for the marc. The combined extracts in each case were concentrated under vacuum at a temperature not exceeding 70°C. The concentrated solution was mixed with three times of its volume with ethanol and the precipitated polysaccharide was washed successively several times with 70% ethanol, 95% ethanol and finally with acetone, then left in acetone overnight. The crude polysaccharide complex was separated by centrifugation, dried under vacuum and kept in a desiccator over anhydrous sodium sulphate<sup>24</sup>. The yield was (10 g).

##### **B- Determination of monomeric composition of crude glycan of *S. oleraceus* L. herb**

The crude polysaccharide complex was subjected to further investigation to determine its monomeric composition according to the following steps<sup>24&25</sup>.

- 1- Partial hydrolysis of the polysaccharide complex .
- 2- Complete hydrolysis of the polysaccharide complex
- 3- Identification of the acidic and neutral hydrolysates using and GC/MS analysis.

##### **[1] Partial hydrolysis of the polysaccharide complex**

- a) The crude polysaccharide (200 mg) was hydrolysed with 10 ml

of 1N sulphuric acid for 7 hours on a boiling water bath.

- b) The partially hydrolysed solution of glycan was cooled to room temperature then neutralized with solid barium carbonate and filtered from barium sulphate through a filter paper and the precipitate of barium sulphate was washed twice with distilled water.
- c) The combined filterates were concentrated under vacuum to minimal volume and three volumes of ethyl alcohol were added.  
The acidic monomers (mainly barium salt of uronic acid) were precipitated and separated from the mother liquor which contains the neutral monomers.
- d) Demineralization of the precipitated acidic monomer was done using cation exchange resin (Dowex 50W-X8).
- e) The acidic and neutral monomers of the partially hydrolysed polysaccharide complex were silylated for GC/MS analysis.

## [2] Complete hydrolysis of the polysaccharide complex

The same previously mentioned procedure for the partial hydrolysis of the polysaccharide was carried out but with increasing the time of hydrolysis up to 24 hours. For further investigation of acidic and neutral monomers GC/MS determination would be carried out.

## [3] GC/MS identification of the neutral and acidic monomers

The produced monomers of glycans of *S. oleraceus* L. were silylated and subjected to GC/MS analysis, using GC Varian equipped with SSQ 7000 mass spectrometer; (1  $\mu$ L) of each sample was injected to DB-5 column (30 m x 0.25 mm), using helium as carrier gas. Column oven temperature was programmed as follows: starting with 100°C for 3 minutes, then increasing at a rate of 8°C/ minute till 300°C and kept isothermal at 300°C for 5 minutes. (total time 33 minutes).

## C- Demineralization of polysaccharide complex

- 1- The dried powder (30 g) of crude glycan was dissolved in minimal amount of distilled water (450 ml).
- 2- Concentrated HCl (4.5 ml) was added to the aqueous solution of glycan (to obtain solution containing 1% HCl).
- 3- Three volumes of 95% ethanol containing 1% HCl gradually was added to precipitate the demineralized polysaccharide complex.
- 4- The precipitate was successively washed with 70% ethanol containing 1% HCl, then washed several times with 70% ethanol until free from chloride ions, then washed with 95% ethanol and finally with acetone, then left in acetone over night. The demineralized polysaccharide

complex was separated by centrifugation, dried and kept in a desiccator over anhydrous sodium sulphate.

#### D- Saponification

- 1- The dried demineralized polysaccharide complex (10 g) was dissolved in (400 ml) distilled water to obtain homogenous solution. 0.1 N sodium hydroxide was added dropwise to obtain solution of pH (11:12).
- 2- The obtained solution was kept at 11°C for 2 hours with occasional shaking, then 18 ml of 5% HCl was added. A gel precipitate was formed, separated by centrifugation and termed fraction I.
- 3- The precipitated fraction I was successively washed first with 1% HCl followed by 30% ethanol, 70% ethanol, 95% ethanol and finally acetone. The washed precipitated fraction I was left over night in acetone, dried, weighed and kept in a desiccator over anhydrous sodium sulphate.
- 4- The non-precipitated fraction which in the mother liquor obtained from step 2 was precipitated by addition of three volumes 95% ethanol left for 2 hours and separated by centrifugation and termed fraction II.
- 5- The precipitated fraction II was successively washed with 70%

ethanol, 95% ethanol and acetone, then left in acetone overnight, separated by centrifugation, dried under vacuum, weighed and kept in a desiccator over anhydrous sodium sulphate<sup>24</sup>.

#### E- Fractionation of demineralized polysaccharide complex using Diethylaminoethyl-cellulose (DEAE-cellulose)<sup>24</sup>

The capacity of DEAE-cellulose was [1 mol equivalent /g. dried material].

Twenty five g of DEAE cellulose were activated by addition of 1L 0.5 N HCl for 30 minutes. after that the supernatant liquid was poured and the procedure was repeated for 3 times. The precipitate DEAE cellulose was separated from the mother liquor and washed with distilled water till neutral reaction and was dried in air. The anion exchange resin was dispersed in 0.5 N solution of phosphate buffer pH 6 and was left for 30 minutes with occasional shaking then was separated by filtration from phosphate buffer and the process was repeated 6 times. The anion exchange resin was washed with distilled water till neutral reaction and dried in air.

- 1- The previously prepared 20 g of DEAE-cellulose in phosphate buffer pH 6 were dispersed in D.W. and the suspension was transferred to column (25 cm x

- 1.5 cm), the column washed with distilled water.
- 2- The concentrated aqueous solution of demineralized polysaccharide complex (0.2 g) [which was prepared by dispersion in distilled water then 0.5 N ammonium hydroxide solution was added dropwise carefully to pH 7.1 to ensure complete solubility] was fractionated on the prepared DEAE-cellulose column. Gradient elution was carried out using (0.1 N - 0.5 N) phosphate buffer, 0.1 N solution NaOH and finally 0.2 N solution of Na OH. Fractions 30 ml each were collected.
  - 3- Elution being monitored spectrophotometrically at 490 nm using phenol-sulphuric acid reagent.

#### **F- Refractionation of fraction I using sodium acetate**

- 1- One gram of fraction I was dissolved in 100 ml distilled water containing 4 ml 1 N NaOH then filtered through filter paper.
- 2- Six ml of 2 N sodium acetate solution was added and the solution was shaken for 24 hours at room temperature.
- 3- A precipitate was formed, separated from the mother liquor by centrifugation.
- 4- To precipitate the other fraction, three volumes of 95% ethanol was added to the mother liquor.

- 5- Each of the separated fractions were successively washed with 70% ethanol, 95% ethanol and acetone, then left in acetone over night. Both fractions were separated from acetone by centrifugation, dried under vacuum and kept in a desiccator over anhydrous sodium sulphate<sup>26</sup>.

#### **G- Refractionation of fraction I using cetyl trimethyl ammonium bromide (cetavlon)**

- 1- One gram of fraction I was dispersed in 10 ml distilled water then 0.5 N ammonium hydroxide solution was added drop wise carefully to pH 7.1 to ensure complete solubility.
- 2- The dissolved fraction I was transferred into 50 ml volumetric flask and the distilled water was added to the mark.
- 3- The solution was mixed with 50 ml 3% cetavlon in water, by gradual shaking white precipitate was formed, separated by centrifugation and washed four times with 50 ml of each with distilled water The precipitate was formed.
- 4- To remove the excess cetavlon, the mother liquor after centrifugation was treated with 15 ml of 5% potassium iodide, and 25 ml of 10% sodium chloride. The precipitate was removed by filtration and the filtrate was extracted with chloroform four times, then the aqueous layer

was concentrated and diluted by adding four times volume ethanol, a precipitate was formed, separated by centrifugation.

- 5- The separated fractions were successively washed with 70% ethanol, 95% ethanol and acetone, then left in acetone over night. Both fractions were separated from acetone by centrifugation, dried under vacuum and kept in a desiccator over anhydrous sodium sulphate<sup>24&26</sup>.

## II- Biological studies

The prepared crude glycan of *S. oleraceus* L. herb was studied for anti-inflammatory and hepatoprotective activities.

### A- Anti-inflammatory effect

Fourty (40) male albino rats, weighing 80-100 g were used in this study. The rats were uniformly hydrated (3 ml water /rat) on the day of experiment by oral gavage, such treatment was reported to reduce the variability of oedema response. The rats were divided into 8 groups; each of 5 animals and the aqueous solution of each glycan was intraperitoneally injected. One hour later, 0.5 ml of 1% carrageenan solution was injected subcutaneously into the right hind paw of each animal in all groups. The left hind paw was injected subcutaneously with 0.5 ml of saline. After 4 hours of induction of inflammation, the animals were

killed and both right and left hind paw of each animal were cut and weighed. The difference in weight between the right and the left hind paw of each animal was corresponding to the extent of the produced oedema<sup>27</sup>.

The percentage inhibition of inflammation was calculated according to the following equation:

% inhibition of inflammation =

$$\frac{\text{paw oedema of (-ve) control} - \text{paw oedema of drug}}{\text{paw oedema of control}} \times 100$$

### B- Hepatoprotective effect

Thirty (30) male albino rats weighing 100-120 g were divided into 6 groups each consisting of 5 animals. The tested substances were intraperitoneally injected according to the following design

- Group 1: Receiving 5 ml/kg b.w. of normal saline.
- Group 2: Receiving 5 ml/kg b.w. of liquid paraffin
- Group 3: Receiving 5 ml/kg b.w. of 10% CCl<sub>4</sub> in liquid paraffin (hepatotoxic group).
- Group 4,5,6: Treated groups. Liver damage in rats was induced according to Klassan and Plae method<sup>27</sup> by the intraperitoneal injection of 5 ml/kg b.w. of 10% CCl<sub>4</sub> solution in liquid paraffin in three different treatments.



**i) Pre-treated group**

Receiving 0.1 g/kg b.w. of aqueous solution of crude glycan one hour before induction of hepatotoxicity.

**ii) Concomitant-treated group**

Receiving 0.1 g/kg b.w. of aqueous solution of crude glycan concomitantly with induction of hepatotoxicity.

**iii) Post-treated group**

Receiving 0.1 g/kg b.w. of aqueous solution of crude glycan one hour after induction of hepatotoxicity.

**C- Hepatoprotective effect of demineralized, fraction I and fraction II glycans separated from crude glycan of *S. oleraceus* L. herb**

The same procedure was adopted using 0.1 g/kg b.w. of demineralized, fraction I and fraction II.

Blood samples were collected 24 hours after injection, by direct withdrawal from the heart. Blood was left to clot and then centrifuged to separate sera. Alanine amino-transferase (ALT) and Aspartate amino-transferase (AST) were assessed according to the procedure described by the instructions of the commercial Kits (Diamond Diagnostic Co., Egypt).

Dissection of rats were done 24 hours after injection and livers from each group were transferred just

after dissection of rats to stoppered jars containing 10% formalin in normal saline and were kept for histopathological study. Paraffin embeded tissue sections were cut at 3-5  $\mu$ m, stained with hematoxylin-eosin stains (Hx and E) to be studied by light microscope.

Statistical analysis: Data are expressed as (mean  $\pm$  S. E. Standard Error).

**RESULTS AND DISCUSSION**

Polysaccharides, or glycans are homo or hetero polymers of monosaccharides joined together by glycosidic linkage. They possess complex structures due to the presence of many types of inter-sugar linkages involving different monosaccharide residues. Polysaccharides are usually isolated by precipitation with alcohol from the water extract of the plant material<sup>28</sup>.

**Determination of monomeric composition of crude glycan of *S. oleraceus* L. herb**

The crude polysaccharide complex was an amorphous powder brownish in color. The pH of 1% aqueous solution was 5.4 It was soluble in water, dilute mineral acids and alkalies and insoluble in organic solvents. Both acidic and neutral monomers of partially and completely hydrolysed polysaccharide complex were subjected to silylation and GC/MS analysis. The

results of GC/MS analysis are listed in Table (1).

The results recorded in Table (1) revealed that the crude *S. oleraceus* L. polysaccharide complex is composed of xylose, arabinose, mannose, galactose, glucose and glucuronic acid. The percentage of each monomer after partial hydrolysis was different from that after complete hydrolysis, as the time of complete hydrolysis was enough to hydrolyse any polymer present in the core of the polysaccharide complex.

#### Fractionation of polysaccharide complex of *S. oleraceus* L. herb

In order to determine the polymeric composition of the polysaccharide complex (fractionation into individual polymer), different methods for fractionation were used. If no change in the monomeric composition of the separated fractions qualitatively or quantitatively that is indicated the homogeneity of the polysaccharide

complex. For fractionation of the polysaccharide complex, demineralization was done first, then the methoxyl group which may be present at the carboxyl group (-COOCH<sub>3</sub>) was removed by saponification.

The obtained demineralized polysaccharide complex was an amorphous powder yellowish brown in color. Starting with (30 g) crude glycan, the yield of the demineralized glycan was (12.5 g). The determined ash content of the powder was (0.4% w/w).

#### Saponification

Using (10 g) of the demineralized glycan, fractions I & II were obtained. Fraction I (6 g) appeared as an amorphous brown powder insoluble in water and dilute acids but soluble in aqueous solution of alkalies. Fraction II (360 mg) appeared as an amorphous white powder soluble in water and aqueous solutions of acids.

**Table 1:** Monomeric composition of *S. oleraceus* L. herb crude glycan as determined by GC/MS analysis.

Monomers	% of monomers in partial hydrolysates	% of monomers in complete hydrolysates
1. Xylose	7.54	4.53
2. Arabinose	4.17	3.07
3. Mannose.	18.1	3.39
4. Galactose	3.54	15.87
5. Glucose	12.18	38.52
6. Glucuronic acid	54.47	34.62

### Fractionation of the demineralized polysaccharide complex using DEAE-cellulose

The demineralized glycan was chromatographed on DEAE-cellulose column. Elution being monitored spectrophotometrically using phenol-sulphuric acid reagent. The results of the absorbance (at 490 nm) of the collected fractions were illustrated in Fig. 1. From the figure the demineralized glycan composed of one polymer (Retention volume: 2520 ml; fraction 84).

### Refractionation of fraction I

Fraction I was further fractionated by different methods:

1- Refractionation using sodium acetate: One gram of fraction I was fractionated to 2 sub-fractions FI-a (180 mg) and FI-b (130 mg).

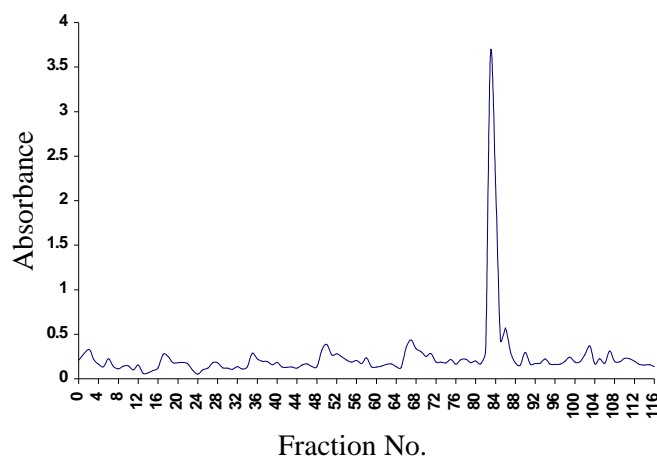
2- Refractionation using (cetyl trimethyl ammonium bromide): One gram of fraction I was fractionated to 2 sub-fractions FI-c (110 mg) and FI-d (20 mg).

### Investigation of the monomeric composition of the separated fractions

The separated fractions were subjected to partial hydrolysis and the obtained acidic and neutral monomers were subjected to silylation and GC/MS analysis. The results revealed that the monomeric composition of various fractions was as follows:

The monomeric composition of the separated fractions

1- Fraction I (acidic) was composed of mannose (27.78%), xylose (9.80%), galactose (9.01%), glucose (29.25%) and glucuronic acid (24.16%).



**Fig. 1:** Elution profile of demineralized polysaccharide complex of *S. oleraceus* L. herb on DEAE-cellulose monitored spectrophotometrically by phenol-sulphuric acid reagent.

- 2- Fraction II (neutral) was composed of xylose (20.55%), arabinose (5.08%), galactose (9.89%) and glucose (64.48%).
- 3- Fraction I-a was composed of mannose (16.21%), xylose (5.55%), galactose (1.83%), glucose (10.37%) and glucuronic acid (66.04%).
- 4- Fraction I-b was composed of xylose (36.55%) and glucose (63.45%).
- 5- Fraction I-c was composed of mannose (72.67%), glucose (16.03%) and glucuronic acid (11.30%).
- 6- Fraction I-d was composed of mannose (62.64%), xylose (6.68%) and galactose (30.68%).

From the results it can be concluded that the monomeric composition of the two sub-fractions in each case differed quantitatively from one method to another. So, the polysaccharide complex of *S. oleraceus* L. herb was an acidic heterogenous polysaccharide mixture

Summary of fractionation of polysaccharide complex and the yields of each fractions are illustrated in scheme I.

### Biological investigations

#### Anti-inflammatory effect of the crude glycan

Winter *et al.*<sup>29</sup> and Capasso *et al.*<sup>27</sup> methods were adopted. The methods are based on the ability of drugs to inhibit the oedema

produced in the hind paw of rats by injecting a small dose of an inflammatory agent such as carrageenan in the plantar tissue of the paw. The degree of inhibition of oedema was measured by the difference in weight between the rat hind paw injected only by the oedemogenic agent and that injected by aqueous extracts of glycans in different dose levels. Results are recorded in Table 2 and illustrated in Fig. 2.

From Table 1 and Fig. 2, the crude glycan of *S. oleraceus* L. herb showed significant anti-inflammatory activity. The percent inhibition of inflammation was increased by increasing the dose till 1 g/kg b.w.

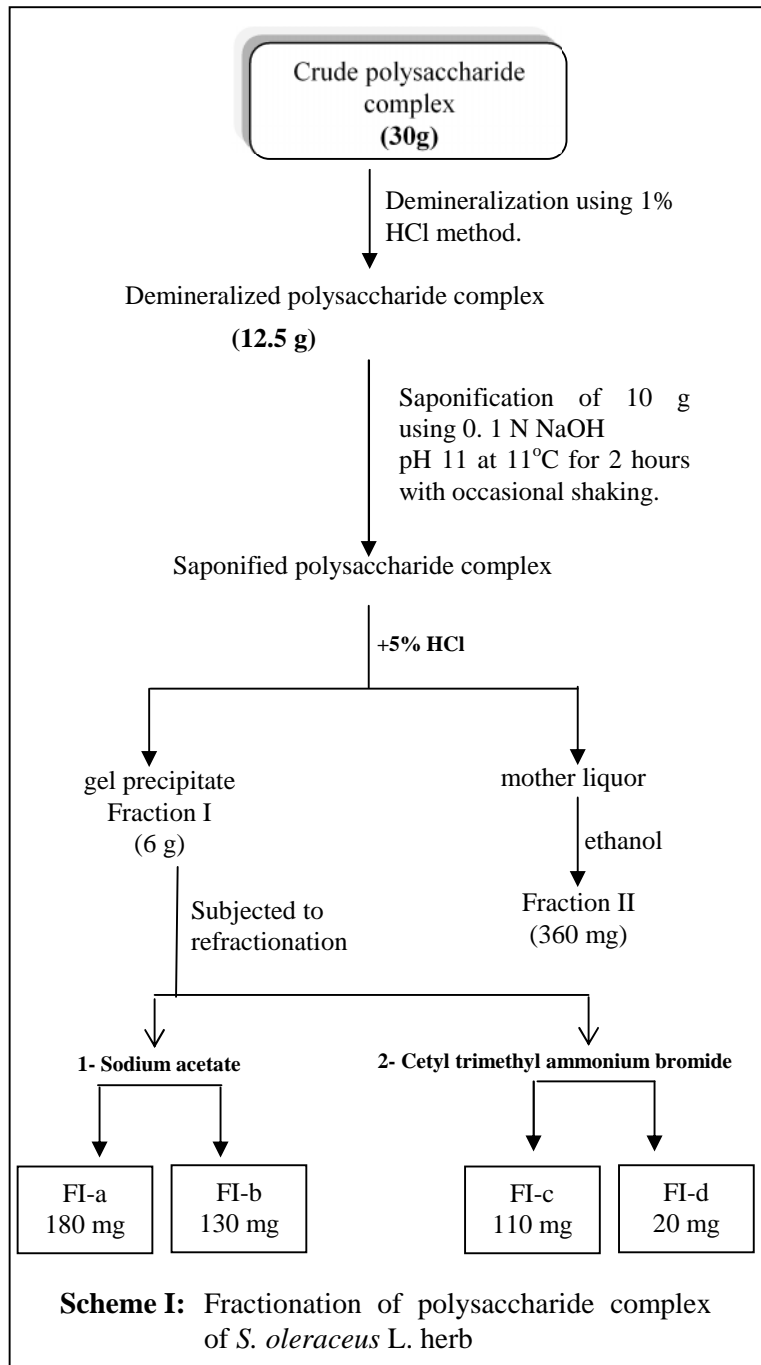
#### Hepatoprotective effect

The use of herbal drugs in the treatment of liver diseases had a long tradition in folk medicine. Herbal drugs usually protect the liver from oxidative injury, promote virus elimination, block fibrogenesis or inhibit tumor growth<sup>10&11</sup>.

#### Two parameters were used for studying the hepatoprotective effect

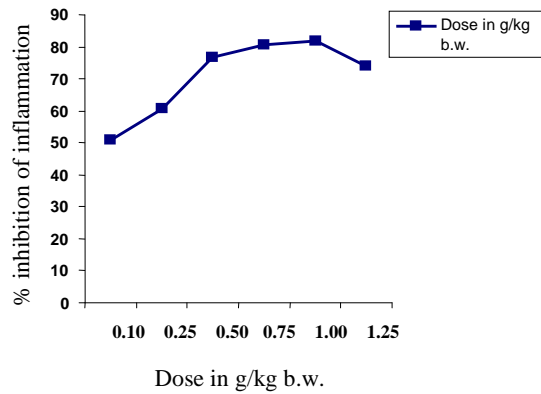
- 1- Determination of serum ALT and AST.
- 2- Histopathological study of the liver.

The intraperitoneal injection of aqueous solution of crude glycan of *S. oleraceus* L. herb significantly



**Table 2:** Percent inhibition of inflammation using different doses of crude glycan separated from *S. oleraceus* L. herb.

Group	Dose g/kg b.w.	Mean differences in rat paws M ± S.E.	% inhibition of inflammation
Control (-ve)	--	0.78±0.004	0.00
Indomethacin	0.005	0.34±0.002	56.41
<i>S. oleraceus</i> L. herb	0.10	0.38±0.003	50.77
	0.25	0.23±0.003	60.77
	0.50	0.18±0.003	76.72
	0.75	0.15±0.003	80.51
	1.00	0.14±0.002	81.79
	1.25	0.20±0.003	74.10



**Fig. 2:** Percent inhibition of inflammation produced by different doses of crude glycan of *S. oleraceus* L. herb.

decreased the ALT and AST serum levels if compared to the normal and hepatotoxic control animals as shown in Tables 3 and 4 and Figs. 3,4 and 5.

The results recorded in Tables (3 and 4) and illustrated in Figs. 3,4 and 5. revealed that;

The intraperitoneal injection of aqueous solution of crude glycans of *S. oleraceus* L. herb significantly decreased the ALT and AST serum levels if compared to the normal and hepatotoxic control animals. The highest values of % inhibition of hepatotoxicity were detected in pre-treated group.

#### **Investigation of the hepatoprotective effect of demineralized, fraction I and fraction II glycans separated from crude glycan of *S. oleraceus* L. herb**

Further investigation of hepatoprotective effect of some of the separated glycans as demineralized, fraction I and fraction II were performed and the results were compared with that of crude glycan.

The results are illustrated in Figs. 6 and 7 and revealed that the hepatoprotective investigations of glycans separated from *S. oleraceus* L. herb have proved that all of these glycans had powerful hepatoprotective action against CCl<sub>4</sub> induced hepatotoxicity in rats. Fraction I glycan (containing glucuronic acid) had the highest protective action.

#### **Histopathological examination**

CCl<sub>4</sub> induced extensive necrotic patches with severe congested central veins and minimal to moderate mononuclear cellular infiltration mainly lymphocytes Fig. 8A.

The intraperitoneal injection of aqueous solution of crude, demineralized, fraction I and fraction II glycans separated from *S. oleraceus* L. herb prevented the massive necrosis and showed very mild central zonal necrosis Fig. 8B. and mild peripheral vacular degeneration Fig. 8C. Control normal hisopathological structure of the liver is shown in Fig. 8D.

In conclusion, the hepatoprotective effect of glycans separated from *S. oleraceus* L. herb was confirmed from the biochemical analysis and histopathological study of liver sections of rats.

Fraction I glycan (acidic fraction containing glucuronic acid) has the highest significant protective action on the hepatotoxicity of CCl<sub>4</sub>, indicating that the presence of uronic acids in the polysaccharide complex may increase the hepatoprotective effect<sup>10</sup>.

The results suggested that the glycans separated from *S. oleraceus* L. herb provided liver protection in rat models of liver damage induced by CCl<sub>4</sub>.

**Table 3:** Effect of crude glycan separated from *S. oleraceus* L. herb on serum ALT in rats with CCl<sub>4</sub> induced hepatotoxicity.

Group	I. Pre-treated group		II. Concomitant treated group		III. Post- treated group	
	ALT serum level U/L M ± S. E.	% inhibition of hepatotoxicity	ALT serum level U/L M ± S. E.	% inhibition of hepatotoxicity	ALT serum level U/L M ± S. E.	% inhibition of hepatotoxicity
Normal (-ve control)	15.42±0.22	--	15.4 ± 0.22	--	15.42±0.22	--
Liquid paraffin	19.16±0.33	--	19.16±0.33	--	19.16±0.33	--
Hepatotoxic (+ve control)	59.90±0.34	--	59.9±0.34	--	59.90±0.34	--
<i>S. oleraceus</i> L. herb	17.00±0.30	71.62	17.58±0.26	70.65	17.34±0.18	71.05

M = Mean S. E. = standard error.

All groups are significantly different from hepatotoxic control at P < 0.05.

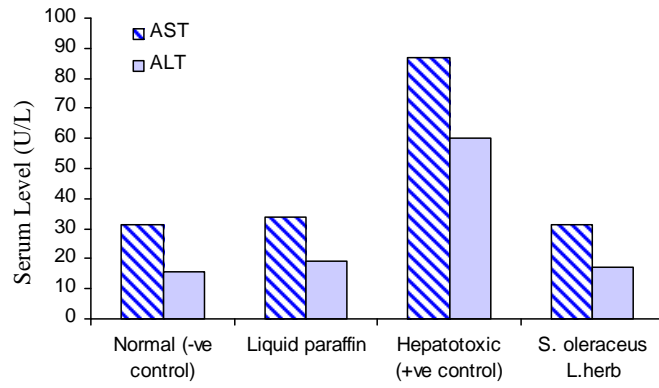
**Table 4:** Effect of crude glycan separated from *S. oleraceus* L. herb on serum AST in rats with CCl<sub>4</sub> induced hepatotoxicity.

Group	I) Pre treated group		II) Concomitant treated group		III) Post-treated group	
	AST serum level U/L M ± S.E.	% inhibition of hepatotoxicity	AST serum level U/L M ± S.E.	% inhibition of hepatotoxicity	AST serum level U/L M ± S.E.	% inhibition of hepatotoxicity
Normal (-ve control)	31.18±0.14	--	31.18±0.14	--	31.18±0.14	--
Liquid paraffin	33.88±0.31	--	33.88±0.31	--	33.88±0.31	--
Hepatotoxic (+ve control)	86.86±0.27	--	86.86±0.27	--	86.86±0.27	--
<i>S. oleraceus</i> L. herb	31.40±0.21	63.85	31.75±0.22	63.44	34.94±0.24	59.77

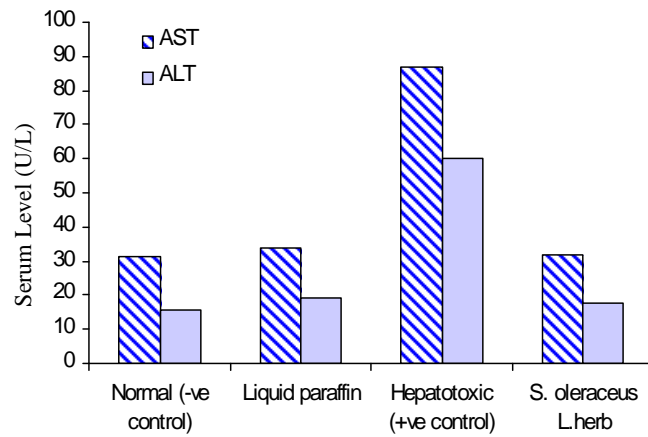
M = Mean S. E. = standard error.

All groups are significantly different from hepatotoxic control at P < 0.05.

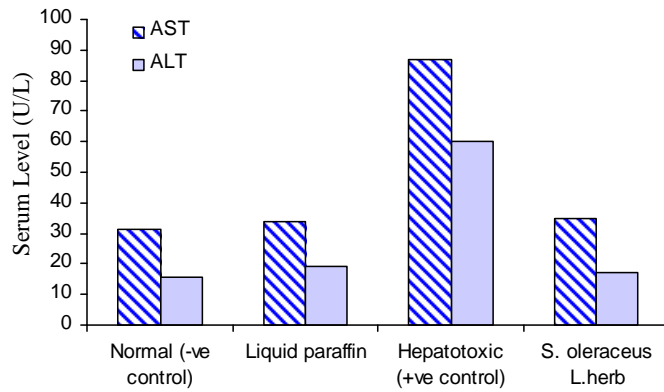




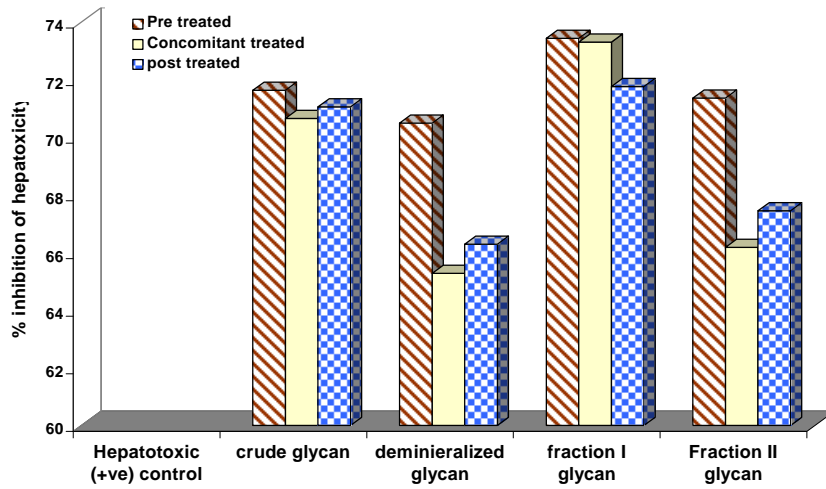
**Fig. 3:** Effect of crude glycan of *S. oleraceus* L. herb on serum ALT and AST levels in "pre-treated group"



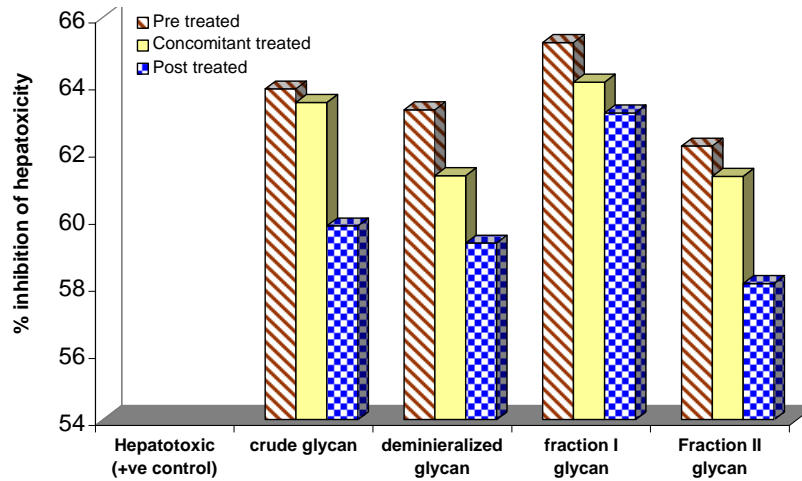
**Fig. 4:** Effect of crude glycan of *S. oleraceus* L. herb. on serum ALT and AST levels in "concomitant-treated group".



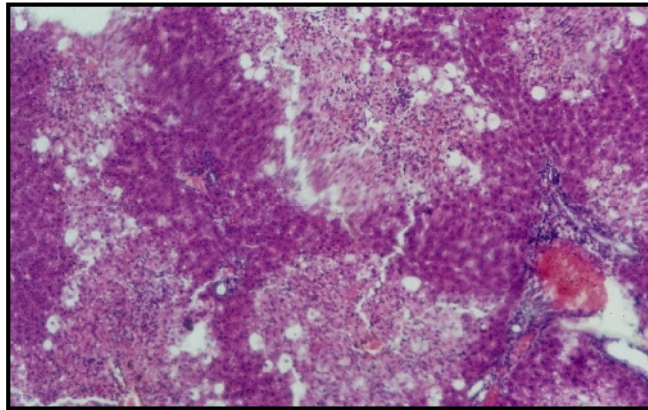
**Fig. 5:** Effect of crude glycan of *S. oleraceus* L. on serum ALT and AST levels in "post-treated group"



**Fig. 6:** Percent inhibition of hepatotoxicity of different glycans of *S. oleraceus* L. herb regarding ALT serum level values.

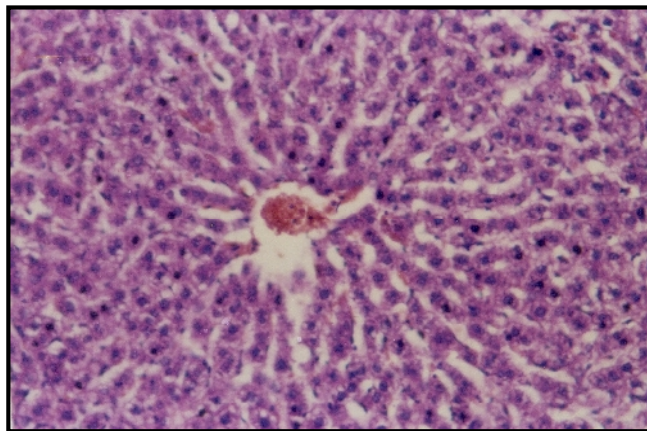


**Fig. 7:** Percent inhibition of hepatotoxicity of different glycans of *S. oleraceus* L. herb regarding AST serum level values.



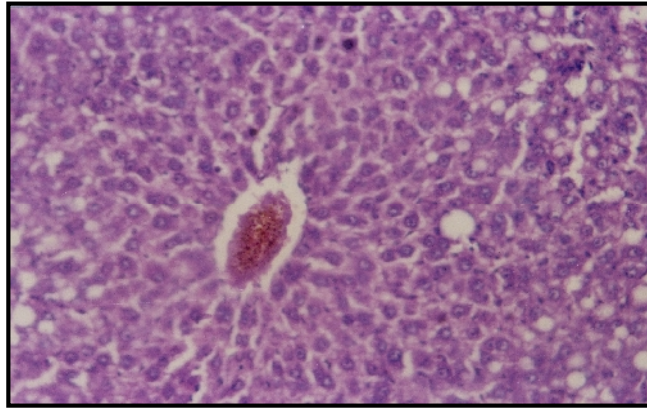
**Fig. 8A:** Liver section from an animal showing extensive necrotic patches with severely congested central vein and minimal to moderate mononuclear cellular infiltration mainly lymphocytes.

[Hx and E X 125]



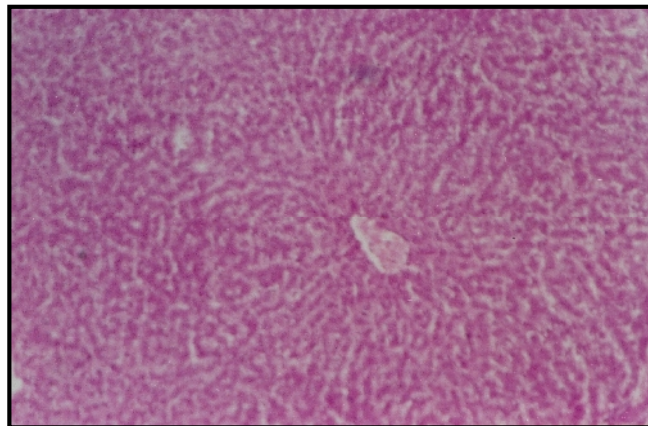
**Fig. 8B:** Liver section from an animal showing very mild central zonal necrosis.

[Hx and E X 250]



**Fig. 8C:** Liver section from an animal showing mild peripheral vacular degeneration.

[Hx and E X 250]



**Fig. 8D:** Liver section from an animal showing mild dilatation of central vein and blood sinusoids.

[Hx and E X 125]

### Acknowledgement

Our sincere thanks and gratefulness are expressed to Prof. Dr. Karima El-Dosoky Professor of Pathology, Faculty of Medicine, Tanta University, for her kind assistance and unlimited help in carrying out the histopathological study in this work.

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