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THE HEPATOPROTECTIVE AND ANTI-FIBROTIC ACTIVITIES OF FREEZE-DRIED SAPONIN AND TANNIN EXTRACTS ON CCl_4 - INDUCED HEPATOTOXICITY IN RATS.

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ABSTRACT

Saponins of defatted soybean seeds and tannins of faba bean hulls were extracted, freeze-dried and the chemical composition was determined before and after extraction and freeze-drying.

The hepatoprotective and anti-fibrotic activities of these freeze-dried extracts on carbon tetrachloride (CCl_4)-induced hepatotoxicity on rats were evaluated.

Repeated administration of CCl_4 subcutaneously (S.C) on rats for 8 weeks significantly elevated serum enzymes activity assessted for liver function, i.e., AST, ALT and ALP. Also, the same administration accumulated and deposited the hepatic collagen protein of rats.

The altered chemical parameters and the accumulated and deposited collagen were significantly benefited by the both freeze-dried extracts administration per orally (P.O). The results show that both freeze-dried saponin and tannin extracts have a hepatoprotective and anti-fibrotic activities against CCl_4 induced hepatotoxicity in rats, but the mechanism and the active compounds of these extracts require further studies.

INTRODUCTION

Legumes have diverse uses and roles in agriculture and environmental protection. (D' Mello and Devendra, 1995).

Legumes play an important role in the traditional diets of many regions except so the world especially, in the Middle Eastern region. In contrast, in Western countries beans tend to play only a minor dietary role despite the fact, that they are low in fat and are excellent sources of protein, dietary fiber, and a variety of micronutrients and phytochemicals (Messina, 1999).

Soybeans have many unique phytochemicals including isoflavones, saponins, phytates, phytosterols, phenolic acids and trypsin inhibitors (Wang and Wixan, 1999). Also, faba bean hulls are rich in proanthocyanidines (PAs) (condensed tannins) (Cabrera and Martin, 1986).

Many phytochemicals have been considered antinutrient in traditional nutritional theory. However, all of these compounds have been cited as important in prevention of degeneration conditions such as heart diseases and cancers *(Wang and Wixan, 1999)*.

Carbon tetrachloride (CCl_4) is a potent toxic agent produces liver injury in many species *(Slater,1984)* and different kinds of hepatic lesions including hepatocellular necrosis and subsequent regeneration or cirrhosis. As the injury spreads within the cell, all the mitochondrial properties and the cellular elements affected (Trivedi &Mowat, 1983).

As a result of increased interest and intensive research activity in food phytochemicals of plant origin, the present investigation was carried out to study and evaluate faba bean tannins and soybean saponins as a hepatoprotective and anti-fibrotic compounds on chronic liver injury and fibrosis induced by chemical toxic agent (CCl_4) .

MATERIALS AND METHODS

Materials

Faba bean hulls and soybean seeds:

Commercial faba bean (*Vicia faba*) hulls were obtained from local market , Giza, Egypt. Soy bean (*Glycin max*) seeds were obtained from Soybean Processing Factory, Food Technology Research Instutute, Agriculture Research Center, Giza, Egypt.

Standards:

Crystalline catechin, saponin and pyrogallol were obtained from Sigma Chemical Co., Saint Louis, Missori, USA. 4-hydroxy trans-proline was obtained from Fluka Chemical Co..

Solvents:

Acetone , ethyl alcohol and propanol were further distillated before use.

Animals:

Thirty-six Winster albino rats were obtained from Organization of Biological Products and Vaccines (OBPV), Helwan Farm, Helwar.-Cairo, Egypt.

Carbon tetrachloride:

Carbon tetrachloride (CCl_4) was obtained from Feinchemie KG., Sebnitz, Germany.

Kits:

Kits of Glutamate – Oxalate – Transaminase (GOT) / (AST), Glutamate– Pyruvate – Transaminase (GPT) / (ALT) and Alkaline Phosphatase (ALP) were obtained from Biodiagnostic Co., 29 El-Tahreer St., Dokki-Giza, Egypt.

Other chemicals:

All other reagents and chemicals were of reagent grade and were used without further purification.

Methods:

Faba bean tannin extraction:

Tannins from commercial faba bean hulls were extracted and freeze-dried according to *Treviño et al. (1992).*

Determination of total phenolic and condensed tannin contents:

Total phenolic and condensed tannin contents of faba bean hulls(T) before extraction and after extraction and freeze-drying((T^1) were determined by the Folin-Denis *(Burns, 1963)* and Vanillin-Hydrochloric Acid *(Broadhurst and Jones, 1978)* methods, respectively.

Soybean saponin extraction:

Saponins from defatted soybean seeds were extracted according to *Ikedo et al. (1996)* then the obtained extract was concentrated and freeze-dried.

Determination of saponin content:

G_6:CCl_4/$T^{\backslash}$

Saponins of defatted soybean seeds(S) and the freeze-dried extract($S^{\backslash}$) were determined by the method of *Hiai et al. (1976)* .

Chemical composition:

The dried faba bean hulls(T), freeze-dried tannin extract($T^{\backslash}$), dried defatted soybean seeds(S) and freeze-dried saponin extract($S^{\backslash}$) were analyzed for crude protein, ether extract, crude fiber and ash according to AOAC (1995).

Animal adaptation and administration protocols:

Animals were fed with a mixture of feed and barley (3:1W:W) until a constant weight was reached 210 to 360 g, then they were housed in individual cages, the houses were kept at 25±5°C, and a 12 hs, light/dark cycle. Then animals were fed with a standard laboratory diet until the end of the experiment. After an adaptation period for 7 days, rats were divided into six homogenous groups each of six as follows:

Group (1): Served as a normal control group, receiving paraffin oil (3mL/.Kg,S.C.) two times per week for eight weeks, and normal saline (5 mL/.Kg,P.O.), four times per week for 8 weeks, at intervals with paraffin oil dose.

Group (2): Served as injured group, receiving 40% CCl_4 (3mL/.Kg,S.C.) in G_1: **control** paraffin oil, two times per week for eight weeks, and normal saline (5 mL/.Kg, P.O.), four times per week for 8 weeks, at intervals with CCl_4 dose.

Group (3): Served as saponin group, receiving paraffin oil (3mL/Kg,S.C) two G_2:CCl_4 times per week for eight weeks, and freeze-dried saponin extract(0.125g /5 $mL.Kg^{-1}$,P.O.), four times per week for 8 weeks, at intervals with paraffin oil dose.

Group (4): Served as tannin group, receiving paraffin oil (3mL/.Kg,S.C.) two times per week for eight weeks, and freeze-G_3:$S^{\backslash}$ dried tannin extract(0.125g / 5mL / Kg,P.O.), four times per week for 8 weeks, at intervals with paraffin oil dose.

Group(5): Served as saponin – CCl4 treatment, receiving 40% CCl_4paraffin G_4:$T^{\backslash}$ oil (3mL/Kg,S.C.) two times per week for eight weeks, and freeze-dried saponin extract (0.125g /5 mL/Kg,P.O.), four times per weeks for 8 weeks, at intervals with CCl_4 dose.

Group(6): Served as tannin – CCl_4 treatment, receiving 40% CCl_4/paraffin oil

G_5:CCl_4/S' (3mL / Kg,S.C.) two times per week for eight weeks, and freeze-dried tannin extract (0.125g /5 mL /Kg,P.O.), four times per week for 8 weeks, at intervals with CCl_4 dose.

(In the present study, we have been adjust the dose of the freeze-dried extracts to mimic the same dose of silymarin, a well known hepatoprotective drug, in the experimental rat model).

Assessment of liver function:

Blood was withdrawn from the inferior vena cava of the eye under diethyl ether anesthesia 24 h., 3, 6, and 8 weeks after all treatments, the blood left to co-agulate at room temperature for one hour, serum was separated by centrifugation at 3000 rpm/4°C/20mins.

Serum glutamate –oxalate- transaminase (sGOT)/(sAST) and serum glutamate- pyruvate-transaminase (sGPT)/(ALT) activities were measured according to *Reitman & Frankel (1957);* serum alkaline phosphatase (sALP) activity was measured according to *Belfied & Goldberg (1971).*

Assessment of anti-fibrotic property and collagen content:

At the end of eight weeks, all animals were killed, liver tissues were removed and liver sections were taken from right lobe.Then dehydrated by 95% ethyl alcohol for 5-6 h, and defatted by acetone for two days. The defatted tissues were dried in an oven at 110°C then grounded into powder,

collagen content and the anti-fibrotic property were estimated by the measurement of the content of hydroxyproline in the powderec livers according to the method of *Woessner (1961)* and modified by *laitinen et al. (1974).*

Statistical analysis:

The data obtained from the biological and biochemical evaluation were statistically analyzed according to *Snedecor and Cochran (1982).*

RESULTS AND DISCUSSIONS

Chemical composition:

Data in Table (1) represent the proximate composition of milled faba bean hulls (T) and defatted soybean seeds (S) before extraction of tannins and saponins.

Table (1): Proximate composition on dry weight basisof milled faba been hulls (T) and defatted soybean seeds (S) of tannins and saponins :

Sample	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	Phytochemicals (g/kg)
T	8.14	0.96	52.58	3.10	Total phenols 46.08
					Tannins20.11
S	48.95	8.16	8.29	8.25	Saponins 39.79

- **Tannins expressed as catechin equivalents.**
- **Total phenols expressed as pyrogallol equivalents**
- **Saponins expressed as standard saponin equivalents.**

Data in Table (2) represent the proximate composition of freeze- dried tannin extract (T') and freeze-dried saponin extract (S').

Table (2): Proximate composition on dry weight basis of freeze-dried tanins extract (T^1) and Freeze-dried saponin extract (S^1):

Sample	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	Phytochemicals (g/kg)
T^1	4.75	0.17	0.00	5.44	Total phenols 937.04
					Tannins 565.41
S^1	15.42	6.24	0.00	14.39	Saponins 420.50

- **Tannins expressed as catechin equivalents.**
- **Total phenols expressed as pyrogallol equivalents.**
- **Saponins expressed as saponin standard equivalents.**

Biological evaluation

Effect of saponin and tannin extracts on elevated serum transaminases:

The mean values of serum aspartate aminotransferase (AST) activities IU/L are shown in Table (3). It is clear that injection of 40% CCl_4-/paraffin oil (3mls/ Kg, S.C.) resulted in 2.99, 2.36, 2.96 and 5.53 folds increase in serum AST activities at 24 h., 3, 6 and 8 weeks, respectively in group (2) as compared to its normal control values.

Table (3): Effect of saponin and tannin extracts on elevated serum aspartate aminotransferase (AST) activities IU/L:

Groups	Experimental period			
	24h.	3 weeks	6weeks	8weeks
G_1 :Control	56.000^{J}±4.517	70.833^{J}±7.330	119.833^{HIJ}±22.26	260.833^{FG}±79.390
G_2: CCl_4	167.833^{HI}±8.180	166.833^{HI}±9.368	355.000^{E}±7.642	1443.333^{A}±64.700
Increment fold	2.99	2.36	2.96	5.53
G_3: S^1	50.000^{J}±2.966	105.833^{HIJ}±13.934	192.500^{HG} ±95.06	595.000^{C}±263.570
G_4: T^1	72.00^{0J}±2.191	87.000^{IJ}±6.899	316.67^{0FE}±36.148	461.000^{D}±101.499
G_5: CCl_4/S^1	128.000^{HIJ}±23.689	127.000^{HIJ}±10.78	288.333^{FE}±14.72	796.667^{B}±142.642
% recovery	23.73%	23.88	18.78%	44.80%
G_6 :CCl_4/T^1	137.167^{HIJ} ±6.463	101.500^{IJ}±5.857	329.333^{FE}±35.48	711.667^{B}±127.971
% recovery	18.27%	39.16%	7.23%	50.69%

LSD P≤0.05=87.493

*** Mean with the same letter (s) are not significantly different.**

The mean values of serum alanine aminotransferase (ALT) activities IU/L are shown in Table (4). It is clear that injection of 40% CCl4/ paraffin oil (3 mls/ Kg, S.C.) resulted in 1.74, 1.63, 1.30 and 4.57 folds increase in serum ALT activities at 24h., 3, 6, and 8 weeks, respectively in group (2) as compared to its normal control values.

Hepatocytes contain many enzymes that may be released into the blood if the cell membranes are damaged. An elevation of serum enzymes such as ALT and AST reflected damage to the hepatic parenchymal cells (Stacey *et al.*, 1993) .This was associated with a number of inflammatory disorders (Sinha & Saran, 1972 and Hoder & Wilkinson, 1980) and massive necrotic lesions of hepatocyte (Rees & Spector, 1961), because of their high concentrations and easy liberation from the hepatocyte cytoplasm (Kirchain & Gill, 1997).

Table (4): Effect of saponin and tannin extracts on elevated serum alanine aminotransferase (ALT) activities IU/L:-

Groups	Experimental period			
	24h.	3 weeks	6weeks	8weeks
G_1 :Control	22.333^{F} ±3.266	27.167^{F}±2.483	63.000^{EDF}±23.630	96.333^{ED}±26.912
G_2: CCl_4	38.833^{EF}±10.187	44.333^{EF}±9.374	81.833^{EDF}±14.865	439.830^{A}±61.157
Increment fold	1.74	1.63	1.30	4.57
G_3: S`	34.500^{EF}±9.116	26.5000^{F}±3.564	120.000^{D} ±58.652	364.167^{B}±163.230
G_4: T`	21.667^{F}±1.966	42.167^{EF}±19.135	119.333^{D}±8.066	354.000^{B}±53.889
G_5: CCl_4/S`	75.333^{EDF}±10.3864	67.50^{EDF}±25.177	119.167^{D}±24.983	302.500^{B}±155.748
G_6 :CCl_4/T`	55.333^{EF} ±2.944	56.000±5.329	217.167^{C}±31.391	333.333^{B}±72.296

LSD P≤0.05=62.106

*** Mean with the same letter (s) are not significantly different**

In the present study, administration of CCl_4 for 8 weeks resulted in a significant elevation of serum AST and ALT activities.

Several researchers reported the elevation of serum transaminases activities following the administration of toxic doses of CCl_4 in rats (*Danhof et al., 1985; Lin et al., 1998; Lind & Gandolfi, 1999; Chen et al., 2000; Lu et al., 2000 and Ohta & Sahashi, 2002).*

Despite numerous investigations, the mechanism of CCl_4-induced liver injury is due to its metabolism by the mixed-function oxidase system in the endoplasmic reticulum of the liver. The first step is a rapid reductive formation of trichloromethyl (CCl_3) radical by complexing with one or more of the cytochrome *P-450 (Recknagel & Glende, 1973).* CCl_3 is thought to induce lipidperoxidation resulting in the release of microsomal carboxylesterase and other enzymes such as aminotransferases into the extracellular compartment, including blood serum *(Zimmerman, 1978)* and this may consistence with the above results concerning the elevation of transaminase eneymes after CCl_4 in-toxicantion.

Data in Tables 3 and 4 show the normal control values of serum AST and ALT activities (IU/L) in group (1). Given freeze-dried saponin extract (0.125g/5mls /kg, P.O.) in group (3) and freeze-dried tannin extract (0.125g/5mls / kg, P.O.) in group (4) 4 times per week for six weeks did not significantly affected serum AST and ALT activities as compared to its normal control values.

In consistence with the above findings, rats fed on high tannin diets showed a considerable hypertrophy of the parotid glands accompanied by a large increase in their content of unique proline-rich proteins, these proteins have a very high affinity for tannins suggesting that function as a primary defence against dietary tannins immediately on their introduction to the digestive tract *(Mehansho et al., 1983).* Also, transaminases activity was not changed by anti-nutritional diet *(Yugarian et al., 1992 and El-Shemy et al., (2000).*

In the 8th week, there was an elevation of serum AST and ALT activity in groups 3 and 4. We therefore suggest that it may be due to an accumulation of freeze-dried extracts in the hepatocytes until the end of the

experimental period resulted in liver lesions which accompanied by an elevation of serum AST and ALT activities up to the normal control values.

Although, condensed tannins and saponins have strong antioxidative activity *in vitro* and can, in small doses, prevent CCl_4 induced liver injury, but larger doses result in liver lesions *(Lin et al., 1998)*.

From Table (3) it is clear that the significant reduction and recovery occurred in serum AST activity was 23.73, 23.88, 18.78 and 44.80% at 24h., 3, 6 and 8 weeks, respectively when freeze-dried saponin extract was administered (0.125g/5mls / kg, P.O.) in group (5) and 18.27, 39.16, 7.23 and 50.69% at 24 h., 3, 6, and 8 weeks, respectively when freeze-dried tannin extract was administrated (0.125g/5mls / kg, P.O.) in group (6) as compared to the injured group (2).

However, these freeze-dried extracts failed to reduce the elevation occurred in serum ALT activity at the 8th week, when CCl_4 was administered for 8 weeks Table (4) in groups (5 and 6).

The significant reduction and recovery occurred in serum AST activities due to administration of freeze-dried saponin and tannin extracts are in harmony with those of other investigators who assessed the hepatoprotective activities of other tannin and saponin extracts on decreasing activities of AST and ALT induced by CCl_4 *(Jeong & Park, 1998; Lin et al., 1998 and Chen et al., 2000)*.

Effect of saponin and tannin extracts on elevated serum alkaline phosphatase (ALP) activity IU/L:

The mean values of serum ALP activities (IU/L) are shown in Table (5). It is clear that injection of 40% CCl_4/paraffin oil (3mls / Kg, S.C.) resulted in 2.5, 2.76, 1.7 and 1.2 folds increase in serum ALP activities at 24h., 3, 6, and 8 weeks, respectively in group (2) as compared to its normal control values.

Table (5): Effect of saponin and tannin extracts on elevated serum alkaline phosphatase (ALP) activity IU/L:-

Groups	Experimental period			
	24h.	3 weeks	6weeks	8weeks
G_1 :Control	86.210^{JI} ±3.615	98.702^{HJI} ±4.91	147.223^{EFG} ±11.950	270.840^{B} ±36.284
G_2: CCl_4	215.857^{CD}±10.650	272.273^{B} ±37.026	246.143^{CB} ±34.114	333.010^{A} ±11.360
Increment fold	2.50	2.76	1.70	1.20
G_3: S	69.610^{J} ±6.150	133.890^{HFG} ±16.202	112.667^{HIG} ±20.151	219.253^{CD} ±44.260
G_4: T	101.108^{HJI} ±33.107	109.352^{HJIG} ±15.958	104.653^{HJI} ±20.797	169.162^{EF} ±54.217
G_5: CCl_4/S	91.288^{J} ±9.257	125.938^{HIG} ±8.261	102.593^{HJI} ±27.879	241.775^{CB} ±75.165
% recovery	57.71%	53.75%	58.32%	27.40%
G_6 :CCl_4/T	221.130^{CD} ±68.227	251.687^{CB} ±29.155	181.755^{ED} ±59.426	271.965^{B} ±50.055
% recovery	2.44%	7.56%	26.16%	18.33%

LSD P≤0.05= 40.27

*** Mean with the same letter (s) are not significantly different**

Hepatocytes contain many enzymes that may be released into the blood if the cell membranes are damage. Elevated levels of alkaline phosphatase indicate injury to biliary apparatus of the liver *(Zimmerman, 1978 & 1982 and Stacey et al., 1993)*. Increase in ALP appears to result from

increased hepatic synthesis of the enzyme rather than leakage from bile duct or failure to clear circulation *(Martin & Friedman, 1998)*.

Drug induced hepatocellular injury is associated with rise in alkaline phosphatase level usually to less than three times the upper limit, unlike the massive increases in ALT and AST *(Schiano & Black, 1998)*.

In the present study, administration of CCl_4 for eight weeks resulted in significant elevation of serum ALP activity.

Several researchers reported the elevation of serum ALP activity following the administration of toxic doses of CCl_4 in rats (*Danhof et al., 1985; Schiano & Black, 1998 and Mohamed Amany, 2002*).

Data in Table (5) show the normal control values of serum ALP activities (IU/L). Given freeze-dried saponin and tannin extracts at a dose of (0.125g/5mls.kg^{-1}, P.O.) four times per week for eight weeks groups (5 and 6) did not significantly affected serum ALP activity (they still in the normal values).

This result is in acceptable with those of *Yugarian et al., (1992) and El-shemy et al., (2000)* who reported that ALP activity was not changed by anti-nutritional diet, but *Lei et al., (1993)* found that plasma alkaline phosphatase activity was slightly decreased linearly with increased dietary phytase activity.

From Table (5) it's also clear that the significant reduction and recovery occurred in serum ALP activity were 57.71, 53.75, 58.30 and 27.40% at 24h., 3, 6 and 8 weeks, respectively when freeze-dried saponin extract was administered (0.125g/5mls kg^{-1}, P.O.) in group (5) and 2.44, 7.56, 26.16, and 18.33% at 24h., 3, 6, and 8 weeks, respectively, when freeze-dried tannin extract was administered (0.125g/5mls.kg^{-1}, P.O.) in group (6) as compared to injured group (2) .

The above findings maybe interpreted the significant hepatoprotective action of these extracts on the reduction of elevated serum ALP activities with small differences indicated that the freeze-dried saponin extract is more efficient than the freeze-dried tannin extract in reducing elevated activity of serum ALP induced by CCl_4.

Effect of saponin and tannin extracts on elevated hepatic collagen:

The mean values of hepatic collagen mg/g dried liver are given in Table (6). It is clear that injection of 40% CCl_4/paraffin oil (3mls. Kg^{-1}. S.C.) two times per week for 8 weeks resulted in a significant elevation in hepatic collagen by 2.7 folds in group (2) compared to the normal control value.

Repeated administration of CCl_4 to rats for 8 weeks (also the occupational exposure to human) induced chronic liver damage and liver cirrhosis or fibrosis as reported by *Perez Tamayo(1983) ; Stacey et al. (1993) and Chen et al.(2000)*.

Collagen degradation might be impaired in liver cirrhosis and it deposited in it leading to a disruption in normal architecture and function (Stacey *et al.*, 1993 and Chen *et al.*, 2000).

In the present study, administration of CCl_4 for 8 weeks resulted in chronic liver damage associated with a significant elevation in hepatic collagen and this in concern with the above researchers.

Despite numerous investigations, the mechanism of CCl_4-induced hepatic collagen deposition or degradation due to the imbalance between synthesis and degradation of it, thus explaining the accumulation of collagen and this process is irreversible. The decreased collagenolysis may be the result of changes involving collagenase and susceptibility of substrate (Chen *et al.*, 2000).

Table (6): Effect of saponin and tannin extracts on CCl_4-induced an elevation of hepatic collagen:-

Groups	Collagen content as mg/g dried liver
G_1 : Control	$6.857^{E} \pm 0.437$
G_2 : CCl_4	$18.527^{A} \pm 1.522$
Increment fold	2.7
G_3 : S`	$8.930^{CD} \pm 1.005$
G_4 : T`	$7.893^{ED} \pm 0.352$
G_5 : CCl_4/S`	$10.383^{B} \pm 0.442$
% recovery	43.96%
G_6 : CCl_4/T`	$10.117^{CB} \pm 0.186$
% recovery	45.39%

LSD P≤0.05= 1.4292

*** Means with the same letter(s) are not significantly different**

Data in Table (6) show the normal control levels of hepatic collagen mg/g dried liver. Given freeze-dried saponin and tannin extracts 4 times per week for 8 weeks per orally resulted in slight increase in hepatic collagen in groups (3 and 4) this may be due to the accumulation of small amounts of these extracts in the hepatocytes within the end of the experimental period which may be lead to liver lesions accompanied by a slight increase in hepatic collagen.

It is clear from Table (6) that the significant reduction and recovery occurred in the elevated hepatic collagen was 43.96 and 45.39% when freeze-dried saponin extract in group (5) and freeze-dried tannin extract in group (6) were given perorally, respectively as compared to the injured group (2).

Phenolic compounds, particularly catechins and those containing a gallate ester, were effective at micromolar concentration at inhibiting proteoglycan, collagen break-down and inflammation accompanied by destruction of the connective tissues *(Adcocks et al., 2002)*. Also saponins particularly, Gypenoside improved the collagenolytic activity, the degradation of newly synthesized collagen and the repair of hepatocyte *(Chen et al., 2000)*, this may be in consistence with our findings.

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النشا ط الواقي و المضاد للتليف الكبدي لكل من مستخلص الصابونيين و التانيين المجففيين على التسمم الكبدي المحدث بواسطة مادة رابع كلوريد الكربـــون فـــي الجرذان.

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صابونينات بذور فول الصويا منزوعة الدهن و تانينات قشور الفـــول البلـــدي تـــم استخلاصـــها و تجفيدها ؛ كما تم تقدير التركيب الكيميائي بها قبل و بعد عملية الاستخلاص و التجفيد.

تم تقييم هذه المستخلصات المجففة من ناحية تأثيرها الوقائي و المضاد للتليف الكبدي و المحدث فـــي الجرذان بواسطة مادة رابع كلوريد الكربون.

أدي الحقن المتكرر لجرذان التجارب بمادة رابع كلوريد الكربون لمدة ثماني أسابيع متواصـــلة إلـــي ارتفاع معنوي شديد في نشاط الإنزيمات المحددة لوظائف الكبد و الموجودة في مصل الدم مثل ALP, ALT AST , . أيضا أدت نفس المعاملة إلى تراكم و هدم بروتين الكولاجين في النسيج الكبدي للجرذان.

الثوابت الكيمائية المتغيرة في مصل الدم و الكولاجين المتراكم و المهدم تحسن تحسنا معنويا نتيجـــة للمعاملة بهذه المستخلصات المجففه.

إن النتائج توضح إن كلا من مستخلص الصابونينات المجفد و التانينات المجفد لديه نـــشاط واقـــي و مضاد للتليف الكبدي المحدث في الجرذان و لكن ميكانيكية و نشاط هذه المستخلصات تحتاج إلي دراســـات أخري مستقبلا.