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The Affirmation of Serum Glycoproteins as Biomarkers for both Valproate Bepatotoxicity and Silymarin Hepatoprotective Effect.

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ABSTRACT

Male Wister albino rats as experimental animals for the induction of acute hepatotoxicity with sodium valproate (Na-VPA). Two different dose levels; low dose of Na-VPA (25 mg. / kg. body weight / day, orally, for 6 months, and high dose of Na-VPA (50 mg. / kg. body weight / day, orally, for 6 months, were used.

To follow the protective effect of silymarin against Na-VPA hepatotoxicity, the animals received silymarin (50 mg. / kg. body weight / day orally, along with each dose level of Na-VPA and for 6 months.

The alteration of serum glycoproteins was followed up at intervals of 1, 2, 4 and 6 months post treatments.

Generally, serum glycoprotein members recorded a virtual sensitivity to both Na-VPA hepatotoxicity and silymarin hepatoprotection. They showed different elevations that ranged from low to moderate and high levels with Na-VPA treatment. So, serum glycoproteins are suggested to be used as an affirmative biomarkers to follow-up Na-VPA hepatotoxicity. On the other hand serum glycoproteins acted as a good signs for the virtual or limited hepatoprotective offered of silymarin against low – or high dose – valproate treatment respectively.

INTRODUCTION

Valproic acid (VPA), 2- propylpentanoic acid or dipropylacetic acid, is one of a series of fatty or carboxylic acids that is highly water soluble. Its low molecular

weight and pka influence its transport across biological membranes while its water solubility favor its absorption (Wilder and Bruni, 1981).

Sodium valproate (Na-VPA) has been widely used as antiepileptic drug and the bioequivalent between VPA and its sodium salt has been demonstrated (Mattson, 1979). Valproate is almost metabolized completely in the liver (Jacobs and Loscher, 1978; Mattson, 1979) and its highest concentration was found in the blood (Dickinson et al., 1979).

Hyperammonemia occuring as a sign of hepatotoxic effect during valproate therapy of higher doses or long-term treatment that may be required to achieve the optimum control of disease (Mattson, 1979; Coulter and Allen, 1980; Thomas, 1980). Moreover, raised levels of serum glutamic oxalacetic and glutamic pyruvic transaminases (Coulter and Allen, 1980) and alkaline phosphatase (Clark et al., 1980) were also recorded.

Silymarin is natural flavonoid from the milk thistle *Silybum marianum*. It is a complex of three isomers; silibinin, silidianin and silichristin (Dehamlow et al., 1996). On the level of human, silymarin protect lymphocytes against DNA damage and cytotoxicity (Duthie et al., 1997) and human colonocytes, in vitro, from DNA damage of hydrogen peroxide (Duthie and Dobson, 1999). In rats, the hepatoprotective effect of silymarin was gained against carbontetrachloride (Rymsa et al., 1990), peracetamol (Muriel et al., 1992) and free radicals (Winwood et al., 1993).

Serum glycoproteins, play an important role in pathobiochemical changes of many liver diseases such as hepatitis, cirrhosis, diabetes, rheumatic disease and bilhariziasis (Khafagy et al., 1972). The concentration of serum protein – bound uronic acid, hexosamine, hexose, L-fucose, N-acetylneuraminic acid were significantly elevated with the liver injury and renal failure so, these-members of serum glycoproteins can be used as indicators of such pathological processes (Djurdjic and Mandic, 1990; Abdel-Ghaffar et al., 1998).

It is well documented that, clinically, with sever epilepsy where Na-VPA high dosage treatment is used, it is essential to monitor serum glutamic oxalacetic and glutamic pyruvic transaminases, platelets and serum valproate level as indices of common side effects (Clark et al., 1980; Jeavons, 1980). So, the present research aimed to study the affirmative degree of serum glycoproteins to be as biomarkers for both Na-VPA hepatotoxicity and silymarin protective effect. Moreover, our investigation was extended to study the dose – response of serum glycoprotein towards Na-VPA toxicity.

MATERIAL AND METHODS

In the present study animals (male Wister albino rats weighting 100-120gm) were maintained on laboratory standard chow diet. They were administrated with sodium valproate, Na-VPA (as Depakene drug, Laboratories LABAZ, Paris, France, that diluted with distilled water) in low dose (25 mg/kg. body weight / day, orally) or high dose (50 mg/kg. body weight / day, orally) for 6 months regularly. Simultaneously, animals received silymarin (Sigma Chemical Co. St. Louis, USA). It was suspended in distilled water and administered as daily dose (50 mg/kg. body weight, orally) from the beginning of the experiment till the end of 6 months. 180 healthy rats used and they were divided into six groups as follow:

I- 30 healthy rats as normal control.

II- 30 healthy rats were orally administered with silymarin (50 mg/kg. body weight /day).

III- 30 healthy rats were orally administered with low Na-VPA dose (25 mg/kg. body weight /day).

IV- 30 healthy rats were orally administered with high Na-VPA dose (50 mg./kg. body weight /day).

V- 30 healthy rats were orally administered with the low Na-VPA dose and silymarin (50 mg-/ kg -/ body weight / day).

VI- 30 healthy rats were orally administered with the high dose of Na-VPA and silymarin (50 mg/kg. body weight /day).

During the experimental period, blood samples for batches of 6 rats from each of the previous animal groups were collected at intervals 1, 2, 4 and 6 months post the beginning of the experiment.

Blood sampling:

The experimental animals under investigation were anaesthetized by inhalation of diethylether. The blood samples were withdrawn from hepatic portal vein and collected in clean centrifuge tubes, centrifuged in cooling centrifuge at 3000 rpm for 15 min to separate blood serum. The separate sera were kept at – 40°C for subsequent analysis.

Biochemical analysis:

Serum glycoproteins were performed according to the following methods, serum uronic acid (Seibert and Atno, 1946), serum hexose (Weimer and Moshin, 1952), serum hexosamine (Winzler, 1955), serum seromucoid (Weimer and Moshin, 1952), serum fucose (Dische, 1949) and serum N-acetylneuraminic acid (Hess et al., 1957).

Statistical analysis:

The mean (± SD) were calculated for each animal group and time point of the experiment. The data were analyzed by analysis of variance (ANOVA) where $P < 0.05$ and then, the significance of difference between control and treated groups were evaluated by Student's t.test (Hine and Wetherill, 1975).

RESULTS

Generally, serum glycoproteins were found to be significantly elevated due to Na-VPA treatment. These elevations were of time – and dose level dependent. The highest elevated levels recorded for serum protein bound hexose that were of 258.60% and 274.88% after 6 months of Na-VPA treatment for low and high dose levels. Although serum sermucoid was less sensitive (145.65 %), than the bounded

hexose with the low Na-VPA dose levels, it recorded absolutely the highest elevation value (377.44 %) with the high Na-VPA dose. Serum protein-bound hexosamine recorded a moderate significant elevation with low and high Na-VPA dose levels. At the end of the treatment period, hexosamine showed elevations of 178.04% and 186.07% with low and high doses of treatment respectively. Bounded uronic acid exhibited moderate elevation (71.70 %) with low Na-VPA dose levels but it doubled (140.29 %) with the highest Na-VPA treatment, after 6 months of treatment. Both serum N-acetylneuraminic acid and bounded fucose showed the lowest sensitivity to Na-VPA treatment where their elevations were of 33.97% and 29.59% with low dose respectively. While with the high Na-VPA dose treatment, they had a moderate elevations with 75.26% and 74.22% after 6 months treatment. (Tables 1-6 and Figs. 1-6).

Silymarin was able to protect against Na-VPA hepatotoxicity where now elevation in serum uronic acid N-acetylneuraminic acid and fucose were observed for the low and high Na-VPA dose levels and throughout 6 months of treatment. However, protein – bound hexose, hexosamine and seromucoid still showed significantelevations (85.47%, 84.62% and 71.86%) especially with the advanced stage of high Na-VPA treatment. (Tables 1-6 and Figs. 1-6).

DISCUSSION

Generally, sodium valproate produces its hepatotoxic effect, especially long–term treatment with high dose, by increasing the liver peroxidation that simultaneously occurred in the hepatic cells with extensive damage (Johnston and Slater, 1982). Hyperpolarization of hepatocyte plasma membrane may also occurred and leading to its high permeability (Harrison and Simmonds, 1982; Plaa and Hewitt, 1982).

The most serious side effects observed during valproate treatment is the acute liver toxicity (Andersen and Stale – Ritland, 1995; Cepelak et al., 1998). In rats, Na-VPA is dose – related hepatotoxin depends on the development of the liver capacity to metabolize relatively large quantities of the drug (Ware and Millward –

Sadler, 1980). Valproate shifted the whole body toward metabolic acidosis resulting in an increase in circulating ketone bodies (Kupperberg, 1982).

Moreover, Schafer and Luhrs (1978) confirmed the presence of about 40% of the VPA dose during chronic administration as valproate glucuronide.

Deviation in liver enzymes activity usually observed shortly after valproate treatment (Gram and Bentsen, 1985; Cepelak et al., 1998). Enhanced activities of serum glutamic oxalacetic and glutamic pyruvic transaminases (Hagen et al., 1979; Coulter and Allen, 1980) and decreased liver alkaline phosphatase activity (Kaplan, 1986; Abd El-Ghaffar and El-Badawy, 2000). Paralleling to an increase of its activity in serum. This was attributed to hepatocyte damage and increased plasma membrane permeability (Plaa and Hewitt, 1982).

Glycoproteins are the serum protein bound metabolites of liver glycosaminoglycans. They are used clinically in the diagnosis of cancer (Shamberger, 1984) acute hepatitis (Nishizono, 1985), gastric ulcer and liver cirrhosis (Sakal et al., 1990) and finally in patients with colorectal carcinoma (Putzki et al., 1992).

The results of serum glycoproteins showed gradual changes, which ranged from mild to severe elevations in the Na-VPA treated animals. This elevation in such a gradual way might be attributed to the stepwise reduction of liver total protein, especially albumin, due to the extensive binding of valproate to this albumin in serum (Mattson, 1979; Bruni et al., 1980). This gradually will decrease the albumin binding capacity for many compounds inside the cells, the rate of cellular replacement, enzymes and coenzymes synthesis and consequently more susceptibility to the toxicant action (Hathcock, 1982; El-Toukhy et al., 1989).

With high dose of Na-VPA treatment, the drug exhibits some tissue binding, especially with liver, due to the release of free metabolites. As a consequence of liver cell damage, a reduced valproate intrinsic clearance was attained (Levy and Allen, 1982). As a result, valproate free fractions level would be increased gradually in such situation leading to gradual stress within the hepatic cells and

hepatic dysfunction. The releasing of liver glycosaminoglycans metabolites into the circulation will occur due to the disturbance of its degradation.

Another sign of Na-VPA toxicity is based on the observation of hyperamonemia as a sign of hepatotoxicity of valproate (Coulter and Allen, 1981; Murphy and Marqurdt, 1982). The authors decided that this hyperamonemia is due to elevate blood concentration of propionic acid that inhibits the carbamly phosphate synthetase, which is the main mitochondrial enzyme in the first step of urea cycle (Gruskay and Rosenberg, 1979). Leading to impairment in urea synthesis. Haltberg et al. (1995) speculated that increase concentration of ammonia is an activator for elevation of serum hexosaminidase activity which is the main glycosaminoglycans degradative enzyme leading to more releasing of its metabolites into serum. This may explain the elevation in glycosaminoglycans metabolism observed in our investigation .

The crucial protective mechanism of silymarin was reported to be due to the inhibition of lipid peroxidation. The major mechanisms involved are free radical scavenging, cytoplasmic membrane - stabilization, and an enhancement of protein synthesis (Leng – Peschlow et al., 1991). So, it improved the functional markers of liver damage (Lang et al., 1990).

In our results, silymarin was able to prevent the disturbance in the serum glycoproteins in case of low Na-VPA dose. This protection was partial in case of high dose.

So, we can suggest that, this protective effect of silymarin against the elevated serum glycoproteins of Na-VPA treatment was due to its stabilization of the cell membrane phospholipide, which is affected with Na-VPA toxicity, the enhancement of protein synthesis is also very important for sinthesis of liver glycosaminoglycans to form proteoglycans. Finally, silymarin may also normalized the enzymatic activities in both liver including those interfere with the glycosaminoglycans degradation enzyme hexosaminidase, that elevated due to Na-VPA hyperammonemia process.

It could be concluded that, serum glycoproteins had grate susceptibility for valproate hepatotoxicity of low or high dose levels of treatment. So, serum glycoproteins members can be used as an affirmative biomarkers for valproate hepatotoxicity. Moreover, these serum glycoproteins acted as good signs for the virtual or limited hepatoprotective offered of silymarin in case of low or high dose of valproate treatment respectively.

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Table (1): Serum protein - bound uronic acid content[(a)] in Na-valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [(b)] and under the two dose levels of Na-VPA.

Treatment	Normal	SIL		Low dose of (Na-VPA)[(c)]				High dose of (Na-VPA)[(d)]			
Type / periods (Months)	Control			VPA		Na-VPA +SIL		Na-VPA		Na-VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	28.33±14.4	30.00±4.05	(+3.69)	43.86±13.80**	(+54.82)	37.80±9.11	(+30.66)	55.60±13.29**	(+96.25)	38.17±0.43	(+31.93)
2	43.22±9.94	44.05±5.31	(+1.90)	74.10±10.71**	(+71.45)	48.23±11.46	(+11.59)	83.78±2.96**	(+93.85)	52.49±9.31	(+21.45)
4	93.25±17.83	90.25±1.20	(-3.22)	156.66±2.78**	(+68.00)	99.51±7.70	(+6.71)	186.98±2.96**	(+100.51)	96.39±7.39	(+3.37)
6	109.01±9.74	115.13±2.94	(+5.61)	187.27±2.57**	(+71.70)	114.08±5.14	(+4.65)	261.95±13.97**	(+140.29)	120.31±9.96	(+10.31)

(a) Expressed as mg uronic acid / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) Non significant (P> 0.05).

(**) Highly significant (P < 0.01).

Table (2): Serum protein - bound hexose content[a] in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [b] and under the two dose levels of Na-VPA.

Treatment / Type / periods (Months)	Normal control	SIL		Low dose of (Na-VPA)[c]				High dose of (Na-VPA)[d]			
				Na-VPA		Na-VPA +SIL		Na-VPA		Na-VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	77.66±8.95	80.01±3.20	(+3.02)	129.33±5.19 **	(+66.33)	78.00±5.65	(+0.43)	131.94±10.90 **	(+69.89)	102.17±4.47 **	(+31.95)
2	96.82±12.20	97.21±5.11	(+0.40)	191.60±22.73 **	(+97.89)	99.83±10.28	(+3.10)	190.00±1.40 **	(+96.24)	126.26±7.22 **	(+30.14)
4	99.49±5.42	98.00±3.22	(-1.49)	290.50±17.67 **	(+197.98)	102.39±10.22	(+2.91)	384.07±15.96 **	(+286.04)	143.15±20.03 **	(+43.88)
6	105.50±5.40	103.73±2.33	(-1.68)	378.33±24.84 **	(+258.60)	116.44±7.56	(+10.37)	395.50±26.16 **	(274.88)	195.67±17.67 **	(+85.47)

(a) Expressed as mg hexose / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) non Significant ($P > 0.05$).

(**) Highly significant ($P < 0.01$).

Table (3): Serum protein - bound hexosamine content[a] in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [b] and under the two dose levels of Na-VPA.

Treatment / Type / periods (Months)	Normal control	SIL		Low dose of (Na-VPA)[c]				High dose of (Na-VPA)[d]			
				Na-VPA		Na-VPA +SIL		Na-VPA		Na-VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	14.86±5.15	16.00±3.02	(+0.076)	* 23.09±4.24	(+55.38)	20.24±7.35	(+36.20)	** 32.00±2.83	(+115.34)	19.30±3.67	(+29.88)
2	19.39±4.03	20.11±2.18	(+0.037)	** 43.94±2.46	(+126.61)	20.24±3.39	(+4.38)	** 55.96±5.35	(+188.60)	20.42±3.24	(+5.31)
4	23.05±0.98	25.32±1.78	(+0.098)	** 54.33±4.04	(+135.70)	24.61±2.22	(+6.76)	** 60.50±7.77	(+162.47)	** 40.81±0.48	(+77.04)
6	26.91±0.24	25.95±1.20	(-0.035)	** 74.82±0.25	(+178.04)	30.15±2.10	(+12.04)	** 77.09±4.93	(+186.47)	** 49.68±1.99	(+84.62)

(a) Expressed as mg hexosamine / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) Non significant (P> 0.05).

(**) Highly significant (P < 0.01).

Table (4): Serum seromucoid content[a] in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [b] and under the two dose levels of Na-VPA.

Treatment / Type / periods (Months)	Normal control	SIL		Low dose of (Na-VPA)[c]				High dose of (Na-VPA)[d]			
				Na-VPA		Na-VPA +SIL		Na-VPA		VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	50.54±0.78	52.00±1.33	(+2.88)	52.67±9.80	(+4.21)	51.84±2.10	(+2.57)	55.47±4.70	(+9.75)	52.96±1.17	(+4.78)
2	50.02±5.93	52.10±2.00	(+4.15)	54.78±11.20	(+9.52)	51.40±7.46	(+2.76)	66.16±4.60	(+32.27)	55.88±3.76	(+11.72)
4	52.28±2.46	53.80±2.34	(+2.91)	135.96±16.80 **	(+160.06)	60.64±12.36	(+15.99)	166.98±4.20 **	(+219.39)	89.27±9.96 **	(+70.75)
6	54.34±6.01	54.83±2.31	(+0.90)	133.47±12.66 **	(+145.62)	60.47±4.78	(+11.28)	259.44±15.88 **	(+377.44)	93.29±5.36 **	(+71.86)

(a) Expressed as mg seromucoid / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) Non significant (P> 0.05).

(**) Highly significant (P < 0.01).

Table (5): Serum N-acetylneuraminic acid content[a] in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [b] and under the two dose levels of Na-VPA.

Treatment / Type / periods (Months)	Normal control	SIL		Low dose of (Na-VPA)[c]				High dose of (Na-VPA)[d]			
				Na-VPA		Na-VPA +SIL		Na-VPA		Na-VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	50.59±0.78	52.63±1.27	(+4.03)	56.20±10.46	(+11.89)	54.00±1.97	(+6.74)	52.40±7.63	(+3.57)	51.96±0.94	(+0.02)
2	51.73±5.91	51.38±1.91	(- 0.67)	56.60±5.37	(+9.41)	55.60±5.37	(+7.98)	57.00±8.40	(+10.18)	59.00±5.53	(+14.05)
4	52.28±8.76	52.75±3.25	(-0.09)	61.25±3.90	(+16.00)	53.80±1.90	(+1.89)	63.14±0.98	(+19.58)	60.10±3.53	(+13.83)
6	50.01±6.20	53.00±3.71	(+5.97)	67.00±7.35	(+33.97)	53.10±0.14	(+6.18)	87.65±14.42 **	(+75.26)	69.50±14.84 *	(+38.97)

(a) Expressed as mg N-acetylneuraminic acid / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) Non significant ($P > 0.05$).

(*) Significant ($P < 0.05$).

(**) Highly significant ($P < 0.01$).

Table (6): Serum protein -bound fucose content[a] in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment [b] and under the two dose levels of Na-VPA.

Treatment / Type / periods (Months)	Normal control	SIL		Low dose of (Na-VPA)[c]				High dose of (Na-VPA)[d]			
				Na-VPA		Na-VPA +SIL		Na-VPA		Na-VPA +SIL	
	Mean ±SD	Mean ±SD	%	Mean±SD	%	Mean ±SD	%	Mean±SD	%	Mean ±SD	%
1	6.71±0.72	6.81±0.12	(+1.49)	6.88±0.12	(+2.53)	6.25±2.30	(-6.86)	7.64±1.89	(+13.85)	6.37±0.26	(-5.06)
2	6.96±1.86	6.90±0.32	(+0.86)	8.23±0.86	(+18.25)	6.04±0.29	(+13.22)	10.72±3.26 *	(+54.2)	7.88±3.61	(+13.22)
4	6.66±0.99	6.85±0.18	(+2.85)	8.22±1.30	(+23.42)	6.81±1.92	(+2.25)	10.96±0.25 **	(+64.76)	7.37±0.042	(+10.66)
6	6.42±0.59	6.80±0.41	(+5.94)	8.32±1.98	(+29.59)	6.58±1.51	(+2.49)	11.23±0.55	(+74.92)	8.50±2.14	(+32.39)

(a) Expressed as mg fucose / 100 ml serum.

(b) Dose level of silymarin treatment = 50 mg./ kg. body weight / day.

(c) Low dose level of Na-valproate treatment = 25mg/ kg. body weight /day.

(d) High dose level of Na-valproate treatment = 50 mg / kg body weight/ day.

( ) Non significant ($P > 0.05$).

(*) Significant ($P < 0.05$).

(**) Highly significant ($P < 0.01$).

Fig. (1): Serum protein–bound uronic acid content in Navalproate (Na-VPA) treated rates with or without silymarin (SIL) treatment and under the two dose levels of Na-VPA.

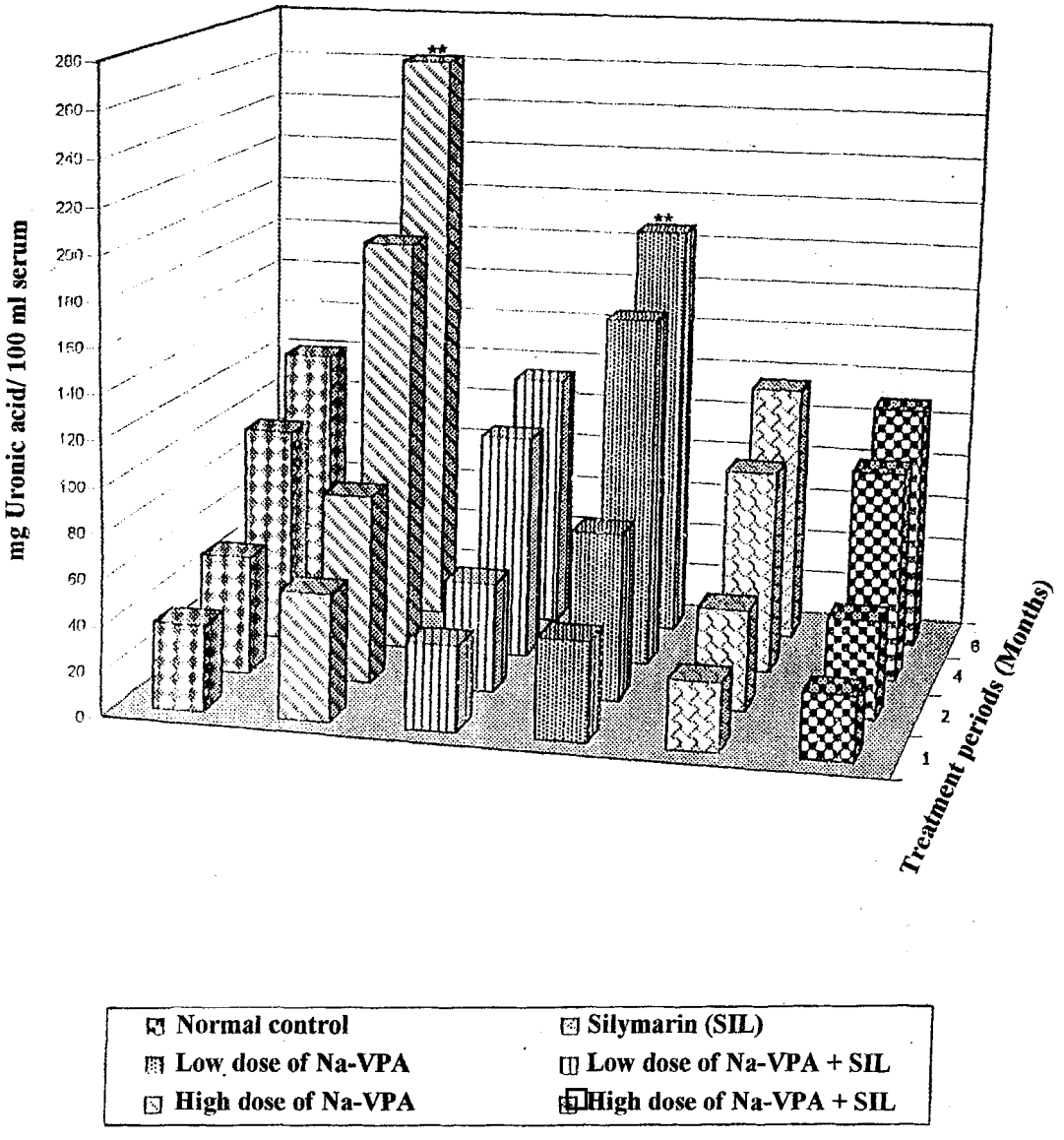


Fig. (2): Serum protein–bound hexose content in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment and under the two dose levels of Na-VPA.

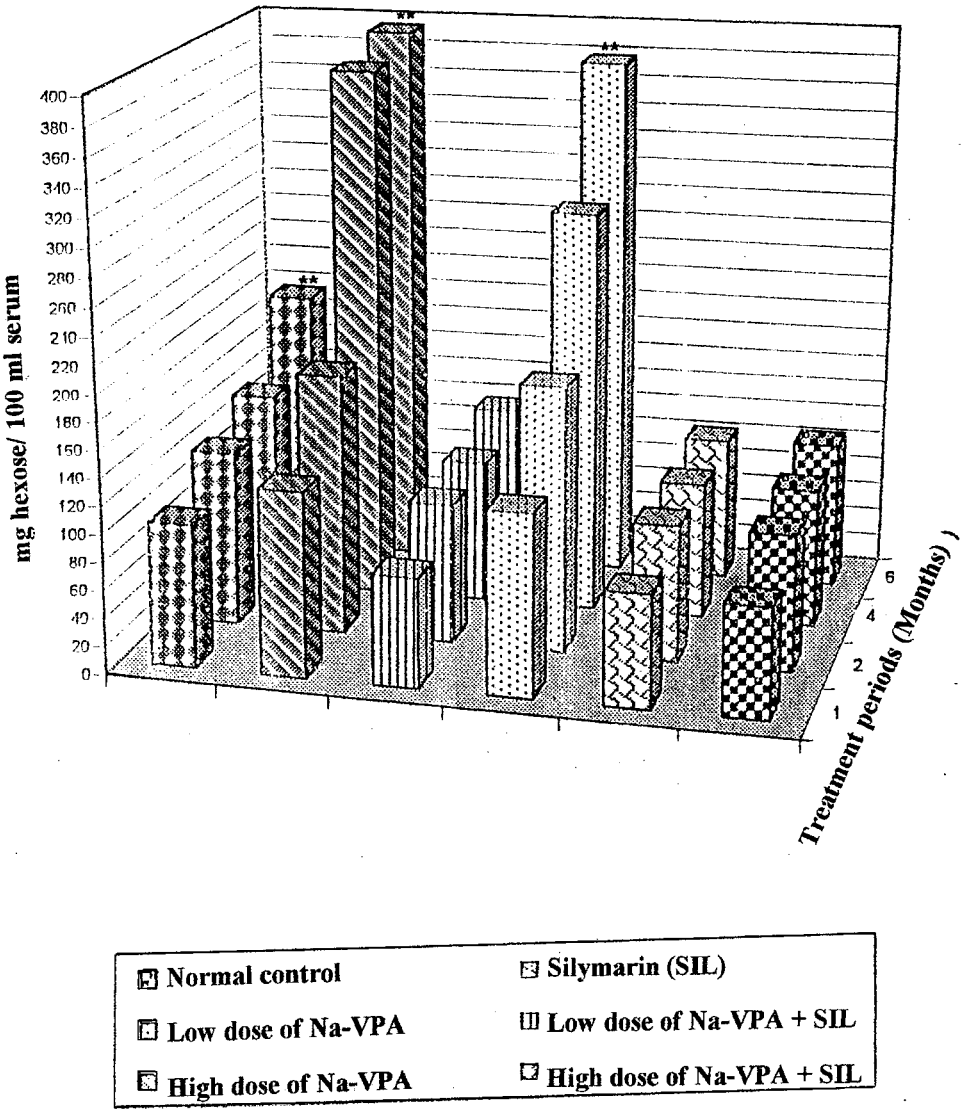


Fig. (3): Serum protein–bound hexosamlne content in Na-Valproate (Na-VPA) treated rates with or without silymarin (SIL) treatment and under the two dose of Na-VPA.

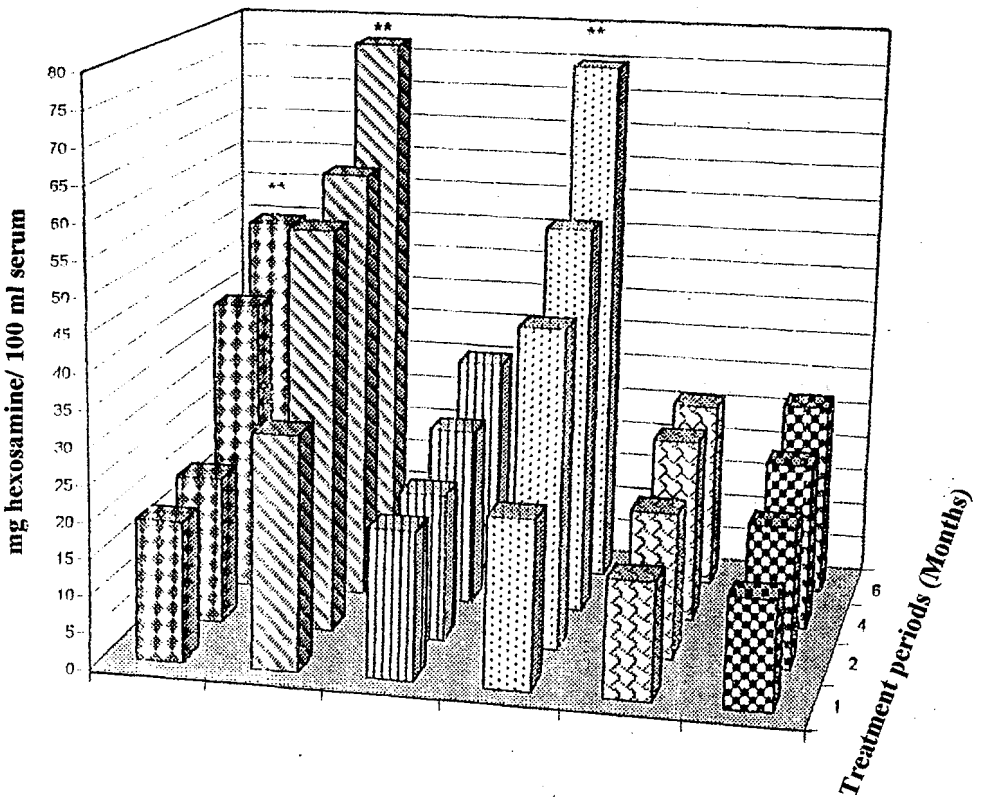


Fig. (4): Serum seromucold content in Na-Valproate (Na-VPA) treated rates with or without silymarin (SIL) treatment and under the two dose levels of Na-VPA.

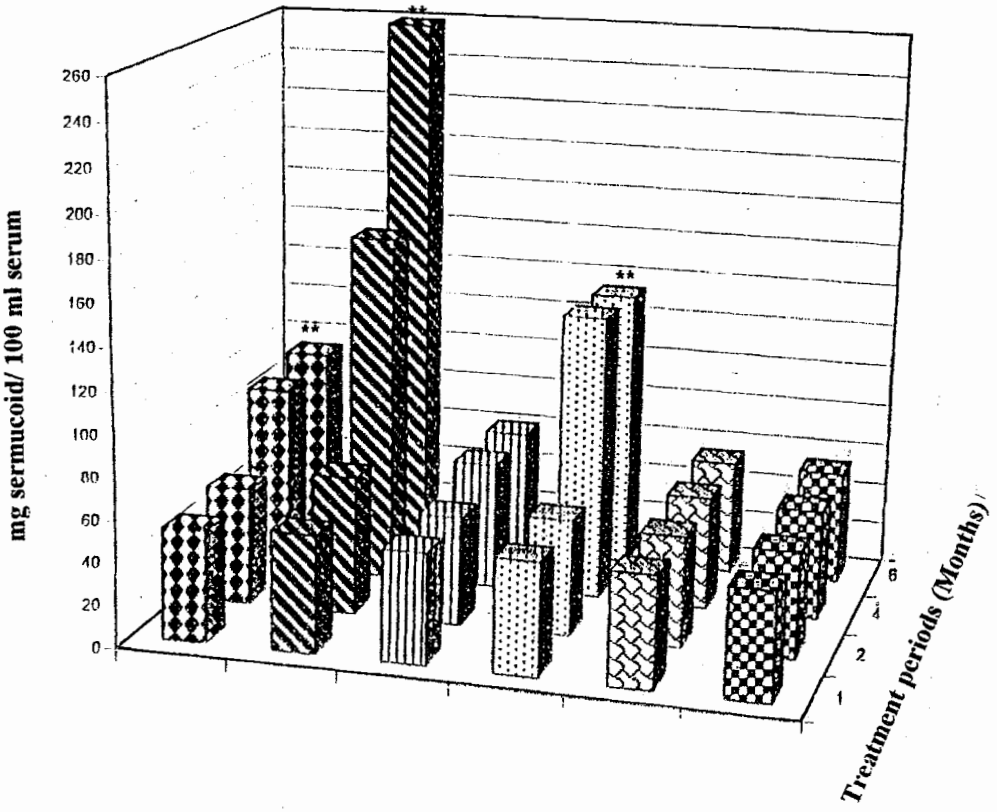


Fig. (5): Serum N-acetyl neuraminic acid in Na-Valproate (Na-VPA) treated rates with or without silymarin (SIL) treatment and under the two levels of Na-VPA.

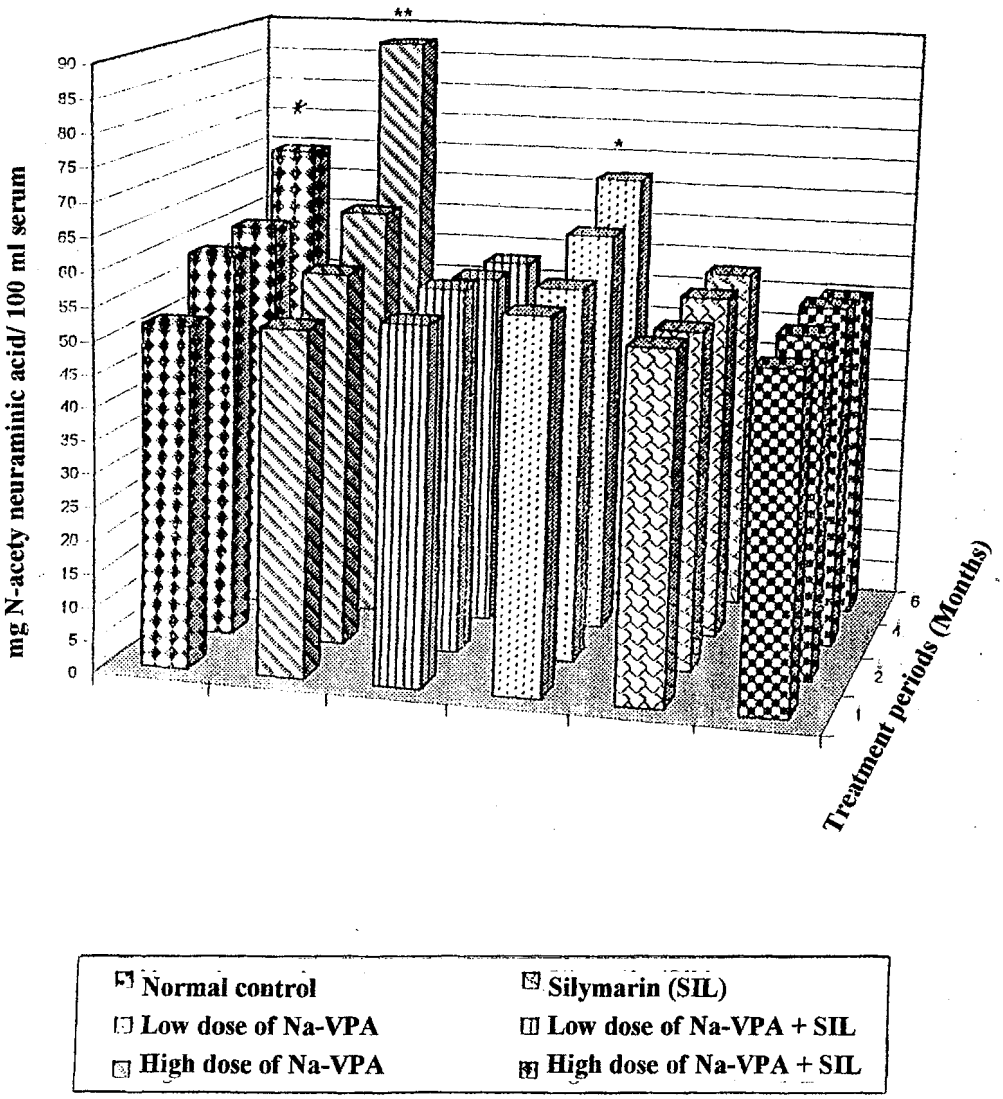
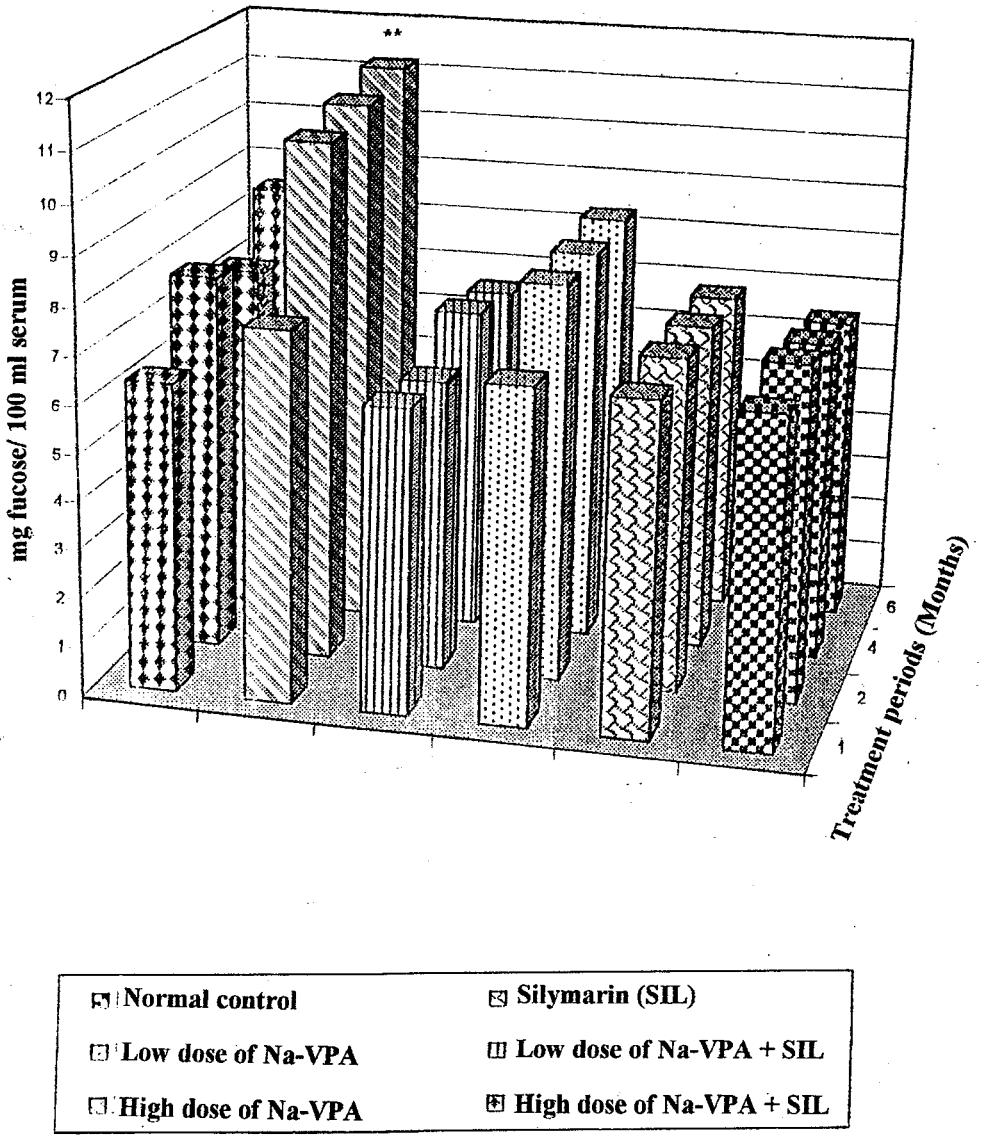


Fig. (6): Serum protein–bound fucose content in Na-Valproate (Na-VPA) treated rats with or without silymarin (SIL) treatment and under the two levels of Na-VPA.



الملخص العربي

مدى إيجابية جليكوبروتينات مصل الدم كمؤشرات حيوية لكل من التأثير السمي للفالبروات والتأثير الوقائي للسليمارين على الكبد .

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أستخدم لهذه الدراسة ذكور جرذان التجارب البيضاء حيث أحدث بها تسمم كبدي مزمن بواسطة استخدام فالبروات الصوديوم .

استخدمت جرعتين مختلفتين من فالبروات الصوديوم هما (٢٥ مجم / كجم من وزن الجسم / يومياً) . حيث تم التناول عن طريق الفم وذلك لمدة ٦ أشهر متواصلة ، (٥٠ مجم / كجم من وزن الجسم / يومياً) بواسطة التناول عن طريق الفم ولمدة ٦ شهور متواصلة .

ولكي نتابع التأثير الوقائي لمادة السليمارين ضد التسمم الكبدي بواسطة فالبروات الصوديوم تلقت حيوانات التجارب السليمارين (٥٠ مجم / كجم من وزن الجسم / يومياً . بالتناول عن طريق الفم لمدة ٦ أشهر متزامنة مع إعطاء كل جرعة من جرعتي فالبروات الصوديوم المذكورة سابقاً .

تم متابعة الاختلال في محتوى مصل الدم من الجليكوبروتينات لفترات ١ ، ٢ ، ٤ ، ٦ أشهر بعد بداية تناول الجرعات ولقد وجد أن وحدات الجليكوبروتينات في مصل الدم قد سجلت تغيرات جوهرية بسبب التسمم الكبدي بالفالبروات . فلقد أظهرت الجليكوبروتينات زيادة في معدلاتها تتراوح من زيادة متوسطة إلى زيادة عالية معتمدة على الجرعات وعدد الأيام من تناول فالبروات الصوديوم . وذلك يوضح مدى إمكانية استخدام جليكوبروتينات مصل الدم كمؤشر حيوي إيجابي للكشف عن مدى التسمم بواسطة فالبروات الصوديوم .

وعلى الجانب الأخر فإن جليكوبروتينات مصل الدم تعمل كمؤشرات جيدة لمدى التأثير الوقائي للسليمارين سواء كان التأثير فعال ضد الجرعة الصغيرة أو محدود ضد الجرعة الكبيرة من فالبروات الصوديوم .