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RESEARCH ARTICLE

Biochemical Effects of glucosamine on glycoproteins of rats with hepatic fibrosis.

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Abstract

Glycoproteins are significantly contributing to the surface properties of the cells. Glucosamine has membrane stabilizing activity and liver protective effect. The aim of this study is to evaluate the alteration of glycoprotein in hepatic fibrosis and the effects of glucosamine administration in rats. The experiment was conducted into six groups of rats; control, thioacetamide (TAA), glucosamine hydrochloride, TAA and glucosamine hydrochloride, glucosamine sulphate and TAA and glucosamine sulphate groups. Serum and liver carbohydrate moieties (total hexose, fucose, hexosamine, and sialic acid) of the glycoproteins were evaluated in rats. Serum and liver glycoproteins were isolated and analyzed by using sodium dodecyl sulphate polyacrylamide gel electrophoresis. The results indicated that liver hexose and sialic acid were lower in TAA rats administrated with glucosamine hydrochloride or sulphate compared to TAA rats. Liver hexosamine of TAA rats administrated with glucosamine sulphate was lower compared to TAA rats administrated with glucosamine hydrochloride. Liver glycoprotein fucose showed a decrease in TAA, TAA& glucosamine hydrochloride or sulphate groups compared to control group. The isolated glycoproteins from serum showed high expression while glycoproteins from liver showed low expression profile in TAA rats compared to control and glucosamine administrated rats. In conclusion, the administration of fibrotic rats with glucosamine hydrochloride or sulfate altered liver glycoprotein especially rich in hexose and sialic acid..

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Introduction:-

Hepatic fibrosis is a process of healing and scarring in chronic liver injury, due to toxins and various infectious, immunologic, and metabolic disorders. A common feature in hepatic fibrosis is increased accumulation of extracellular matrix (ECM) constituents including glycoprotein such as fibronectin, laminas, proteoglycans and collagens. Increased synthesis of ECM components usually follows the activation of hepatic stellate cell (HSC) (**Pinzani *et al*., 1998; Eng *et al*., 2000**) and probably other matrix producing liver-derived myofibroblast-like cells (**Cassiman *et al*., 2002**). Also a decrease in collagen degradation during the fibrogenic process contributes to matrix accumulation (**Arthur *et al*., 2000**).

Thioacetamide (TAA) is a potent hepatotoxin, which is frequently used to produce an experimental model to study the mechanisms involved in the progression of hepatic disease and the impact of various drugs on this progression. It induces cytotoxic injury through a two-step bioactivation, which is mediated by the microsomal cyrtochrome P450 isozyme E1 or flavin containing monooxygenase systems to TAA sulphoxide and further to a reactive metabolite, TAA-S, S-dioxide which causes lipid peroxidation at the plasma membrane level (**Hessien *et al*., 2008**).

Glycoproteins are important groups of compounds involved in the cellular function as mediators of immunological specificity (**Pakkir *et al*., 2012**). The Carbohydrates moieties of glycoproteins have also been implicated in the transport of metabolites across cell membranes (**Thirunavukkarasu and Sakthisekaran, 2003**).

Glucosamine a naturally occurring derivative of glucose is an important component of proteoglycans and glycoproteins, essential constituents of many eukaryotic proteins. Also it is a component of many biological important systems widely spread in nature. Many nutrimental, biological and pharmaceutical effects of glucosamine, such as application in osteoporosis treatment and wound healing promoting by enhancing hyaluronic acid synthesis (**Mccarty, 1998**) have been widely reported and glucosamine is easily absorbed and of excellent properties (**Lippiello, 2003**). Glucosamine, a constituent of glycosaminoglycans, has been used widely to treat human osteoarthritis (OA). Moreover, glucosamine inhibits the neutrophil functions, thereby possibly exhibiting anti-inflammatory actions in OA (**Chu *et al*., 2006**). It is a part of the connective tissues, membranes, lipopolysaccharides, mucopolysaccharides and participates in detoxic function of the liver and kidneys. Also, glucosamine and its derivatives possess antiinflammatory, liver-defending, antihypoxic and other pharmacological activities (**Zupanets *et al*., 1990; Jamialahmadi *et al*., 2014**).

It was found that glucosamine prevent the development of the hepatocyte cytolysis syndrome and normalize the cholate and glycogen synthetic functions of the liver. Also, D-glucosamine exhibited a significant anabolic effect that also supplemented its hepatoprotective activity (**Sal'nikova *et al*., 1990**). Sulphated glucosamine improved cellular antioxidant potential by controlling oxidative damage that could be important for its therapeutic potential in osteoarthritis. Furthermore sulphated glucosamine had been used in treatment of human macrophages and chondrocytes and inhibited radical stimulated oxidation of membrane proteins, DNA and lipids depending on its dose (**Mendis et al., 2008**). The aim of the present study was to evaluate the biochemical effects of glucosamine on glycoprotein contents in thioacetamide induced hepatic fibrosis in rats.

Material and Methods:-

Experimental Animals:-

Sixty adult males Albino Wistar rats aged 4-6 weeks weighting 200 ± 20g were purchased from the breeding unit of Egyptian Organization for Biological Products and Vaccines (Helwan, Cairo) were used in this experiment. All rats were fed a commericial diet and water *ad libitum* and maintained under controlled conditions of temperature (22±1ºC) and humidity with a 12 h light/dark cycle

Rats were divided equally into six groups

Group I (Normal control group): Rats received normal rat food.

Group II (TAA group): Rats were injected with TAA 200 mg /kg body weight intraperitoneal three times a week for a month (Fan *et al*., 2007).

Group III (glucosamine hydrochloride group): Rats were intragastric administrated with 500 mg glucosamine hydrochloride /kg body weight every day for a month (**Yan *et al*., 2007**).

Group IV (glucosamine sulphate group): Rats Control rats were intragastric administrated with 500 mg glucosamine sulphate /kg body weight, every day for a month (**Yan *et al*., 2007**).

Group V (TAA & glucosamine HCl group): Rats were injected with TAA 200 mg /kg body weight intraperitoneally three times per week in combination with glucosamine HCl (500 mg/kg body weight) daily for a month.

Group VI (TAA & glucosamine sulphate group): Rat were injected with TAA 200 mg /kg body weight intraperitoneally three times per week in combination with glucosamine sulphate (500 mg/kg body weight) daily for a month.

Rat experimentation was consistent with the guidelines of Ethics by the Guide for the Care and Use of Laboratory Animals in accordance with animal care committee of the Faculty of Science, Tanta University, and Tanta, Egypt. At the end of experiment rats were anesthetically sacrificed. Serum was isolated from blood by centrifugation for 10 min. at 4000 r.p.m and kept at -20ºC until assay. Rat livers were resected and divided into two parts; one of them was fixed in formalin, other part was stored in aluminum foil at - 20ºC until assay.

Evaluation of Biochemical parameters:-

Serum levels of ALT, AST and alkaline phosphatase activities and serum albumin levels were measured by using commercial kit (Vitro Scient, Germany). Mucoprotein content of serum was determined according to **Weimer and**

Moshin (1952). Briefly, the protein or protein bound carbohydrate left in solution by 1.8 M perchloric acid and precipitated by phosphotungstic acid. The sediment was dissolved and determined by using Folin method.

The hepatic collagen content was measured as described **Bergman and Loxley, (1963)** and was modified by **Medugorac (1980)**. Briefly, 2.5 ml of tissue homogenate was mixed with 2.5 ml of 6 N HCl and heated in oven at 110^{o}C for 16 hours, and neutralized with 4M of sodium hydroxide then 2 ml of clear filtrate was taken and mixed with 1 ml of oxidant solution consisting of sodium acetate trihydrate, trisodium citrate dihydrate, citric acid and isopropanol. After 4 minutes incubation at room temperature, 2 ml of Ehrlich's reagent was added and the tubes were incubated in water bath at 60^{o}C for 21 minutes. Absorbance of test sample was measured at 558 nm and the level of hydroxyproline was determined using standard of hydroxyproline.

Glycoproteins were isolated from serum by precipitation using 95% ethanol and their sugar moieties were estimated. Serum hexose and fucose levels were estimated according to **Djurdjić, and Mandić (1990)**. Briefly 0.02 ml of serum, 0.08 ml saline then 2.5 ml of 95 % ethanol were added and centrifuged at 2000 rpm for 10 minutes. The precipitated proteins were suspended in 0.5 ml of distilled water and 2.5 ml H_2SO_4 were added. The tubes were placed in boiling water bath for 3 minutes then cooled. 0.5 ml of cysteine phenol reagent was added. The tubes were left for 1 hour in ice. The absorbance was measured at 398 nm and 490 nm corresponding to fucose and hexose levels respectively.

Liver hexose was determined according to **Thirunavukkarasu and Sakthisekaran (2003)** after hydrolysis of glycoprotein according to **Selvendiran *et al.* (2006)**. Briefly 1 ml of 10 % liver homogenate (prepared in 0.9% NaCl) was added to test tube containing 1 ml of 2N HCl and the tubes were sealed. The sealed tubes were kept in boiling water bath for 16 h for complete hydrolysis and neutralized with 4M of sodium hydroxide and made up 10 ml with distilled water then filtered. 0.5 ml of filtrate was added to 4.25 ml of orcinol – H_2SO_4 reagent and the tubes mixed well. The tubes were covered and placed in water bath at 80^{o}C for 15 min then cooled and color allowed to develop in the dark for 25 min and measured at 540 nm. Concentration of hexose in liver tissue (mg/g tissue) was calculated using the standard galactose.

Serum Protein bound hexosamine was estimated according to **George and Chandrakasan (1996**) after precipitation of serum glycoproteins using 95% ethanol. In a nutshell 5 ml of 95% ethanol were added to 0.1 ml of serum, mixed well centrifuged for 15 minutes, decanted and the precipitate was suspended in 5 ml of 95% ethanol then the tubes were centrifuged and supernatant was discarded. 2 ml of 3 N HCl were added to the precipitated proteins and hydrolyzed in a boiling water bath for 4 hours. The hydrolysate was neutralized with 3 M NaOH and diluted to 10 ml with distilled water. 0.5 ml of acetylacetone reagent was added to 0.5 ml aliquots. The tubes were heated in a boiling water bath for 15 minutes and cooled followed by addition 2.5 ml of 95% ethanol and tubes were mixed. 0.5 ml of Ehrlich's reagent was added and diluted to 5 ml with 95% ethanol. The absorbance was read after 30 minutes at 530 nm. Concentration of serum hexosamine (mg/100 ml Serum) was calculated using standard glucosamine level.

Liver hexosamine liver was estimated according to **George and Chandrakasan (1996)** as described previously after hydrolysis of glycoprotein according to **Selvendiran *et al.* (2006)**.

Sialic acid in the liver homogenate, serum and isolated liver glycoproteins was determined according to **Niebes, 1972**. In serum, after precipitation of glycoproteins, they were hydrolyzed with 1 ml of 0.1N H_2SO_4. Then sialic acid in hydrolysate is heated with 0.25 ml of 0.025M periodic acid and excess periodate is reduced by 0.25 ml of 4%sodium arsenite with liberation of iodine that disappear within 1-2 min. then pink color is developed after boiling with 2 ml 0.1N thiobarbituric acid and is more soluble in 5 ml acidified n-butanol containing 5% 12N HCl which extract the color in upper organic layer. The absorbance of color is measured at 550 nm. For estimation of liver glycoprotein sialic acid, 1 ml of sample was hydrolyzed with 1 ml of 0.1 N H_2SO_4 at 80^{o}C for 1 hr and the samples were centrifuged at 2000 g for 3 min then 0.25 ml of 0.025 M periodic acid was added to 0.5 ml of hydrolysate. The procedure were completed as described previously for serum protein bound sialic acid and the concentration of sialic acid (mg/g tissue) was calculated using standard sialic acid.

Isolation of rat serum and liver glycoproteins:-

Isolation of serum glycoproteins was carried out according to **Ananthasamy *et al*. (1965).** In brief sodium acetate was added to the serum to give a final concentration of 0.1 M. An equal volume of saturated ammonium sulphate solution was added and the serum kept in the cold for 16 hr. The precipitate obtained on centrifugation at 10000g for 30 min. was discarded. The supernatant solution was adjusted to pH 4.8 by adding 1N HCl and kept in the cold for 18 hr. The precipitate was removed by centrifugation at 10000 g for 30 min. The supernatant solution was adjusted to pH 3.5 with 1 N HCl. The clear supernatant solution obtained after centrifugation at 10000 g for 30 min. then solid ammonium sulphate was added to give 100% saturation. The mixture was stirred well and left in the cold for 48 hr. The precipitate obtained on centrifugation at 10000g for 30 min. was dissolved in water. The obtained samples were dialysed against distilled water and left overnight.

This isolation of liver glycoproteins was carried out according to **Josić *et al*., (1989)**. Briefly the liver (1 g) was cut into small pieces and suspended in 10 ml of 5 m M Tris-HCl (pH 7.2) and 1 m M calcium chloride and homogenized. The centrifugation at 1000 g for 10 min was carried out to remove nuclei. The enriched membrane fraction was obtained through centrifugation at 40000 g for 35 min. The supernatant after centrifugation was discarded. The pellet was suspended in 3 ml 10 m M Tris-HCl (pH 7.4) containing 1 mM Magnesium chloride, 155 mM sodium chloride, 1 mM calcium chloride and 1% (w/v) Triton X-100. The protein content in the isolated fractions and in homogenates was determined according to Folin method as described previously. Furthermore hexose, fucose, hexoamine and sialic acid were determined in glycoprotein fractions as described later in liver tissue.

Sodium Dodecyl Sulphate Polyacrlamide (SDS-PAGE) Gel Electrophoresis of isolated serum and liver glycoproteins was performed using 10% gel, 8 and 20 µg protein /well respectively according to **Laemmli (1970)**.

Statistical analysis:-

Group means (± standard deviation) were computed by student's paired two tailed *t* test using GraphPad software and $p<0.05$ was considered statistically significant.

Results:-

Serum ALT, AST and ALP activities were significantly increased in TAA, TAA &glucosamine hydrochloride and TAA & glucosamine sulphate groups compared to control group**.** There was significant decrease in ALP activity of TAA& glucosamine hydrochloride or TAA& glucosamine sulphate groups compared to TAA group. Serum mucoprotein of TAA& glucosamine hydrochloride group was significantly increased compared to TAA group while serum mucoprotein of TAA& glucosamine sulphate was significantly decreased compared to TAA& glucosamine hydrochloride group. Liver collagen of rats intoxicated with thioacetamide and administrated either with glucosamine hydrochloride or sulphate was significantly decreased compared to TAA group **(Table 1)**.

Serum sialic acid levels showed significant decrease in TAA & glucosamine sulphate and TAA & glucosamine hydrochloride groups as compared to TAA group **Fig. (1)**.

Liver sialic acid showed extremely significant increase whereas Liver hexose and isolated liver fraction hexose showed a highly significant decrease in TAA, TAA& glucosamine hydrochloride or sulphate groups compared to control group **Fig (2C and 3)**. In addition there was a significant increase in liver hexose in glucosamine hydrochloride or glucosamine sulphate groups compared to control group (**Fig 2A**). Liver fucose of glucosamine hydrochloride group was significantly increased compared to control group **Fig. (2B)**. Liver fucose and isolated Liver fraction fucose results showed extremely significant decrease in TAA, TAA& glucosamine hydrochloride or sulphate groups compared to control group **Fig. (3)**. There was a significant decrease in liver hexosamine of TAA & glucosamine sulphate group compared TAA& glucosamine hydrochloride while liver isolated fraction hexosamine was significantly increased in TAA & glucosamine sulphate group compared to TAA& glucosamine hydrochloride group **Fig.(3**).

The ratio of liver glycoprotein fraction hexose to liver hexose levels was significantly decreased ($p<0.05$) in glucosamine hydrochloride group compared to control while this ratio was significantly increased in TAA &glucosamine sulphate group as compared to TAA& glucosamine hydrochloride group (**Fig 4**). The ratio between liver glycoprotein fucose and liver fucose concentrations was significantly decreased in glucosamine hydrochloride

group as compared to control group. Also, the results showed that the ratio of isolated liver fraction hexosamine to liver hexosamine levels in TAA & glucosamine sulphate was significantly increased ($p<0.001$) as compared to control or TAA or TAA & glucosamine hydrochloride groups. **Fig. (4)**. It was indicated that the ratio between liver glycoprotein fraction sialic acid and liver sialic acid concentrations are significantly ($p<0.001$) increased in glucosamine hydrochloride group while decreased in TAA intoxicated groups as compared to control group. In addition this ratio was significantly increased in TAA& glucosamine sulphate group compared to TAA group ($P<0.01$) and TAA & glucosamine HCl ($p<0.05$) as shown in **Fig. (4)**.

The gel electrophoretic analysis of the protein fractions obtained in the isolation of the serum glycoprotein was shown in **Fig. (5)** .Four major glycoproteins were determined in all fractions with a molecular weight of 70, 58 and 48, 32 KDa. It was indicated that the four isolated glycoproteins are enriched in TAA treated groups compared to control groups. There was a pronounced difference between the serum glycoproteins of normal and TAA intoxicated rats. Bands 1, 3, 4 of molecular weights 70, 48and 32 KDa respectively are enriched in TAA treated rats. Also band of 70 KDa was enriched in TAA intoxicated rats and treated with glucosamine either hydrochloride or sulphate compared to glucosamine hydrochloride or sulphate groups respectively. Also the expression of glycoproteins with molecular weights of 58 and 48 KDa was decreased in rats intoxicated with TAA and treated with glucosamine compared to TAA group.

After polyacrylamide gel electrophoresis in sodium dodecyl sulphate the glycoproteins of the liver plasma membrane fraction were separated into about 10 bands as shown in **Fig. (6**). The molecular weights of bands 5, 6 and 7 and 10 were estimated to be about 60, 58, 55 and 46 KDa respectively. It was indicated that the most isolated glycoproteins are diminished in TAA treated groups compared to control groups with high expression of all isolated protein in TAA & glucosamine hydrochloride or sulphate groups compared to their control and TAA group.

Discussion:-

Fibrosis is a chronic disease that may progress to cirrhosis, which consequently result in hepatic failure (**Pinzani *et al.*, 2008**). Therefore, prevention and treatment of liver fibrosis is a critical step preventing liver cirrhosis and failure. The goal of our study was to evaluate the effect of glucosamine either hydrochloride or sulphate on serum and liver glycoprotein sugar moieties in TAA induced liver fibrosis.

Glucosamine acts as antioxidant (**Xing *et al.*, 2006**) and enters in the formation of glycoproteins (**Ma *et al.*, 2002)** which undergo alteration in its glycosylation during liver diseases **(Blomme *et al.*, 2009).**

Chronic administration of TAA effectively caused liver injury, dysfunction and fibrosis without affecting other organs and leads to repeated apoptosis, necrosis and regeneration of hepatic cells, which leads to regenerative nodules and subsequently adenomas and carcinomas of hepatic cells in rodents (**Gervasi *et al.*, 1989**; **Wang *et al.*, 2012**).

Serum Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) activities are reliable indicators for assessing liver cellular lesions because they are excessively released into the bloodstream after disruption of hepatocellular membrane (**AL-Attar, 2011**). The aminotransferases liver enzymes (ALT and AST) are commonly used as diagnostic markers for damage and necrosis of liver cells. However, ALT is more specific for liver injury than AST which is present in a diversity of different tissues (**Pratt and Kaplan, 2000**). Furthermore, ALT is present only in the cytosol while AST present in both the mitochondria and cytosol of hepatocytes (**Thapa and Walia, 2007**).

In the present study serum ALT, AST and ALP activities showed significant increase in rats injected with TAA and rats injected with TAA in combination with either glucosamine hydrochloride or glucosamine sulphate compared to control group. Also a decrease in ALT, AST and ALP activities in rats intoxicated with thioacetamide during injection of glucosamine was found.

Similar to our findings **Al-Attar (2011)** reported an elevation in serum liver enzymes, e.g. AST, ALT, and GGT in rats injected with TAA (300 mg/kg twice weekly for 10 weeks). Also, **Aydin *et al.* (2010)** reported that administration of 200 mg TAA/kg body weight i.p. Twice a week for three months to female wistar rats significantly increased plasma ALT and AST activities.

In this study, serum mucoprotein was measured as protein and it was found that serum mucoprotein concentration was significantly increased in TAA rats injected with glucosamine hydrochloride**,** while decreased in TAA rats administrated with glucosamine sulphate compared to control. Glucosamine administration in TAA intoxicated rats showed significant decrease in collagen levels compared with thioacetamide group, this is in compatible with other study of **Campo *et al*. (2004)** in which treatment with glycosaminoglycans hyaluronic acid and chondroitin-4-sulphate, especially when in combination, successfully limited collagen deposition in the hepatic tissue in a subchronic rat model of carbon tetrachloride-induced liver fibrogenesis and this confirmed the antioxidant and antifibrogenic effects of glycosaminoglycans.

In this study the effect of TAA and glucosamine treatment on glycoproteins were assessed by measuring the levels of sugar moieties in serum, liver and isolated glycoproteins from liver including hexose, fucose, hexosamine and sialic acid.

The previous studies have been indicated that of human proteins are co or post-translationally modified by mono- or oligosaccharides. The glycoprotein saccharides contribute physicochemical properties, assist in intracellular protein folding or increasing stability against proteolytic activity (**Moncada *et al*., 2003; Westerlind, 2012; Nakata, 2015)**. Glycoproteins exert key role in mediating cell surface function including cell–cell recognition, cellular adhesion, binding and clearance of serum glycoproteins and metabolic transport among others (**Pakkir *et al*., 2012**).

Glucosamine could be converted to uridine diphosphate N-acetyl glucosamine. This sugar is then used for O-linked glycosylation of several proteins, including chromatin proteins; transcription factors, nuclear pore proteins, and certain cytoskeletal proteins, leading to alteration in their biological activity (**Kearse and Hart, 1991**).

In addition, glucosamine has been played an important role in the detoxification of kidney and liver and have liver-protecting and antimicrobial activities *in vivo* (**Muzzarelli, 1993**). Such properties of glucosamine led scientists to hypothesize that hexosamine would be involved in the glycosylation of cytoplasmic and nucleoplasmic proteins, which is a regulatory modification (**Reason *et al*., 1992**).

In the present analysis serum, liver and isolated liver fraction hexose were decreased in all TAA intoxicated groups when compared to control group. This is an indication of active degradation of glycoprotein in TAA induced hepatic fibrosis as in the study of **George and Chandrakasan (1996)** which was carried out on rats hepatic fibrosis induced by dimethylnitrosamine and caused a decrease in liver and serum protein bound hexose which was due to an active degradation of glycoprotein moiety in dimethylnitrosamine induced hepatic fibrosis. In their simultaneous study they have noticed an increase in the activity of β-glucosidase and D-galactosidase both in liver and serum, which could also support the decreased hexose moiety of glycoprotein in liver and serum (**George and Chandrakasan, 1996**).

There was decrease hexose in liver glycoprotein fraction of rats intoxicated with TAA and administrated glucosamine either hydrochloride or sulphate compared to TAA group and this indicates the ameliorating effect of glucosamine on liver hexose level in hepatic fibrosis. It is evident that hexoses are associated more directly with collagen as compared to hexosamine or sialic acid (**Sharon, 1975**) and the increase in the concentration of total hexose may be explained in the presence of increased collagen content and synthesis in fibrotic conditions (**Venkatesan *et al*., 1998**).

L-fucose is a monosaccharide normally present in low levels in the rats serum. It is however, a common structural component of glycoproteins and is present in the non-reducing end of the sugar chain of glycoconjugates and an important constituent of blood group specific antigen (**Sakai *et al*., 1990**). Morever, fucose containing glycans play important roles in many biological responses such as protein folding and antigen processing (**Becker and Lowe, 2003**).

Serum fucose of TAA rats treated with glucosamine hydrochloride or glucosamine sulphate was high compared to control group. These results are similar with **Sathish Kumar *et al*. (2004)** who found that serum free and protein bound fucose concentrations were increased in the patients with liver diseases (hepatitis B related disease, alcoholic liver disease and acute liver diseases) versus control. Liver and liver glycoproteins fucose of TAA rats, TAA rats administrated with glucosamine hydrochloride and TAA rats or glucosamine sulphate was decreased as compared with control groups on contrary to **George and Chandrakasan (1996)** who observed the increase in liver glycoprotein fucose moiety levels on all days of dimethylnitrosamine treatment in rats except on 21st day.

The possible reasons for this increase in serum fucose may arise from the degradation of fucosylated proteins and further action of fucosidases (**Jezequel-Cuer *et al.*, 1992**). Increase in fucosidase activity has been shown in certain pathological conditions including liver. α-L-fucosidase, a lysomal enzyme acts in fucose containing glycoconjugates which are increased in liver cirrhosis (**Cai *et al.*, 1998**). In addition increased fucosylation of proteins has been shown in tissues with certain pathological conditions such as liver disease which may be due to increase fucosyltransferase activity (**Sakai *et al.*, 1990**).

Glucosamine is used in cell cultures to stimulate hexosamine flux and protein O-glycosylation, but at greater concentrations than those in human plasma following oral dosing (**Ali *et al.*, 2011**). Sialic acid is found in many body tissues and fluids and plays a role in the vital function of humans **(Sillanaukee *et al.*, 1999).** The presumed functions of sialic acid include stabilizing the conformation of glycoproteins and cellular membranes, this is due to its negative charge, assisting in cell–cell recognition and interaction, and serving as chemical messengers in tissue and body fluids; impacting transmembrane transportation mechanisms; affecting the function of membrane receptor molecules by developing binding sites for ligands, antibodies, enzymes, microbes, etc., or by blocking such; affecting the functioning, stability, and survival of glycoproteins in blood circulation; and regulating the permeability of glomeruli basement membrane (**Schauer *et al.*, 1995).**

In the current study it was found that liver sialic acid level was increased in thioacetamide groups compared to control group and these results were in accordance with the study for **George and Chandrakasan (1996)** who studied glycoprotein metabolism in dimethylnitrosamine induced hepatic fibrosis in rats and found that liver sialic acid was significantly increased in rats treated with dimethylnitrosamine, while in the present study both serum and liver sialic acid were decreased in rats intoxicated with TAA and administrated with glucosamine as compared to TAA group and this demonstrates changes in glycosylation in liver fibrosis after administration of glucosamine.

Increased sialic acid concentrations have been measured experimentally in CCl_4 or acetaminophen-induced liver toxicity (**Wu *et al.*, 2006; Yapar *et al.*, 2007**). Furthermore, it was determined that sialic acid concentrations increase in the hepatic disorders (**Cylwik *et al.*, 2007**).In a study of **Niethamer *et al.* (2012)**, It was found that oral administration of glucosamine besides N-acetylmannosamine in glucosamine myopathy was improved and this was linked with the sialic synthesis pathway.

Moreover, it was found that serum protein bound hexosamine levels showed significant decrease in rats injected with TAA compared to control group and this in contrary to **George and Chandrakasan (1996)** who found that liver hexosamine levels showed significant increase in liver hexosamine level on the 14^{th} and 21^{st} day of dimetylnitrosamine injection.

Furthermore there was a decrease in liver hexosamine of rats intoxicated with TAA and administrated with glucosamine sulphate compared to rats intoxicated with TAA and administrated with glucosamine hydrochloride. Liver glycoproteins hexosamine of TAA group showed a significant decrease when compared to control group.

Kesava Reddy and Dhar (1991) George and Chandrakasan (1996) have demonstrated alteration in glycoprotein concentration during inflammatory conditions, liver fibrosis, malignancy and in tumorous human lung tissue.

Administering 7.9 g/kg of body of weight ethanol every day to Wistar rats for 60 days resulted in a significant increase of liver hexosamine, fucose, and sialic acid levels and a significant reduction of total hexoses level as compared with those of the control rats (**Senthilkumar and Nalini, 2004**).

Serum Glycoproteins with a molecular weights of 32, 48 and 58 KDa were decreased in rats intoxicated with TAA and treated glucosamine either hydrochloride and sulphate as compared to control and TAA group. Also liver glycoproteins were separated and then the separated fractions were applied to SDS -polyacrylamide gel electrophoresis of isolated liver glycoproteins fractions showed variation in density of four bands between different groups. The molecular weights of the four distinct bands were about 46, 55, 58 and 60 KDa. These bands were diminished in TAA groups compared to control groups and enriched in TAA& glucosamine groups compared to TAA groups. Our results were in accordance of study of **Sorrell *et al.* (1983)** who indicated that liver slices prepared from chronic ethanol-fed rats display both impaired synthesis and secretion of glycoproteins and proteins and these defects are further potentiated by acute ethanol administration.

In conclusion, glucosamine administration in TAA induced hepatic fibrosis in rats could ameliorate hepatic fibrosis by alteration of the glycoprotein expression and sugar moieties especially sialic acid and hexose.

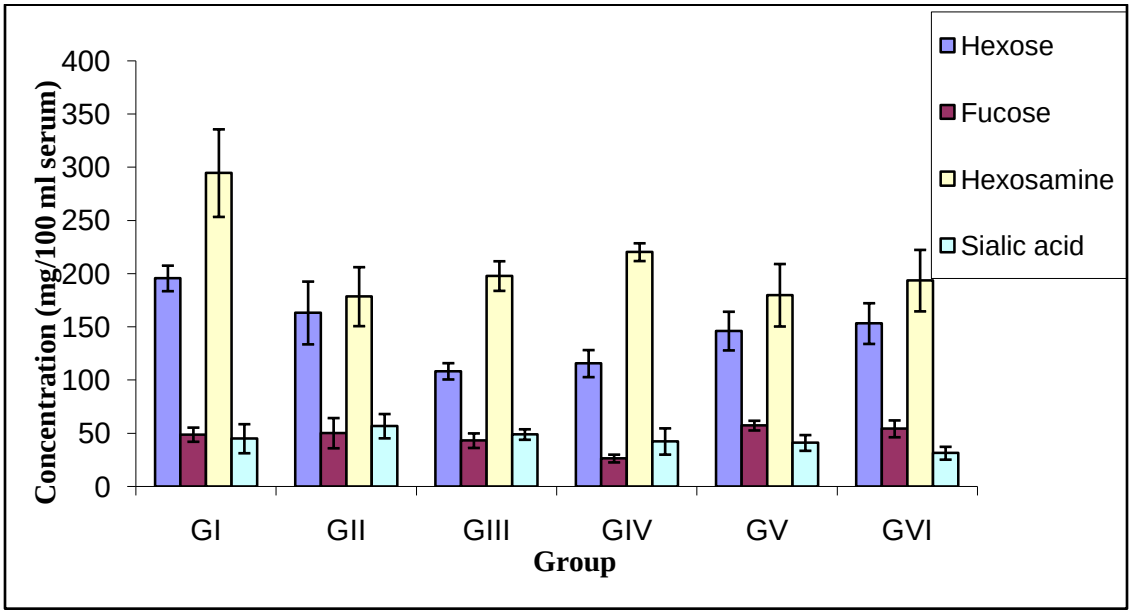
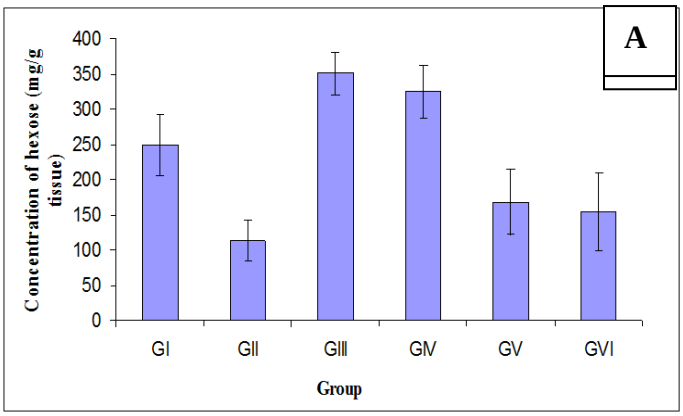
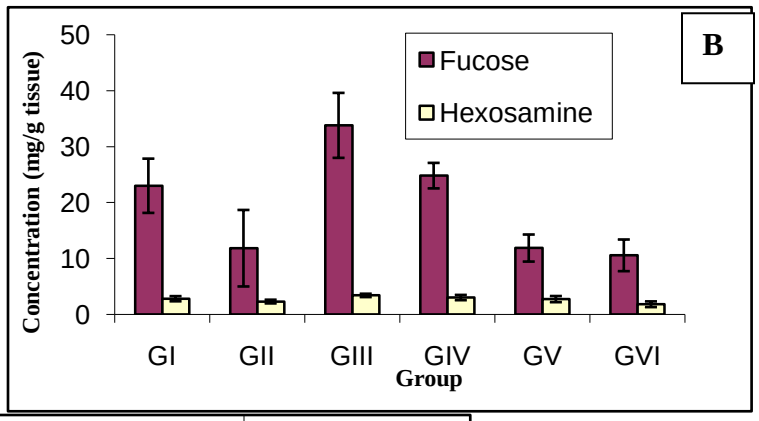


Fig. (1): Serum glycoprotein sugar moieties levels in male rats of studied groups





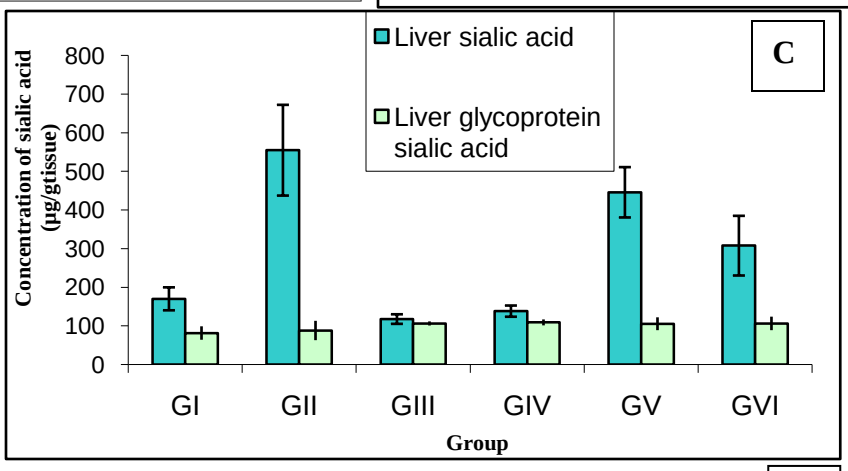


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Fig. (2): Liver sugar moieties in different rat groups. (A) Liver hexose level (B) Liver fucose and hexosamine levels (C) Liver and liver glycoprotein sialic acid levels

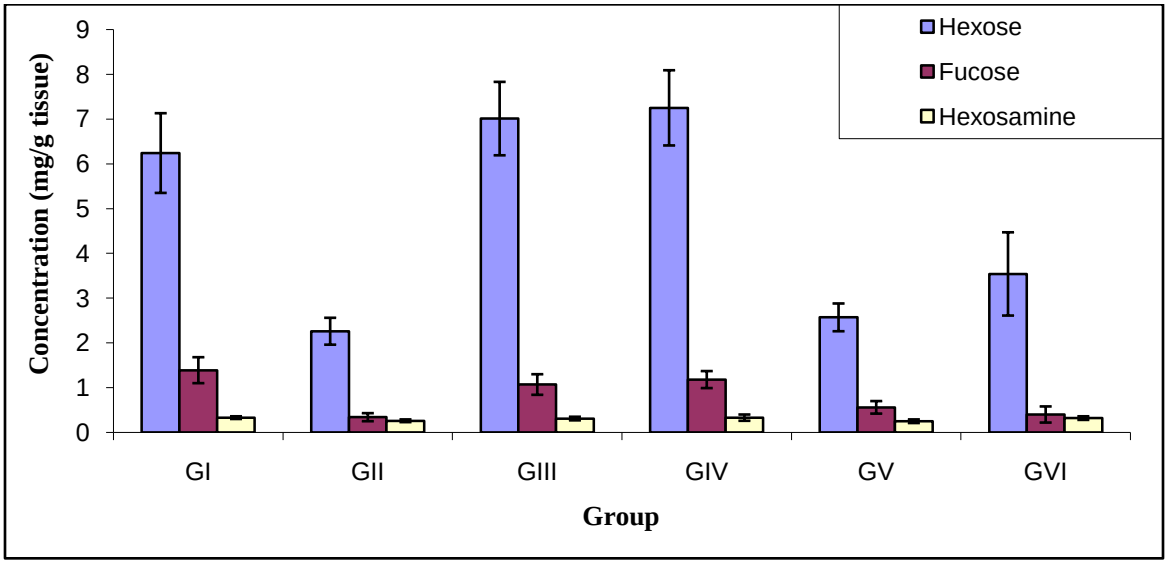

Fig. (3): Liver glycoproteins hexose, fucose and hexosamine levels in male rats of studied groups.

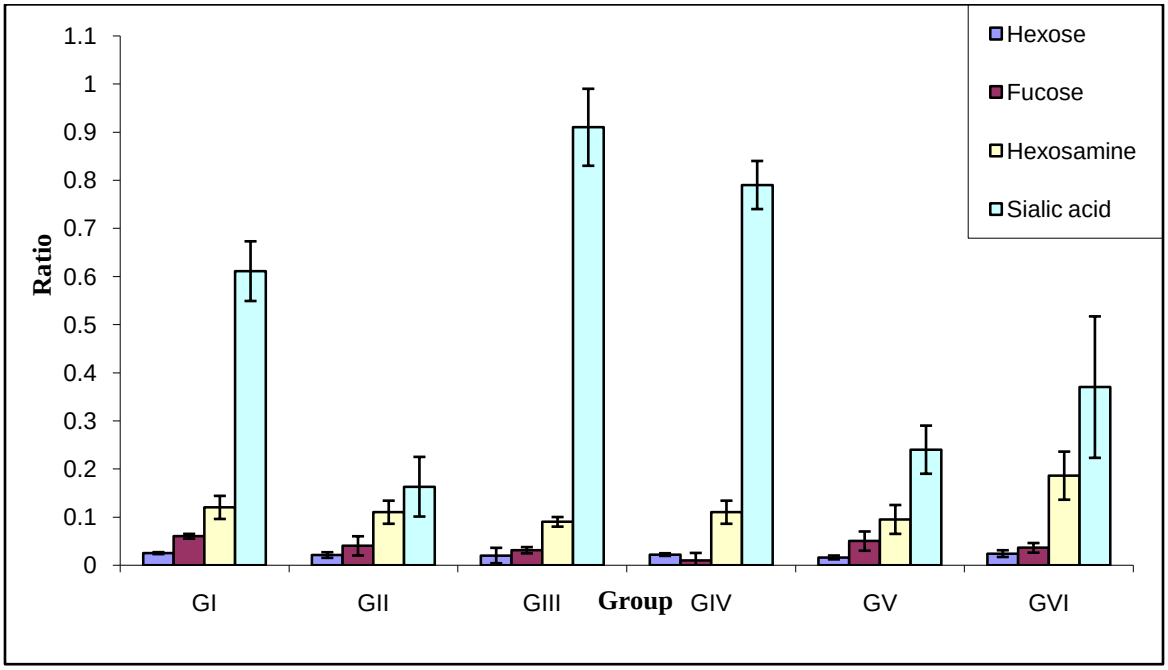

Fig. (4): The ratio between liver and liver glycoprotein sugar moieties levels in male rats of studied groups.

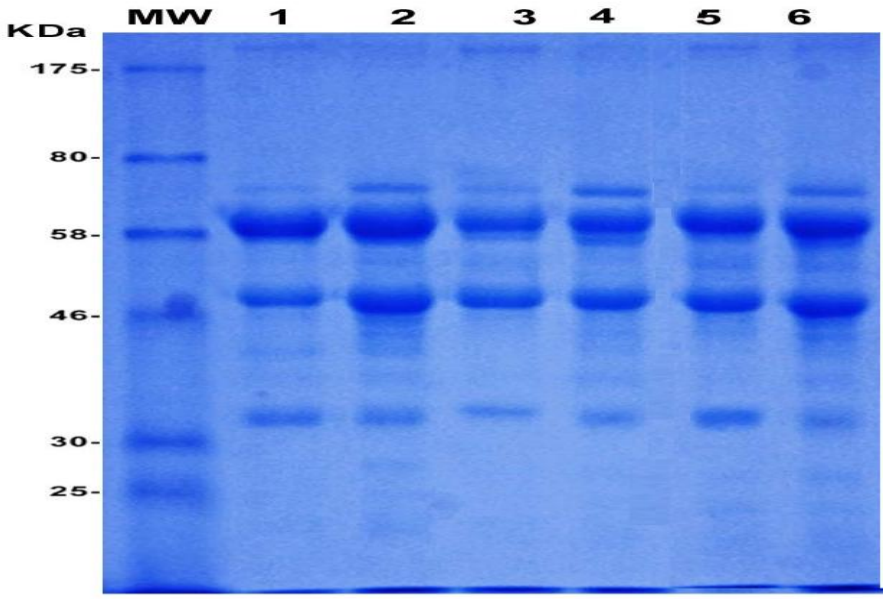

Fig. (5): SDS-PAGE of serum glycoproteins. MW lane, molecular weight marker Lane 1 control, Lane 2 TAA group, Lane 3 glucosamine hydrochloride group, Lane 4 TAA &glucosamine hydrochloride group, Lane 5 glucosamine sulphate group and Lane 6 TAA & glucosamine sulphate group.

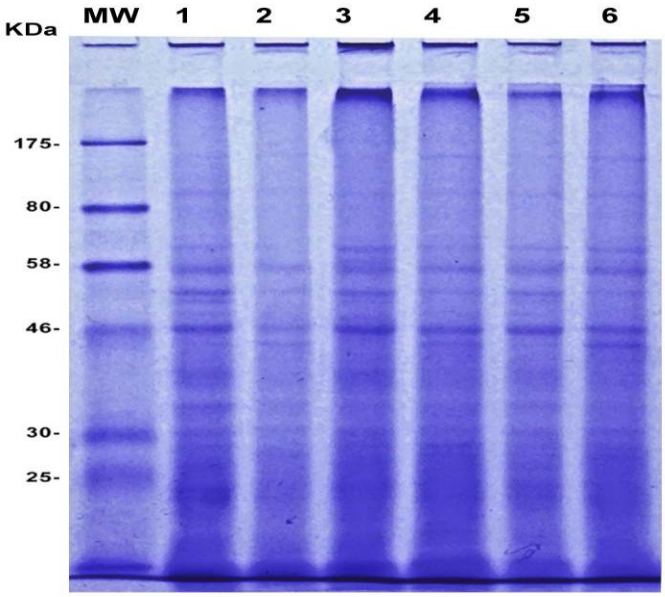

Fig. (6): SDS-PAGE of liver plasma membrane glycoproteins. MW lane, molecular weight marker Lane 1 control, Lane 2 TAA group, Lane 3 glucosamine hydrochloride group, Lane 4 TAA &glucosamine hydrochloride group, Lane 5 glucosamine sulphate group, Lane 6 TAA & glucosamine sulphate group.

Table (1): Serum ALT, AST, ALP activities, and albumin mucoprotein levels and liver collagen of male rats in the studied groups

Parameter / Group	ALT activity (U/l)	AST activity (U/l)	ALP activity(U/l)	Serum albumin (g/dl)	Mucoprotein (mg/ dl)	Liver collagen (µg/g tissue)
Group I (control)						
Range	20.7-42.9	50 – 74.8	45.6-127.2	3.1 – 4.0	165.3 – 246.9	92- 262.2
Mean±SD	29.3 ± 5.9	62.1±7.76	83.7 ±20.75	3.6±0.27	212.7±25.35	199.1±42.89
Group II (TAA)						
Range	32 - 48.4	83.9 – 113.9	408.3-844.6	2.4 – 3.1	112.9 – 187.98	740.5 – 1474.1
Mean±SD	41.9 ± 3	104.5±13.11	631.5±138.7	2.7±0.28	155.9±31.22	1017 ±238.41
%change	+42.8	+68.3	+654	-24.6	-26.7	+410.8
p value	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001
Group III (Glucosamine HCl)						
Range	20.7 - 34.8	55.7 – 89.5	121.3-200	2.9 – 3.5	137.2 – 238.8	200.1 – 308.2
Mean±SD	28.6 ± 4.14	72.8 ± 10.74	184.2±25.01	3.2±0.22	183.3±36.1	267.9±31.56
%change	-2.49	+17.2	+120	-10.14	-13.8	+34.5
p value	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
Group IV (Glucosamine sulphate)						
Range	27.5 - 34.1	60.1 – 88.7	105-230	2.1 – 3.5	122.6- 209.6	186.3 – 402.6
Mean±SD	31.2 ± 2.1	67.8±9.54	191.4±39.75	2.8±0.42	176.6±37.41	331.7±59.29
%change	+6.5	+9.2	+128.6	-21.4	-16.9	+66.6
p value	N.S.	N.S.	<0.05	<0.001	N.S.	N.S.
Group V (TAA+glucosamine hydrochloride)						
Range	29.3 – 40.7	82.2 – 100	299-388	2.2 – 3.6	189.1 – 375.4	340.4 – 922.2
Mean±SD	36.4 ± 4.13	94.5±6.4	347.8±73.3	3.1±0.5	265.7±83.72	536.5±224.68
%change	+24.18	+52.3	+315.5	-11.3	+24.9	+169.4
p value	<0.01	<0.001	<0.01	N.S.	<0.001	<0.01
p value*	N.S.	N.S.	<0.001	N.S.	<0.001	<0.001
Group VI (TAA+glucosamine sulphate group)						
Range	35 - 45	84.35 – 110.87	300-500	2.34 – 3.66	111.3 – 198.24	347.24 – 919.86
Mean±SD	39.1 ± 3.53	94.9±8.18	426.3±69.6	2.9±0.44	154.2±37.48	557.2±202.51
%change	+33.3%	+52.8	409.3	-16.95	-27.5	+179.8
p value	<0.01	<0.001	<0.001	N.S.	N.S.	<0.05
p value*	N.S.	N.S.	<0.001	N.S.	N.S.	<0.001
*p*** value	N.S.	N.S.	N.S.	N.S.	<0.001	N.S.

p value versus control
p value vs TAA*
*p** vs TAA & glucosamine hydrochloride*

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