

## Effect Of Glucosamine On The Healthy Status Of Diabetic Rats

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### ABSTRACT

This search focuses on glucosamine as a drug using in the treatment of diabetes, especially in Egypt. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In Egypt it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Therefore information on antioxidant effects of these medicinal drugs is also included and investigated. Diabetes is a chronic metabolic disorder characterized by altered carbohydrate, fat and protein metabolism, and an increased risk of multiple complications. Effect of glucosamine at doses of 1, 1.5 and 2 g/kg b.w on alloxan-induced diabetic rats were studied, Sprague-Dawley albino rats (30 male), weighing 150 ±5 g were divided into 5 groups and administered glucosamine daily for 28 days. Blood samples were taken from each rat and tested for blood glucose, total cholesterol, (LDL), (HDL), triglycerides, urea, uric acid, creatinine levels and liver enzymes activities. Blood glucose, triglycerides, total cholesterol, LDL, urea, uric acid, creatinine and liver enzymes activities (AST and ALT) were significantly increased, while HDL, was significantly decreased compared with the negative control rats. Treating diabetic rats with 1, 1.5 and 2 g/kg b.w caused a significant improvement in these biochemical measures and the best results were achieved by using 2 g/kg b.w followed by 1.5 and 1 g/kg b.w, respectively.

**Keywords:** glucosamine, treatment of diabetes, diabetes complications, total cholesterol.

### 1. Introduction:

Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by defective insulin secretion and / or action, resulting in long term multi-organ complications (Caughron and Smith, 2002.). Chronic hyperglycemia causes damage to the eyes, heart, kidneys, nerves, and blood vessels (Lebovitz, 2001). The current review focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses. On the other hand, high glucose level was found to increase the production of free radicals, as determined by cell damage markers. Increased oxidative stress has been implicated in the pathogenesis of diabetic complications and reduced levels of antioxidants are found in blood and tissue in both human and experiments diabetes (Cuncio et al., 1995; Baynes & Thorpe, 1999 and Koleva et al., 2002).

Glucosamine (C<sub>6</sub>H<sub>13</sub>NO<sub>5</sub>) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine is part of the structure of the polysaccharides chitosan and chitin, which compose the exoskeletons of crustaceans and other arthropods, cell walls in fungi and many higher organisms. Glucosamine is one of the most abundant monosaccharides (Horton and Wander, 1980). Glucosamine is found in almost all human tissues but is highest in concentration in the liver, kidney and cartilage. It is the most fundamental building block required for the biosynthesis of various compounds including glycolipids, glycoproteins, glycosaminoglycans, hyaluronate and proteoglycans, which are all compounds intimately involved with joint structure and function. Glucosamine is also an essential component of cell membranes and cell surface proteins as well as interstitial structural molecules that hold cells together. Directly or indirectly, glucosamine plays a role in the formation of articular surfaces, tendons, ligaments, synovial fluids, skin, bone, nails, heart valves, blood vessels and mucous secretion within the digestive, respiratory and urinary systems. (De-los et al., 2000). Anderson et al. (2004) found

that fasting plasma glucose values decreased slightly for subjects after oral glucosamine for 6 weeks. There were no adverse effects of oral glucosamine administration on blood, urine or fecal parameters. glucosamine is safe under current conditions of use and does not affect glucose metabolism. The aim of this study is to examine and understand, the effect of different levels of glucosamine (1, 1.5 and 2 g/kg b.w) on serum glucose, lipid profiles, and liver and kidney functions in induced diabetic rats.

## 2. MATERIALS AND METHODS

### 2.1. Materials:

The studied sample is glucosamine was purchased from of Pharmacy's Dakahlia, Governorate, Egypt. Male albino rats (150±5g b.wt. each) of Sprague Dawley strain, the animals were obtained from Research Institute of Ophthalmology, Giza, Egypt. Alloxane was obtained from Sigma chemical company and used as a diabetogenic agent.

### Methods:

Sprague-Dawley albino rats weighing 150 ±5g b.wt were purchased from the Research Institute of Ophthalmology, Giza, Egypt. All rats were fed on basal diet for one week (adaptation period). The basal diet consisted of casein (10%), cellulose (5%) salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to Campbell, 1963. After the adaptation period, diabetes was induced by intraperitoneally injection of 150 mg/kg body weight of alloxane according to the method described by Desai and Bhide (1985). Blood samples were collected after 48 hr. of injection and glucose levels were determined. Rats with blood glucose level higher than 250 mg/dl were considered to be diabetic. Five groups of rats (6 rats each) were studied according to the following scheme for 28 days: (1) negative control (non diabetic rats), (2) positive control (untreated diabetic rats), (3) diabetic rats fed on basal diet administrated orally glucosamine 1 g/kg b.w, (4) diabetic rats fed on basal diet administrated orally glucosamine 1.5 g/kg b.w and (5) diabetic rats fed on basal diet administrated orally glucosamine 2 g/kg b.w (the used levels were according to (ADRAC/ADRU, 2008). Blood samples were collected from orbital plexus venous into centrifuge tubes and the serum was separated and stored at 20° C for analysis.

### 3. Biochemical Analysis:

Serum glucose levels were determined according to the method described by Tietz (1976). Serum total cholesterol (Allain, 1974). (high- density lipoprotein cholesterol (Gordon and Amer, 1977) and low-density lipoprotein were determined according to the methods of Lee and Nieman, (1996). Aspartate amino transferase (AST) determined according to the methods of Henry, (1974). and alanine amino transferase (ALT) activities were calorimetrically determined according to the method of Yound, (1979). Serum urea was measured by the method of carawy, (1955). Serum creatinine was estimated by the method of Bohmer, (1971) Uric acid was determined according to the method described Carawy, (1955) using spectrophotometer.

### 4. Statistical analysis

Standard error and ANOVA test followed by the student *t*-test for significant difference. Statistical significant difference was defined as  $P < 0.05$  (Snedecor and Cochran, 1976).

## 5. RESULTS AND DISCUSSION

Effect of different levels of glucosamine on body weight and blood glucose levels in diabetic rats are shown in Table (1). Alloxane -induced diabetic rats gained on average less body weight than the negative control rats over the whole period of the study. Final body weight of the negative control rats was significantly higher than positive control and all the diabetic groups. The reduction in body weight for the three doses of glucosamine was significantly lower than the negative control rats. Increasing in body weight than the positive control due to administration of 1.5 and 2 g/kg b.w glucosamine. It was significant between group 2 g/kg and 1.5 & 1 g/kg b.w., these results are in agreement with the results mentioned by (De-los et al., 2000). This found a significant decrease in body weights of the rats administered glucosamine compared with negative control group. From Table (1), blood glucose levels were

increasing in diabetic rats compared with the negative control rats. The significant increase in the levels of blood glucose in alloxane –induced diabetic rats could be due to it is a beta cytotoxic induces chemical diabetes through damaging insulin-secreting cell (Hecht et al., 1973). At the end of the experiment, blood glucose levels in diabetic rats dosed with 1, 1.5 and 2 g/kg b.w decreased significantly than those of the diabetic control rats. The blood glucose levels as a result of treatments were  $290.2 \pm 4.6$ ,  $269.1 \pm 0.5$  and  $200.6 \pm 2.7$  for 1, 1.5 and 2 g/kg b.w respectively. The dose of 2 g/kg b.w is the best for controlling blood glucose level while was significantly different than the others. In this respect, Adams (1999) found that the glucosamine at the level 1.5 g/kg b.w significantly lowered the blood sugar level of hyperglycemic rats. Also Marshall (1991) observed that glucosamine showed a clear hypoglycemic effect in diabetic rats. Also may be mediated through stimulation of insulin release resembling the oral hypoglycemic drugs or peripheral glucose utilization (Rossetti, 1993). This may be due to the advanced the hypothesis that routing of incoming glucose through the hexosamine biosynthetic pathway plays a role in the development of insulin resistance.

Table (2) represents the effect of glucosamine on serum lipid profiles in diabetic rats. Diabetes results in a significant increase in total cholesterol level compared with the non diabetic rats. Treating diabetic rats with 1, 1.5 and 2 g/kg b.w glucosamine showed a significant reduction in serum total cholesterol level compared with the positive control rats. Glucosamine (2 g/kg b.w) was significantly different when compared with the negative control rats for total cholesterol levels. From data in the same table (2), it could be observed that LDL-cholesterol levels decreased significantly in diabetic rats compared with the positive control rats as a results of diabetes whereas, in group administrated 2 g/kg b.w, there is no significant between this group and the negative control (Miles, 1998). When diabetic rats administered with 1, 1.5 and 2 g/kg b.w, the levels of lipid profiles were significantly reduced compared with the positive control rats. The highest reduction was achieved by using 2 g/kg b.w followed by 1.5 and 1 g/kg b.w glucosamine respectively, for the tested lipid profile. Diabetes caused a significant decrease in HDL-cholesterol level when compared with the negative control rats. Treating diabetic rats with 1.5 and 2 g/kg b.w glucosamine caused a significant increase in the levels of HDL-cholesterol compared with the untreated diabetic rats McNamara, (1996). The highest increase in HDL-cholesterol level was achieved by using 1.5 and 2 g/kg b.w glucosamine. Milewski, (2002) reported that administration of glucosamine results in decreased plasma triacylglycerol and butyrate levels in alloxane treated rats. Setnikar and Rovati, (2001) found significant decrease triglycerides levels after the intraperitoneally injection of glucosamine in hypertriglyceridemic rats. While the plasma totals cholesterol levels showed no significant difference in relation to baseline levels. These results suggest decreased the serum cholesterol, LDL-cholesterol compared with control rats Anderson et al. (2004). Data presented in Table (3) show the effect of 1, 1.5 and 2 g/kg b.w on kidney functions in diabetic rats. Serum urea, uric acid and creatinine levels (mg/dl) which were the bio-chemical parameters that are related to kidney functions increased significantly in diabetic control rats compared with the negative control rats as results of diabetes. This may be due to the hyperglycemia which caused damage to kidneys (Miles, 1998). Treating diabetic rats with glucosamine caused a significant reduction in serum urea. Uric acid and creatinine levels compared with diabetic control rats. The highest reduction was achieved by 2 g/kg b.w followed by 1.5 and 1 g/kg b.w respectively. The significant increase in serum urea, uric acid and creatinine levels suggests renal malfunction. Creatinine levels are indicators of renal functions, with increased levels appearing in the event of significant impairment (De-los et al., 2000). There is considerable evidence that increased oxidative stress may participate in the pathogenesis of diabetic complications, including nephropathy (Milewski, 2002). This shows that with significant increase in the levels of kidney markers, about 75% of the nephron might have been damaged (Milewski, 2002). From the above mentioned data, it could be concluded that, all tested diabetic groups which were administered glucosamine with different levels (1, 1.5 and 2 g/kg b.w) improved their renal functions. Table (4) shows the effect of 1, 1.5 and 2 g/kg b.w glucosamine on liver functions in diabetic rats. Diabetes resulted in a significant decrease in liver enzymes aspartate amino transferase (AST) and alanine amino transferase (ALT) activities compared with the positive control rats as a result of diabetes and oxidative stress which reduced liver functions. Significant increases of AST and ALT as shown in the results suggest possible necrotic injury of the liver or cholestasis with hepatocellular necrosis (Nicolosi et al., 2004). Liver enzymes activities were decreased to the normal levels found in the negative control rats after treatment with 1, 1.5 and 2 g/kg b.w.

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**Table 1:** Effect of 1,1.5 and 2 g/kg b.w glucosamine on body weight and blood glucose levels in diabetic rats.

Animal groups parameters	C-	C+	1g /kg b.w	1.5 g/kg b.w	2 g /kg b.w
BWG g/100g	42.27 <sup>a</sup> ±2.51	29.41 <sup>b</sup> ±7.56	30.12 <sup>b</sup> ±3.46	33.17 <sup>b</sup> ±4.82	38.58 <sup>a</sup> ±5.71
Blood glucose	89.8 <sup>e</sup> ±0.87	302.1 <sup>a</sup> ±1.2	290.2 <sup>b</sup> ±4.6	269.1 <sup>c</sup> ±0.5	200.6 <sup>d</sup> ±2.7

**Table 2:** Effect of 1,1.5 and 2 g/kg b.w glucosamine on serum triglycerides level and lipid profiles in diabetic rats (mg/dl).

Serum lipids	C-	C+	1g /kg b.w	1.5 g /kg b.w	2 g /kg b.w
Total cholesterol	86.26 <sup>e</sup> ±1.19	181.2 <sup>a</sup> ±2.15	176.1 <sup>b</sup> ±0.13	161.2 <sup>c</sup> ±0.12	140.7 <sup>d</sup> ±3.21
HDL-cholesterol	53.94 <sup>a</sup> ±0.12	39.87 <sup>c</sup> ±1.15	40.89 <sup>c</sup> ±0.04	43.94 <sup>b</sup> ±0.05	47.92 <sup>b</sup> ±0.03
LDL-cholesterol	20.2 <sup>d</sup> ±1.17	46.9 <sup>a</sup> ±4.34	36.1 <sup>b</sup> ±0.91	31.5 <sup>c</sup> ±0.74	23.1 <sup>d</sup> ±0.91

**Table 3:** Effect of 1,1.5 and 2 g/kg b.w glucosamine on kidney functions in diabetic rats (mg/dl):

Parameters	C-	C+	1g /kg b.w	1.5 g /kg b.w	2 g /kg b.w
Creatinine mg/100ml	0.69 <sup>d</sup> ±1.31	1.96 <sup>a</sup> ±0.21	1.82 <sup>a</sup> ±3.14	1.57 <sup>b</sup> ±0.07	1.13 <sup>c</sup> ±0.21
Uric Acid mg/100ml	2.35 <sup>c</sup> ±0.15	2.95 <sup>a</sup> ±0.22	2.82 <sup>a</sup> ±1.2	2.65 <sup>b</sup> ±1.00	2.36 <sup>c</sup> ±0.5
Urea Nitrogen mg/100ml	25.2 <sup>d</sup> ±1.15	48.46 <sup>a</sup> ±0.1	42.76 <sup>b</sup> ±1.2	37.15 <sup>c</sup> ±1.3	33.18 <sup>c</sup> ±2.1

**Table 4:** Effect of 1,1.5 and 2 g/kg b.w glucosamine on liver functions in diabetic rats (mg/dl).

Parameter	C-	C+	1g /kg b.w	1.5 g /kg b.w	2 g /kg b.w
AST(U/L)	25.1 <sup>b</sup> ±0.27	34.2 <sup>a</sup> ±0.11	30.5 <sup>a</sup> ±0.56	26.7 <sup>b</sup> ±0.21	23.1 <sup>b</sup> ±0.15
ALT(U/L)	19.8 <sup>b</sup> ±0.31	28.9 <sup>a</sup> 1.51±	26.4 <sup>a</sup> ±2.52	22.7 <sup>b</sup> ±2.5	19.7 <sup>b</sup> ±4.25

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