

## The Role of Abnormal Ketone Bodies During Insulin Deficiency in Streptozotocin Induced Diabetes

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### ABSTRACT

Diabetes Mellitus (DM) is the most common metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. This study aimed to determine the efficiency of treatment with different doses of insulin through comparing body weight and biochemical parameters such as liver function tests (aspartate transaminase (AST), alanine transaminases (ALT), alkaline phosphatase (ALP); glucose level; insulin level; pancreatic enzymes (amylase and lipase) activities; lipid profile (cholesterol, triglycerides, high density lipoproteins, low density lipoproteins) as well as determination of Beta hydroxybutyrate level ( $\beta$ -HB) which provide useful information for diagnosing and managing of diabetes complications in serum of streptozotocin-induced diabetic rats among the experimental groups. Experimental animals were induced diabetes with a single injection of streptozotocin (STZ) at a dose of 75 mg/kg body weight. Seventy five male rats were divided into five equal groups each of fifteen. Control group (group I), diabetic group (group II), insulin treated diabetic groups (group III, IV and V) which received a single daily dose of one unit of insulin per each (50, 100 or 200 mg) rise in blood sugar, respectively. Obtained results revealed that body weight, different biochemical parameters and ketone bodies level were re-established in diabetic rats treated with affected dose of insulin. We concluded that treatment with sufficient dose of insulin is effective to maintain the biochemical parameters at or near normal level and also, we concluded that determination of  $\beta$ -HB was found to be useful in establishing the diagnosis and avoidance of diabetic ketoacidosis (DKA). Thus,  $\beta$ -HB can be a sensitive metabolic marker to estimate the adequacy of insulin therapy.

**Keywords:** Diabetes mellitus, ketone bodies, insulin, pancreas, amylase, lipase, lipid profile.

### INTRODUCTION

Diabetes mellitus (DM) is a global epidemic disease with an estimated worldwide prevalence of 246 million people in 2007 and forecasts to rise to 300 million by 2025<sup>1</sup>. Currently, there are over 150 million diabetics worldwide, and this is likely to increase to 300 million or more by the year 2025 due to increased sedentary lifestyle, consumption of energy-rich diet and obesity<sup>2</sup>. In the USA the prevalence is estimated to increase from 4.0 to 7.2% (or 29 million) between year 2000 and 2050<sup>3</sup>; consequently, diabetes presents a major challenge to health care systems around the world<sup>4</sup>. In Egypt, the prevalence of type 1 diabetes mellitus is 0.13-0.4% according to the International Diabetes Federation<sup>5</sup>. The global prevalence of DM in the year 2010 among adults has been estimated to be 6.4%. It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes<sup>6</sup>. DM is a complex metabolic disorder characterized by hyperglycemia. The hyperglycemia is caused as a consequence of a deficiency in insulin in type 1 diabetes (IDDM). Molecular pathophysiological mechanisms that precede hyperglycemia, or are observed with the clinical

symptoms of DM, include, among others, alterations in lipid and amino acid metabolism<sup>7</sup>, changes in hormone levels including insulin. Insulin is an anabolic hormone that stimulates protein, glycogen, and lipid synthesis, and inhibits lipolysis and gluconeogenesis. The actions of insulin may be classified as immediate, intermediate or long-term action<sup>8</sup>. The complications of DM include cardiomyopathy, vasculopathy, neuropathy, nephropathy and retinopathy, and are major causes of morbidity and mortality<sup>9</sup>. Ketone bodies (acetoacetate (AcAc), 3- $\beta$ -hydroxybutyrate ( $\beta$ -HB) and acetone) are energy-rich compounds that transport energy from the liver to other tissues. During periods of glucose deficiency, ketone bodies play a key role in sparing glucose utilization<sup>10</sup> and reducing proteolysis<sup>11</sup>. The brain cannot utilize fatty acids for energy when blood glucose levels become compromised. In this case, ketone bodies provide the brain with an alternative source of energy<sup>12</sup>. Ketone bodies are present in small amounts in the blood of healthy individuals during fasting or prolonged exercise. Abnormally large quantities of ketone bodies are found in the blood of individuals who are experiencing diabetic

ketoacidosis, alcoholic ketoacidosis, salicylate poisoning, and other rare conditions<sup>13</sup>. Streptozotocin which is a naturally occurring nitrosourea induced diabetes of experimental animals, a model similar to human insulin-dependent diabetes mellitus, because of its toxic effects on islet beta cells<sup>14</sup>. Therefore, The present study was carried out in attempt to determine if any complications related to the pancreas or liver function tests can be changed during treatments with variable or unaffected amounts of insulin in IDDM-induced in rats. Moreover, the study was further extended to establish the prevalence of ketosis in type 1 diabetes with casual hyperglycemia and to describe the relationship between glycemia and ketonemia in type 1 diabetes.

## MATERIALS AND METHODS

### *Experimental animals*

Seventy five adult male Sprague-Dawley rats weighed about 150-200 g were obtained from the Animal House Laboratory of the National Research Centre, Giza, Egypt. They were housed in plastic cages under standard laboratory conditions at temperature of  $24 \pm 3$  °C with 12–hours light-dark regimen and monitored for the duration of the study. They were provided with standard diet and tap water. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

### *Experimental design*

After one week of acclimatization, animals were divided into five groups each of 15 rats. Group I served as untreated control was given citrate buffer as a drug vehicle only. Group II, III, IV and V rats were starved overnight and injected intraperitoneally with a single dose of Streptozotocin (STZ) [75 mg/kg body weight, Sigma chemical Co., USA] in 0.1M citrate buffer (pH 4.5) as described by Aughstien and Mohammed<sup>15</sup> and then the rats were followed up for one week. Group II rats served as diabetic group and rats were killed after one week. Group III, IV and V were treated subcutaneous with Insulatard human insulin purchased from (Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark) as follow; Group III: received a single daily dose of one unit of insulin per each 50 mg rise in blood sugar for one week. Group IV: received a single daily dose of one unit of insulin per each 100 mg rise in blood sugar for one week. Group V: received a single daily dose of one unit of insulin per each 200 mg rise in blood sugar for one week. At the end of the experiment all insulin treated diabetic rats were killed two hours after the last injection.

### *Samples collection*

At the end of the experiment the animals were anesthetized and blood samples were drawn from retro-orbital plexus into eppendorf tubes. The samples were allowed for clotting at room temperature and then centrifuged at 3000 r.p.m for 15 minutes; the serum was separated and kept in clean stoppered vials at -20 °C until assay. Another part of blood samples were collected in heparinized or EDTA tubes for measuring glucose. The serum was used to measure the activities of liver enzymes (AST, ALT, ALP), blood glucose levels, insulin concentration,  $\alpha$ -amylase

activity, lipase activity, ketone bodies levels and lipid profile (cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol). While; body weights of each rat were determined at the onset of the experiment and every other day following STZ injection.

### *Biochemical analysis*

Serum ALT, AST and ALP activities were measured in sera of the rats by using commercially available kits obtained from Biodiagnostic, Egypt. Serum insulin was measured according to the radioimmunoassay method using Coat-A-Count Insulin kit obtained from Siemens Healthcare Diagnostics GmbH (Germany). The  $\alpha$ -amylase and lipase activities were determined using kit obtained from DTN-Dia Technology, Chema (Italy). The  $\beta$ -hydroxybutyrate concentration was determined in serum by  $\beta$ -hydroxybutyrate assay kit purchased from BioVision, San Francisco, USA. Serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were determined in rats sera using kits obtained from Biodiagnostic, Egypt.

### *Statistical analysis*

The data obtained in the present study were presented as mean  $\pm$  S.E. The significance of the difference between the means was calculated according to the Student (*t*-test). The difference between two means was considered significant at  $P < 0.05$ .

## RESULTS

The results of the current study revealed that the activities of AST, ALT and ALP are significantly increased ( $P < 0.001$ ) in diabetic group received STZ alone and diabetic groups treated with insulin (groups III, IV, V) when compared with control group (table 1). While AST, ALT and ALP activities significantly decreased ( $P < 0.001$ ) by [(69,54 and 36%); (79,68 and 40%); (76, 68 and 56%)] respectively in the groups treated with insulin groups III, IV, V respectively as compared to diabetic group. Also, (table 1) summarizes the mean data of body weights in all experimental animals at the beginning and at the ending of experiment. The results of our study showed that at the beginning of the experiment the body weight was significantly increased ( $P < 0.01$ ) in diabetic group received STZ alone and there was no significant change ( $P > 0.05$ ) in body weight in diabetic groups treated with insulin groups III, V. On the other hand, there was high significant decrease in body weight in group IV treated with insulin ( $P < 0.001$ ) when compared to control group. While, there was no significant change ( $P > 0.05$ ) in body weight in groups (III, V) when compared to diabetic group. While there was very high significant decrease ( $P < 0.001$ ) in body weight in treated group IV compared to diabetic group. At the end of the experiment we found that there was very high significant decrease ( $P < 0.001$ ) in body weight in diabetic group received STZ alone while the body weight in diabetic groups treated with insulin groups IV, V was slightly decreased ( $P < 0.05$ ) when compared to control group; also there was no significant change ( $P > 0.05$ ) in body weight in group III when compared to control group. Also, at the end of the experiment there was very high significant increase in body weight ( $P < 0.001$ ) in

Table 1: Liver function tests (AST, ALT and ALP) as well as the change of body weight in different groups.

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/ml)	Mean body weight (g)	
				Beginning	Ending
I Control	13.04 ± 0.35	23.60 ± 0.37	76.30 ± 0.69	156.00±1.11	193.00±2.28
II Diabetic	147.90 ± 3.74 <sup>a*</sup>	177.70 ± 2.50 <sup>a*</sup>	456.70± 19.40 <sup>*</sup>	165.00±3.07 <sup>a**</sup>	141.00±8.48 <sup>a*</sup>
III Treated 1	45.60 ± 0.85 <sup>ab*</sup>	37.86 ± 0.59 <sup>ab*</sup>	109.70 ± 1.78 <sup>ab*</sup>	158.00±4.00 <sup>ab****</sup>	191.00±5.86 <sup>ab*</sup> , ****
IV Treated2	68.30 ± 0.52 <sup>ab*</sup>	57.38 ± 1.54 <sup>ab*</sup>	146.50± 2.91 <sup>ab*</sup>	134.00±2.94 <sup>ab*</sup>	181.00±5.40 <sup>ab*</sup> , ***
V Treated3	94.50 ± 1.29 <sup>ab*</sup>	106.70±1.85 <sup>ab*</sup>	203.00±1.92 <sup>ab*</sup>	163.00±6.69 <sup>ab****</sup>	183.00±4.82 <sup>ab*</sup> , ***

Data were represented as mean±SE. Statistical significance: \* p< 0.001; \*\* p<0.01; \*\*\* p<0.05; \*\*\*\* p>0.05. (a) As compared to control group ; (b) As compared to diabetic group.

Table 2: The levels of glucose (mg/dl), insulin and β-hydroxybutyrate in different groups.

Groups	Glucose level ( mg / dl )		Insulin level ( IU/ml)	β-hydroxybutyrate (mmol/L)
	Beginning	Ending		
I Control	127.00 ± 4.16	125.00 ± 1.01	19.10 ± 2.62	0.67 ± 0.03
II Diabetic	395.00 ± 13.47 <sup>a*</sup>	384.00 ± 26.80 <sup>a*</sup>	2.23 ± 1.02 <sup>a*</sup>	5.19 ± 0.136 <sup>a*</sup>
III Treated 1	452.00 ± 39.95 <sup>ab*, ***</sup>	131.00 ± 15.45 <sup>ab*, ****</sup>	294.73 ± 16.48 <sup>ab*</sup>	1.59 ± 0.053 <sup>ab*</sup>
IV Treated2	479.00 ± 20.10 <sup>ab*</sup>	153.00 ± 20.78 <sup>ab*, **</sup>	138.65 ± 14.62 <sup>ab*</sup>	2.72 ± 0.174 <sup>ab*</sup>
V Treated3	417.00 ± 14.14 <sup>ab*, ****</sup>	205.00 ± 20.33 <sup>ab*</sup>	112.99 ± 15.49 <sup>ab*</sup>	3.99 ± 0.053 <sup>ab*</sup>

Data were represented as mean±SE. Statistical significance: \*P < 0.001; \*\*P < 0.01; \*\*\*P < 0.05; \*\*\*\*P > 0.05. (a) As compared to control group; (b) As compared to diabetic group.

diabetic groups treated with insulin (groups III, IV, V) when compared with diabetic group. The results of the present study as mentioned in (table 2) showed that at the beginning of the experiment the glucose level was significantly increased (P < 0.001) in diabetic group received STZ alone and diabetic groups treated with insulin (groups III, IV, V) when compared with control group. While the glucose level was moderately increased (P < 0.05) by (14%) in diabetic group treated with insulin group III and significantly increased (P < 0.001) by (21%) in diabetic group treated with insulin group IV when compared to diabetic group. While there was no significant change (P > 0.05) in the glucose level in treated group V compared to diabetic group. At the end of the experiment we found that the glucose level was significantly increased (P < 0.001) by (207%) in diabetic group received STZ alone while the glucose level in diabetic group treated with insulin group IV was significantly increased (P < 0.01) by (22%) when compared to control group; also glucose level in diabetic group treated with insulin group V was significantly increased (P < 0.001) by (64%) as compared to control group. In addition, we found that there was no significant change (P > 0.05) in glucose level in diabetic group treated with insulin group III when compared to control group. Also, at the end of the experiment we found that the glucose level was significantly decreased (P < 0.001) by (66, 60 and 47%) respectively in groups treated with insulin (III, IV, V respectively) as compared to diabetic group. On the other hand, data presented in table (2) showed that the insulin level is significantly decreased (P < 0.001) in diabetic group received STZ alone, while

the insulin level is significantly increased (P < 0.001) in diabetic groups treated with insulin (groups III, IV, V) when compared with control group.

The insulin level significantly increased (P < 0.001) by (132, 62 and 51 fold) respectively in groups treated with insulin (groups III, IV, V respectively) as compared to diabetic group. Moreover, the β-hydroxybutyrate level was found to be significantly increased (P < 0.001) in diabetic group received STZ alone and diabetic groups treated with insulin (groups III, IV, V) when compared with control animals. The level of β-hydroxybutyrate in diabetic groups treated with insulin is significantly (P < 0.001) decreased by (69, 50 and 23%) respectively as compared to diabetic group (table 2). Amylase and lipase activities were measured as an index of pancreatic function. Data presented in fig. (1) showed that the amylase activity was significantly decreased (P < 0.001) in diabetic group (group II) and diabetic groups treated with insulin (groups III, IV, V) when compared with control group. On the other hand, there was a significant (P < 0.001) increase in the amylase activity in diabetic groups treated with insulin (group III and IV) in comparing to diabetic group by (17.7 and 4.4%), respectively. While there was no significant change (P > 0.05) in the amylase activity in group V compared to diabetic group. The activity of lipase in all experimental groups was shown in figure (2), and the results revealed that lipase activity was significantly decreased (P < 0.001) in diabetic group (group II) and diabetic groups treated with insulin (groups III, IV, V) when compared with control group. While, there was a significant (P < 0.001) increase in the lipase activity in

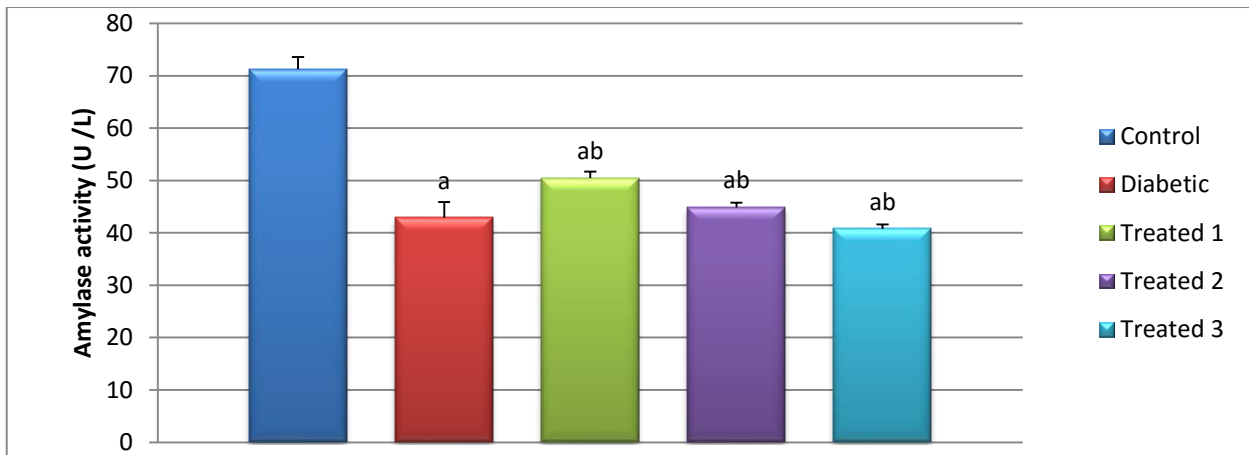


Figure 1: The activity of amylase as mean values  $\pm$  SE of different groups. <sup>a</sup> as compared to Control group, <sup>b</sup> as compared to diabetic group.

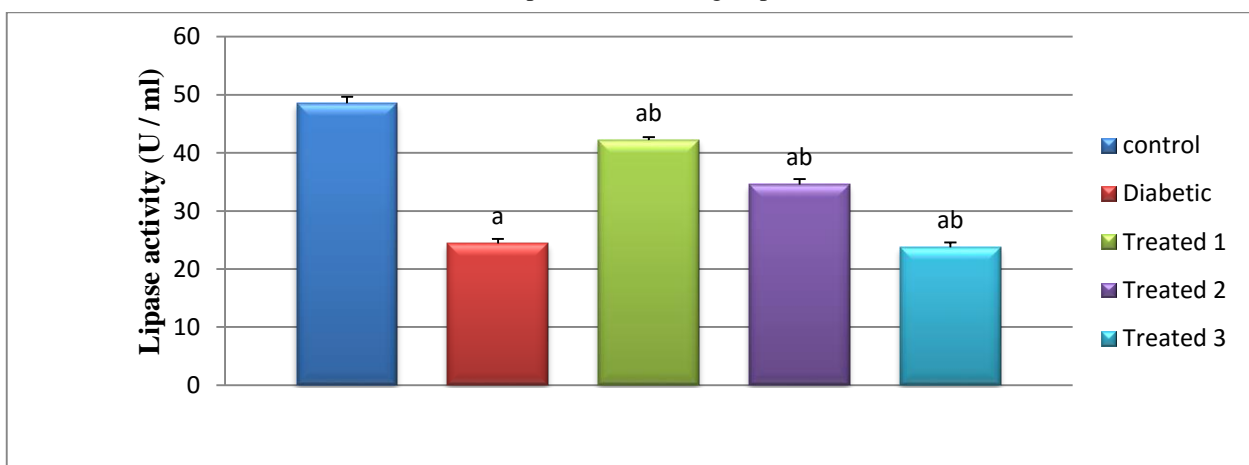


Figure 2: The activity of lipase as mean values  $\pm$  SE of different groups. <sup>a</sup> as compared to Control group, <sup>b</sup> as compared to diabetic group.

diabetic groups treated with insulin (group III and IV) in comparing to diabetic group by ( 73 and 41.5%), respectively. While there was no significant change ( $P > 0.05$ ) in the lipase activity in group V compared to diabetic group. The serum lipid profile of control, STZ-induced rats and insulin-treated diabetic rats are shown in table (3). It was found that the levels of TC, TG and LDL-cholesterol were significantly increased ( $P < 0.001$ ) in all groups received STZ either alone or in diabetic groups treated with insulin (groups III, IV, V) when compared with control group. On the other hand, there was a significant decrease ( $P < 0.001$ ) in the level of HDL-cholesterol in all groups in comparing to control group. The levels of TC, TG and LDL-cholesterol in diabetic groups treated with insulin (group III, IV, V) were significantly ( $P < 0.001$ ) decreased by (49,39.5 and 27%); (33,19.5 and 8%); and (68,55 and 37%) respectively as compared to diabetic group. Meanwhile, the level of HDL-cholesterol in diabetic groups treated with insulin (group III, IV, V) were significantly ( $P < 0.001$ ) increased by (33, 17 and 3%) as compared to diabetic group.

## DISCUSSION

In the present study, the activities of liver enzymes (AST, ALT and ALP) were significantly increased ( $P < 0.001$ ) in

diabetic group (group II) and diabetic groups treated with different doses of insulin (groups III, IV, V) when compared with control group (group I). These results are in-agreement with Elizabeth and Harris<sup>16</sup>, who reported that as a result of damage or toxicity to the liver, these enzymes may leak from the hepatocytes into the circulation where their activities become elevated. Therefore, the elevated activities of AST and ALT in serum of STZ-induced diabetic rats suggest hepatocellular damage.

Meanwhile, the data showed an increased activity of ALP in serum of STZ-induced diabetic rats (group II). ALP considered an indicator of biliary function, cholestasis and hepatic function<sup>16</sup>. In addition these results were in line with Zafar et al.<sup>17</sup> who illustrated that experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space and from there into the peripheral blood. On the other hand, a significant decrease in body weight at the beginning of the experiment were reported in diabetic (group II) and diabetic groups treated with insulin (group III, IV, V) when compared to control group which was reversed by insulin treatment in (group III, IV, V) reaching a value near to the control group at the end of the experiment. While the diabetic group (group II) showed a

Table 3: Lipid profile parameters (TC, TG, LDL and HDL) in serum of different groups.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL- cholesterol (mg/dl)	HDL- cholesterol (mg/dl)
I Control	65.70±1.01	57.30±0.90	18.90±1.19	35.30±0.39
II Diabetic	156.30±0.82 <sup>a*</sup>	112.90±3.87 <sup>a*</sup>	111.80±1.32 <sup>a*</sup>	21.90±0.33 <sup>a*</sup>
III Treated 1	79.50±1.04 <sup>ab*</sup>	75.30±1.71 <sup>ab*</sup>	35.30±1.3 <sup>ab*</sup>	29.16±0.30 <sup>ab*</sup>
IV Treated2	94.50±1.14 <sup>ab*</sup>	90.80±1.84 <sup>abc*</sup>	50.70±1.04 <sup>ab*</sup>	25.60±0.19 <sup>ab*</sup>
V Treated3	113.80±1.49 <sup>ab*</sup>	103.80±1.28 <sup>ab*</sup>	70.50±1.54 <sup>ab*</sup>	22.50±0.26 <sup>ab*</sup>

Data were represented as mean±SE. Statistical significance: \*  $p < 0.001$ . (a) As compared to control group ; (b) As compared to diabetic group.

significant decrease in the body weight at the end of the experiment when compared with control group. Our results were in accordance with Haldar et al.<sup>18</sup> who reported that the characteristic loss of body weight associated with diabetes is due to increased muscle wasting or loss of muscle proteins due to hyperglycemia. Rats treated with insulin showed significant improvement in body weight as compared to the diabetic rats suggesting a protective role of insulin on muscle wasting. Furthermore, Habibuddin et al.<sup>19</sup> also reported that the animals treated with STZ showed loss of their body weights because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. The results of our study revealed that at the beginning of the experiment, glucose level was significantly increased in diabetic group received STZ alone (group II) and diabetic groups treated with insulin (groups III, IV, V) when compared with control group (group I). While at the end of the experiment we found that the glucose level was significantly ( $P < 0.001$ ) increased by (207%) in diabetic group received STZ alone while the glucose level in diabetic group treated with insulin (group IV) was significantly increased ( $P < 0.01$ ) by (22%) when compared to control group; also glucose level in diabetic group treated with insulin (group V) was significantly increased ( $P < 0.001$ ) by (64%) as compared to control group. In addition, we found that there was no significant increase ( $P > 0.05$ ) in glucose level in diabetic group treated with insulin (group III) when compared to control group. This can be explained by the fact that, Glucose-6-phosphatase is a crucial enzyme for the final step of gluconeogenesis or glycogenolysis in which it catalyzes the hydrolysis of glucose-6-phosphate to glucose and phosphate. Glucose is transported out of the liver to increase blood glucose concentration. Normally insulin inhibits the hepatic glucose production by suppressing Glucose-6-phosphatase and fructose-1,6-bisphosphatase enzyme activities<sup>20</sup>. These results were in agreement with Kumar et al.<sup>21</sup> who observed significant increase in blood glucose level in diabetic rats. That may be due to the destruction of pancreatic beta cells by STZ. The elevation of glucose in STZ treated rats is due to an oxidative stress produced in the pancreas, which resulted in a single strand break in pancreatic islets DNA<sup>22</sup>. Hyperglycemia can lead to the depression of the natural antioxidant system<sup>23</sup>. Also, the results showed that the insulin level is significantly decreased ( $P < 0.001$ ) in diabetic group received STZ alone (group II), while the insulin level is significantly ( $P <$

0.001) increased in diabetic groups treated with insulin (groups III, IV, V) when compared with control group. The liver is the major site of ketogenesis and the extrahepatic tissues are responsible for the further metabolism of ketone bodies. The two main ketone bodies are AcAc and  $\beta$ -HB, while acetone is the least abundant ketone body<sup>24</sup>. As lipid cascade activation takes place, due to the lack of insulin, carbohydrate metabolism is blocked by depressed glucose utilization, and gluconeogenesis is stimulated. The consequence is additional availability of acetyl CoA, and reinforcement of ketone body formation. Measurement of  $\beta$ -HB is important as it is the major ketone in diabetic ketoacidosis (DKA), in a proportion of 10–1 with respect to the other ketone bodies<sup>25</sup>. Small amounts of ketoacids are normally formed, but clinically, a significant increase in ketone bodies in the blood can occur under conditions of diabetes (diabetic ketoacidosis), starvation or malnutrition (starvation ketosis), chronic alcoholism (alcoholic ketoacidosis), infection, trauma or some kinds of poisoning<sup>26</sup>. In diabetes, ketoacidosis is brought about by an insulin deficiency, which causes an increase in free fatty acids (which is also closely related to the release of lipolytic hormones), increased transport of free fatty acids into the mitochondria and a decrease in ketone use in the tissues<sup>27</sup>. Ketosis, excessive production of ketone bodies, it occurs secondary to a relative or absolute deficiency of insulin leading to intense lipolysis. This lipolysis releases large amounts of free fatty acids that reach the hepatic mitochondria and result in the overproduction and release of large amounts of AcAc,  $\beta$ -HB and acetone into the blood<sup>28</sup>. There for it could be concluded that insulin has a positive effect, when used in a sufficient dose, in ameliorating the epidemic effect of diabetes on different biochemical parameters. Furthermore, this study supports the opinion that the presence of ketosis, detected by  $\beta$ -OHB levels, together with hyperglycemia, must be taken into account for proper monitoring and therapeutic control of diabetes. Most investigators define normal  $\beta$ -HB levels in diabetic patients as being below 0.5 mmol/l, hyperketonemia above 1mmol/l and the risk of DKA above 3 mmol/l<sup>25</sup>. In cases of very severe insulin deficiency, the serum concentration of these ketone bodies can exceed 25 mM<sup>24</sup>. A delay in the diagnosis and treatment of DKA is associated with a significant increase in patient morbidity and mortality<sup>29</sup>. Our results showed that the  $\beta$ -hydroxybutyrate level was found to be significantly increased ( $P < 0.001$ ) in diabetic group received STZ alone (group II) and diabetic groups treated with insulin (groups

III, IV, V) when compared with control animals. This in line with Wahid et al.<sup>30</sup> results who showed that in type 1 DM hyperglucagonemia was marked leading to increase lipolysis and elevated circulatory free fatty acid level. Elevated FFA level leads to increase formation of acetyl CoA and ultimately increase formation of ketone bodies. Accumulation of ketone bodies produces ketoacidosis<sup>31</sup>. Meanwhile, Reichard et al.<sup>32</sup> reported that, ketogenesis was increased in insulin-deficient subjects. The increased activity of hormone sensitive lipase causes a breakdown of triglyceride into glycerol and FFA. Glycerol is a substrate for gluconeogenesis and FFA serve as precursors of the keto acids in DKA. In the liver FFAs are oxidized to ketone bodies, a process predominantly stimulated by raised level of glucagon in DKA. Also, Shah et al.<sup>33</sup> stated that in severe diabetes mellitus the rate of ketone bodies utilization also declines making the ketosis worse because insulin is said to increase ketone uptake in muscle. Ketosis results in fall in arterial blood pH which is more marked in diabetic ketoacidosis. The amylase content of the pancreas is a reflection of exocrine function. Measurement of serum amylase is useful to determine the pathogenesis of many diseases including (acute pancreatitis, pancreatic tumors, diabetic ketoacidosis and kidney dysfunction)<sup>34</sup>. In the present study, the activity of pancreatic amylase in serum was significantly decreased ( $P < 0.001$ ) in diabetic group received STZ alone (group II) and diabetic groups treated with insulin (groups III, IV, V) when compared with control group. Our data are in line with Hirotani et al.<sup>35</sup> who reported that 40 mg/kg STZ markedly decreased serum and pancreatic amylase activities and Aughsteen et al.<sup>36</sup> who reported that low serum amylase is also associated with insulin deficiency in patients with type 1 diabetes. As insulin affects basal and stimulatory amylase secretion via the islet-acinar axis by binding of insulin to its receptor on acinar cells and stimulates amylase secretion through various pathways Barreto et al.<sup>37</sup>. The initial drop in the serum  $\alpha$ -amylase activity may be interpreted by the impaired pancreatic exocrine secretion due to a decrease in the insulin stimulatory action<sup>38</sup>, such changes possibly being due to endocrine, exocrine and paracrine changes in the parenchyma of the pancreas Mori et al.<sup>39</sup>. In addition, Nakajima et al.<sup>34</sup> reported that, low serum amylase levels may reflect metabolic abnormalities and abnormal glucose metabolism, both of which are associated with impaired insulin action due to insulin resistance and/or inadequate insulin secretion. A large number of morphological, biochemical and immunohistochemical studies on streptozotocin-induced diabetes in the experimental animals have demonstrated a reduction in pancreatic enzymes activity<sup>15</sup>. The findings were attributed to the insulin depletion caused by beta cell damage, which was reversible upon insulin administration. In the present study, It was found that the lipase activity was significantly decreased ( $P < 0.001$ ) in diabetic group (group II) and diabetic groups treated with insulin (groups III, IV, V) when compared with control group. These results are in agreement with Aughsteen et al.<sup>36</sup> (2005) who illustrate an impairment of pancreatic enzyme activity in both types of diabetes. The reduction in lipase activity in

type 1 diabetes corresponded with the duration of illness and serum insulin level. In the meantime, Semakula et al.<sup>40</sup> results revealed a lowered serum lipase on insulin dependent diabetic patients that thought to be due to reduced acinar cell function in the vicinity of insulin-depleted islets. Our study showed that the levels of total cholesterol, triglycerides and low density lipoprotein (LDL) were significantly increased ( $P < 0.001$ ) in all groups received STZ either alone (group II) or in diabetic groups treated with insulin (groups III, IV, V) when compared with control group. While, there was a significant decrease ( $P < 0.001$ ) in the level of HDL-cholesterol in all groups in comparing to control group. As diabetes mellitus is heterogenous metabolic disorder characterized by disturbance of carbohydrate and lipid metabolism. It leads to dyslipidemia, the abnormalities in lipid metabolism generally lead to elevated levels of serum lipids and lipoproteins that in turn, play an important role in the occurrence of premature and severe atherosclerosis<sup>41</sup>. This was confirmed by Tomkin<sup>42</sup> who reported that, the rise in serum triacylglycerols, cholesterol and LDL-cholesterol levels indicate derangement of lipid metabolism and increased incidence of cardiac dysfunction in diabetic rats. Elevation of serum lipids indicates either the defective removal or overproduction (or both) of one or more lipoproteins. This study shows that insulin administration significantly augmented serum HDL cholesterol in rats with STZ-induced diabetes. This finding is advantageous since HDL-cholesterol is responsible for the transportation of cholesterol from peripheral tissues to the liver for metabolism. Insulin thus has the potential to prevent the formation of atherosclerosis and coronary heart disease, two secondary complications of severe diabetes mellitus<sup>43</sup>. Also, Pari and Latha<sup>44</sup> reported that STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, triglycerides, LDL and VLDL and decreases in HDL. In the meantime, this results are in agreement with Pari and Venkateswaran<sup>45</sup> who reported that, excess fatty acids in the plasma produced by the STZ-induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess triglycerides formed in the liver, may be discharged into the blood in the form of lipoproteins. There for it could concluded that insulin has a positive effect, when used in a sufficient dose, in ameliorating the epidemic effect of diabetes on different biochemical parameters. Furthermore, this study supports the opinion that the presence of ketosis, detected by  $\beta$ -HB levels, together with hyperglycemia, must be taken into account for proper monitoring and therapeutic control of diabetes.

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