

# Anti-diabetic effect of $\beta$ -amino Butyric Acid in Streptozotocin induced Rats

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## ABSTRACT

**Aim:** Using Beta amino butyric acid (BABA) in experimental induced diabetes mellitus male Sprague Dawley rats (*Rattus norvegicus domestica*) by Streptozotocin (STZ) injection.

**Materials and Methods:** Diabetic rats received intraperitoneal injection of BABA (100 or 200 mg/kg body weight /d) daily for 3 weeks. After the end of the trial period, blood samples were collected and serum was isolated to measure the concentrations of glucose, insulin, MDA, SOD. In addition, a comet assay was performed for hepatocytes.

**Results:** Diabetes results in a significant elevation in glucose, MDA levels and increase percentage of high damage for DNA in comet assay. The results also revealed a significant decrease in insulin levels, SOD as well as a decrease in non-damaged DNA percentage in the comet assay, at the probability level ( $P < 0.05$ ). While the treatment with the non-protein amino acid BABA in both concentrations lead to a significant decrease in the levels of glucose, MDA in addition to a reduction in percentage of high-damaged DNA in comet assay in addition to a significant elevation in the level of insulin, SOD and rise in non-damaged DNA percentage in the comet assay ( $P < 0.05$ ).

**Conclusions:** Our study revealed BABA would be useful for diabetes therapy through improving of impaired glucose metabolism and recovering oxidative stress. It also may have antioxidant properties likewise results indicate that the preventive effects may inhibit lipid peroxidation as a result of its antioxidant nature. also indicated that BABA was able to reduce DNA damage in male diabetic rats' liver.

**Keywords:** Beta-amino butyric acid, Diabetes, Streptozotocin, Insulin, MDA, Comet Assay.

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## INTRODUCTION

Diabetes mellitus (DM) is not a single disorder, it's a syndrome known as metabolic syndrome, in which different mechanisms lead to deficiency in insulin secretion, insulin action, or both and persistent hyperglycemia [1]. Chronic hyperglycemia promotes the development of various complications, such as neuropathy, nephropathy, retinopathy and it also increase the risk of cardiovascular disorder [2]. The control of hyperglycemia is the most promising approach for preventing or delaying DM complication. We may prevent its complication by attenuation of oxidative stress because recent studies have suggested that oxidative stress is closely associated with diabetes and the development of various complications [3].

Beta amino butyric acid (BABA) is a non-protein amino acid, it is one of the free compounds rarely exist in nature, well known for its ability to stimulate plant resistance against a wide range of pathogens such as Viruses, Bacteria, Oomycetes and Nematodes [4]. Recently, it has been proven that it can changes blood and immune in healthy rat cells, On the other hand, no toxic effect has appeared in these variables [5]. It also demonstrated the ability of this acid to stimulate rat resistance against *P. aeruginosa* [6].

For the scarcity of studies on the effect of this acid on animals and due to its characteristics, this study aimed to identify the effectiveness of this acid and its anti-diabetic effect in diabetic male rats induced by STZ through measuring of biochemical values such as levels of blood glucose, insulin, Lipid Profile, MDA and SOD, along with determination of the amount of damaged DNA in hepatocytes using comet assay.

## MATERIAL & METHODS

**Materials:** BABA & STZ were supplied by (Sigma Aldrich, Germany).

## Laboratory Animals

In this experiment, 28 Sprague dawley male rats were used, bought from the animal house of the college of Veterinary Medicine University of Tikrit at the age of (8-10) weeks with a weight range (180-200) g. and kept in a wire-bottomed cages and maintained in an air conditioned (19-21°C) room and 60 % humidity with a 12h/12h light dark cycle. The rats were fed on special provender consists of (32.5% wheat, 20% barley, 25% corn, 10% animal protein, 1.5 animal fat, 10% skimmed milk powder, 1% salt) and water supplied. After several days of adaptation, STZ (50mg/kg body weight) dissolved in cold phosphate buffer saline (pH 7.2) was injected intraperitoneal following an over night fast. One week after injection, blood glucose measured using On Call Plus™ Meter from tail vein blood. Rats with blood glucose level  $\geq 250$  mg/dl were considered diabetics. BABA was dissolved in distilled water and daily injected intraperitoneal into rat at doses 100 mg/kg and 200 mg/kg body weight/day for 3 weeks.

## Experimental design

Rats divided into four groups, control group had received distilled water, control STZ group which is a STZ-treated rats received distilled water, STZ + BABA group was a STZ-treated rats received 100mg/kg BW of BABA and STZ + BABA group which were a STZ-treated rats received 200mg/kg BW of BABA.

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After the end of the experiment period, animals anesthetized and blood was drawn from them by stabbing the heart. Blood placed in 10ml test tubes and then placed in a cooled centrifuge for 10 minutes at a speed of 3500 rpm to obtain blood serum. Later serum kept in freezer until performing the proper tests. Animals were kindly killed, liver was removed and washed with normal saline (0.9% NaCl) for preparation of the comet assay.

### Biochemical tests

Glucose levels was measured using a commercial kit (Glucose-TR, Spinreact Company, Spain), Insulin (Accu-Bind Insulin ELISA, Monobind Inc. USA), MDA (Malanodialdehyde Microplate assay kit, CohesionBiosciences, China), SOD (Superoxide dismutase Microplate assay kit, Cohesion Biosciences, China).

The concentrations of total cholesterol, triglycerides and high-density lipoproteins in blood serum were calculated using a ready-made test kit from the French company, Biolabo.

The cholesterol concentration of very low-density lipoproteins was calculated by<sup>[7]</sup> method and according to the following equation:-

$$\text{VLDL - C Concentration } \left( \frac{\text{mg}}{\text{dL}} \right) = \text{TG concentration}/5$$

The cholesterol concentration of low density lipoproteins was calculated by<sup>[8]</sup> method and according to the following equation:-

$$\text{LDL - C Conc. } \left( \frac{\text{mg}}{\text{dL}} \right) = \text{Cholesterol Conc.} - (\text{HDL - C} + \text{VLDL})$$

### Comet Assay

Comet assay used in order to determine DNA damage. A ready-made test kit (Trevigen Company, USA). Alkaline Comet assay will detect single and double-stranded DNA breaks, the majority of a basic site as well as alkali labile DNA adducts. Fifty randomly selected cells were counted per sample to quantify the comet cell. The scored was calculated from the ratio of (L/W) comet to determine the comet index (CI). Scored range from 1.2 to 2 considered low DNA damage (LD), from 2.1 to 3 medium DNA damage (MD), and up to 3 high DNA damage (HD)<sup>[9]</sup>.

### Analysis Statistical

A variation analysis test (ANOVA) conducted at probability significant level ( $P < 0.05$ ) using the statistical program SPSS (VER. 16).

### RESULTS

#### Effect of BABA on levels of Blood Glucose and Insulin

Statistical Analysis ( $P < 0.05$ ) in (Figures 1 & 2) showed a significant increase in blood glucose level and a significant decrease in insulin level in the diabetic group, while the results indicated a significant decrease in the level of blood glucose and a significant increase in the level of insulin in the groups treated with the amino acid BABA in both concentrations.

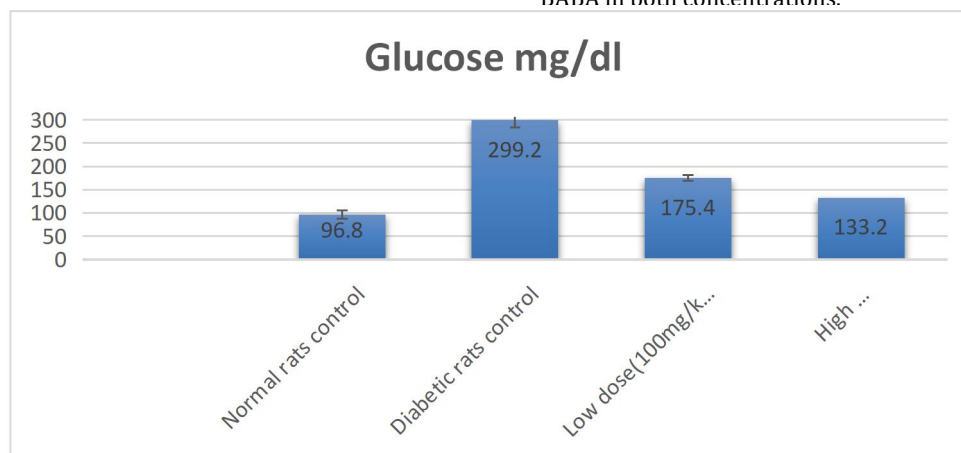


Figure 1: Effect of BABA on levels of Blood Glucose

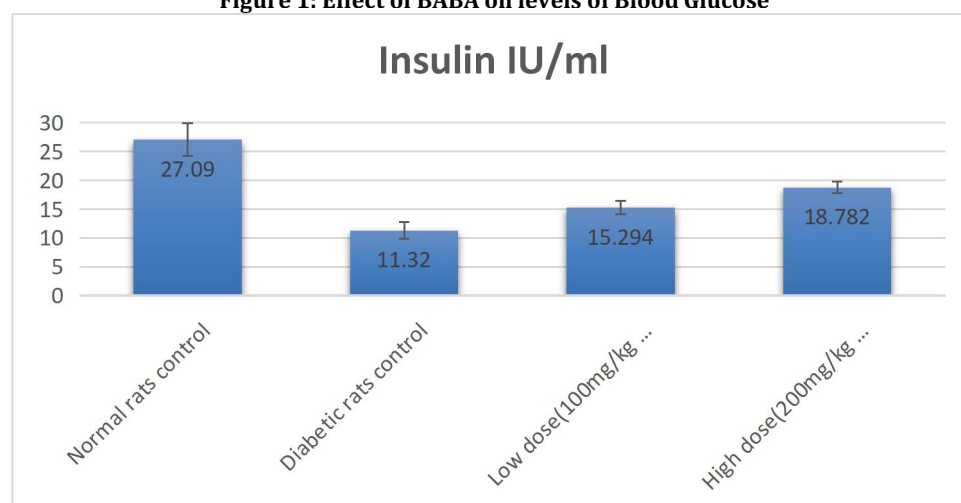


Figure 2: Effect of BABA on level of Blood Insulin

### Effect of BABA on levels of SOD & MDA

The results as in the figures (3 and 4) showed a significant ( $P < 0.05$ ) increase in MDA levels and a

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significant decrease in SOD levels in serum of the induced diabetic control animals group, while the results showed a significant decrease in MDA level and a significant increase in SOD level in the serum of the two groups of

induced diabetic animals treated with BABA, as indicated there were non-significant differences between high dose treated group and the normal control group.

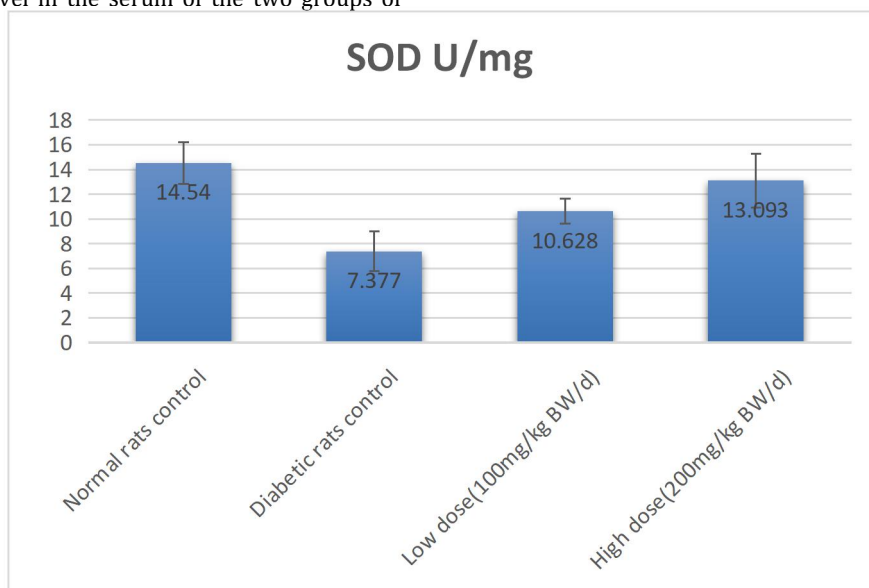


Figure 3: Effect of BABA on levels of SOD

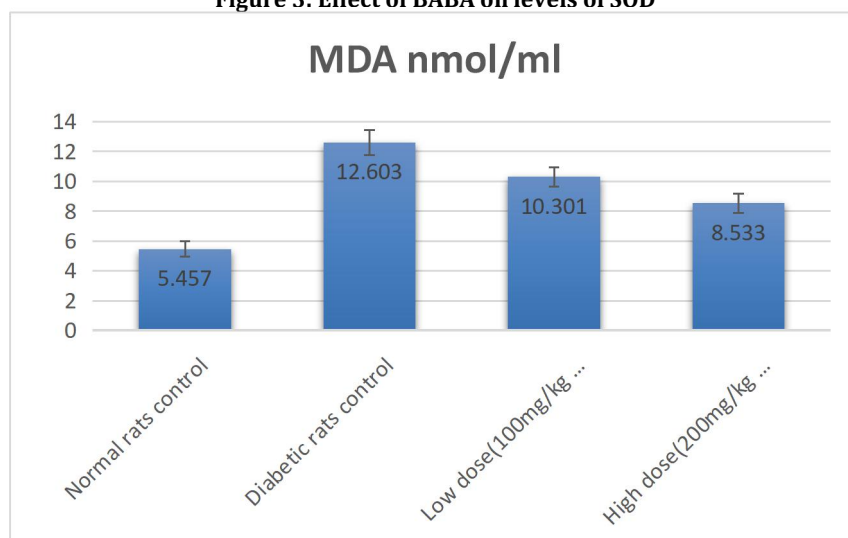


Figure 4: Effect of BABA on levels of MDA

### Effect BABA in Lipid Profile

The current results (table 1) showing a significant increase ( $P < 0.05$ ) in of TC, TG, LDL-C, VLDL-C and a decrease in HDL-C levels from diabetic rats. Furthermore, a significant decrease ( $P < 0.05$ ) in TC, TG, LDL-C levels between the diabetic animals group treatment with BABA in both 1<sup>st</sup> and 2<sup>nd</sup> concentration. On the other hand, it

displays a significant decrease in the group treated with 2<sup>nd</sup> concentration. Moreover, presents a significant increase in diabetic group that received BABA with the 1<sup>st</sup> concentration and non-significantly increase in the 2<sup>nd</sup> concentration treated group.

Table 1: Effect of BABA in Lipid Profile

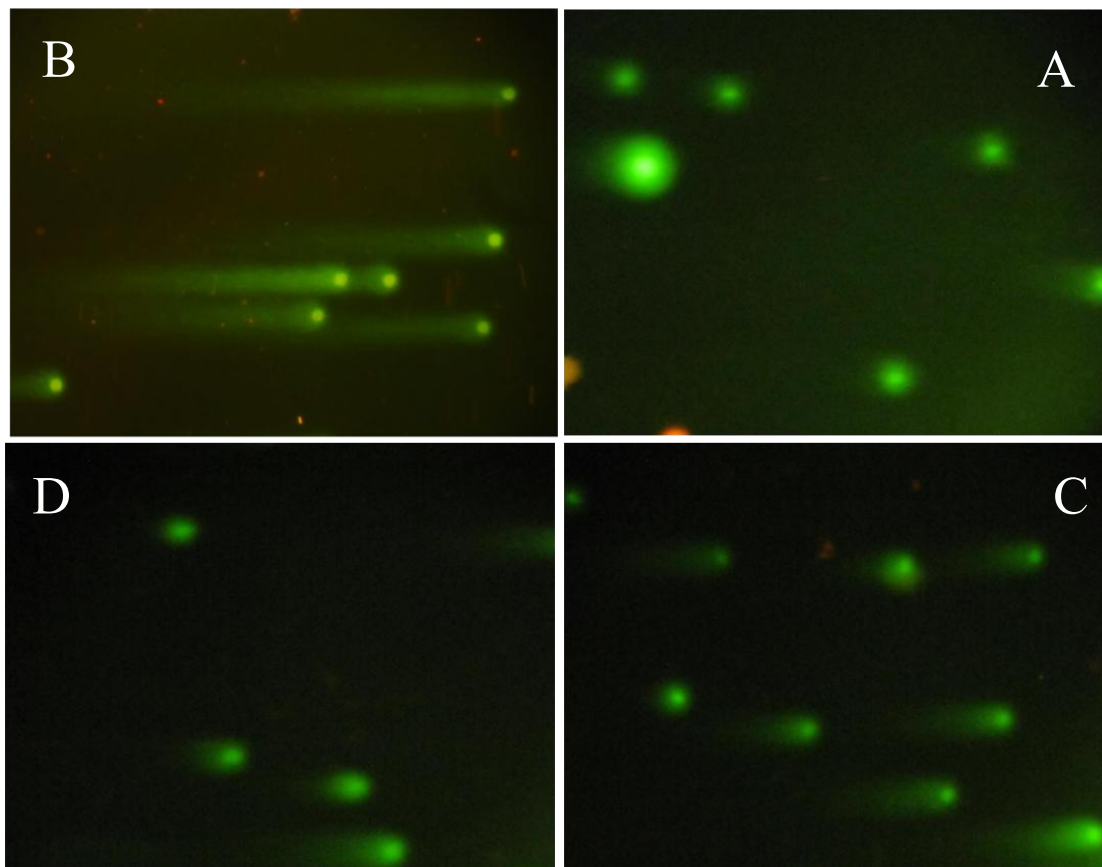
Parameters Groups	TG mg/dl (mean±SD)	HDL mg/dl (mean±SD)	Total Chol. mg/dl (mean±SD)	LDL- C mg/dl (mean±SD)	VLDL-C mg/dl (mean±SD)
Control	A 60.35±3.80	A 25.810±3.348	A 96.28±5.69	A 58.40± 6.30	A 12.071± 0.759
Streptozotocin	B 127.67±4.40	B 19.934±1.411	B 158.20±5.51	B 112.73± 5.95	B 25.526± 0.868
Treatment 1	C 116.23±3.25	A 24.290±3.413	C 132.96±6.14	C 85.43± 7.92	B 23.246± 0.650
Treatment 2	D 93.89±7.63	B 21.310±2.706	A 114.81±4.99	D 74.72± 6.97	C 18.779± 1.527
LSD	5.59	1.746	6.18	5.637	3.071
P-value	0.00037	0.018	0.00019	0.00032	0.00021

### BABA effect in using Comet Assay

The damage in the DNA was evaluated using a score software analysis image comet, as the important criteria used to know the percentage of damage in the DNA was the severity of the injury, and the ratio of high damage, medium damage, low damage, no damage.

### DISCUSSION

**Figure 5. Showing (A) Normal non-comet cell nucleus normal control group; (B) Cell nuclei with high, medium and low comet in diabetic rats without treatments; (C) and (D) Improvement of nucleus from in diabetic rats treated with BABA (100mg/Kg and 200mg/Kg respectively)**



The reason for the current results is may due to the fact that given STZ was has attacked pancreatic  $\beta$ -cells by alkylation of DNA, leading to reduce DNA synthesis and reduce insulin release, that caused an elevation of high blood sugar levels. It could also have been a reason for the development of insulin resistance and disruption of insulin cellular receptor functions, thereby stopping the process of cell glucose reception and activating the processes of decomposing glycogen and the formation of glucose from non-carbohydrate sources. This result agreed with [10].

As for the reason for the low blood glucose level and the high level of insulin in groups treated with non-protein amino acid BABA, may be due to its ability to stimulate insulin secretion by pancreatic  $\beta$ -cells, or the reason rely to the amino acid anti-oxidant properties and thus eliminates free radicals and increasing the consequence of insulin in tissues cells, improving of glucose entry into it.

The reason for rising in MDA level and reduction in SOD level in serum of diabetic animals is that the diabetes caused by STZ increases the oxidative stress due to hyperglycemia which causes an increase the formation of free radicals. High level of oxidation causes an increase in the level of fats, as diabetes is accompanied by an

increase in the level of lipid peroxides and ROS, MDA is a byproduct of lipid peroxide that causes cell damage by interconnecting the membrane components that contain amino groups and making the membrane fragile [11]. Diabetes also reduces the major antioxidant enzymes SOD and CAT that play an important role in clearing the toxic medium for incomplete oxidation [12]. These results were consistent with the results of other researchers [3, 11]. On the other hand, the reason for the low MDA level and high SOD level in the affected groups and treated with BABA, it may be to BABA ability to prevent oxidative stress by raising the antioxidant enzymes such as SOD, SOD that protects cell against ROS by scavenging those damages in the membrane and biological structures [13]. Thus SOD acts as the main defense against ROS and prevents further free radicals' production.

Results presented high levels of TC, TG, LDL, VLDL and low HDL level in diabetic rats serum. These results were consistent with the result of [11]. The reason of elevated levels probably because the diabetic rat has no ability to consume glucose as an energy source leading to lipolysis stimulation in fatty tissues then free fatty acids are released [14], or perhaps the reason is due to an increase in the activity of the cholesterol acyl transferase, which is responsible for cholesterol absorption in intestine,

leading to stimulate this enzyme when an insulin absent<sup>[15]</sup>. In addition, diabetics and low insulin produce lipase an enzyme responsible for hydrolysis of triglycerides and as a result, triglycerides rose and low HDL levels are observed<sup>[16]</sup>.

The reason for the decline in TC, TG, LDL, VLDL levels associated with an increase in HDL level in BABA treated groups is due to the antioxidant activity of BABA and increase in SOD activity, which suppresses and removes free radicals from the body and reduce oxidation of LDL-C leading to decline in cholesterol and triglycerides concentrations thus reduces VLDL-C production in liver lowering its concentration in blood serum.

It has been cleared from the results of our current study that the highest percentage of DNA breaks in diabetic and non-treated group throughout the experiment period, as it revealed from the figure (5), the length of the tail resulting from the migration of DNA material to the outside of the nucleus due to its damage<sup>[17]</sup>. This results were comparable with previous result<sup>[18]</sup>. The reason for DNA damage is due to the oxidative stress and the formation of free radicals due to diabetes and causing severe damage to large cellular particles, especially DNA. Besides direct DNA damage, ROS may also indirectly damage DNA by interacting with lipids, proteins and other cellular components to produce electrophilic types that can interact with DNA<sup>[19]</sup>.

Although diabetes causes many serious problems, either directly or indirectly, Grindel and colleagues indicated that good treatment can prevent oxidative stress and DNA damage that is caused by diabetes<sup>[20]</sup>. Therefore, results of two infected animals' groups treated with BABA, showed a significant decrease in the percentage of DNA breakage, as the high damage percentage (long tail) reduced in these two groups, revealed that the highest rate of decline was in highly dose treated. Thus, the decrease is caused by the ability of BABA to reduce free radicals formed by diabetes. In addition of antioxidant enzymes activation such as SOD that protect DNA from oxidative damage<sup>[21]</sup>.

## CONCLUSIONS

The results of the current study are revealed that low and high dose of BABA would be useful for reducing the progression of diabetes or its complication through improving of impaired glucose metabolism and enhanced oxidative stress. It has also been proven that BABA may have antioxidant properties that will be beneficial for therapeutic purpose and the results indicate that the preventive effects of BABA may cause inhibition of lipid peroxidation as a result of its antioxidant nature. The results of the current study also indicated that BABA was able to reduce DNA damage in male diabetic rats' liver. It is worth mentioning that the results of the current study demonstrated that the high dose (200 mg/kg) of BABA was more positive compared to the low dose (100 mg/kg).

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**Conflict of Interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Ethics:** This article obtained ethical approval from the Central Approval Committee of Research Ethics in Al-Anbar University.

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