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Probiotic Fermented Bitter Melon Juice as a Potential Adjuvant Therapy for Type 2 Diabetes: Evidence from Animal Model Research

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Abstract

Charantin, an antioxidant with anti-diabetic properties that act as a -glucosidase inhibitor, is found in the bitter melon Mordica charantia (MC). Lactic acid's antioxidant capabilities were enhanced by fermentation using Lactobacillus fermentum. The intention of the study was to compare various treatments to acarbose and examine how each affects blood sugar levels and Superoxide dismutase (SOD) levels before and after therapy—a technique for experimentation. A total of 28 male Sprague-Dawley rats were utilized. Streptozotocin (STZ) at a dose of 60 mg/kg was combined with nicotinamide at a dose of 120 mg/kg to cause type 2 diabetes. The animals were randomly randomized into four groups after STZ induction for three days. For a total of 28 days, acarbose (45 mg/100 feed), MC (15 ml/kg BW), and fermented MC (10 ml/ kg BW) were administered. The paired t-test was used to analyze how the data from the pretest and posttest differed. Glucose and Superoxide dismutase SOD concentrations were determined by spectrophotometer and enzyme-linked immunoassay (ELISA), respectively. Fixed at 0.05 was the threshold of significance results. Blood sugar levels in the MC and fermented MC group was significantly lower than those in the acarbose group during fasting and after meals. SOD values considerably rose in the groups that received MC and fermented MC but not to the same extent as in those that received acarbose. Acarbose and fermented MC have advantages, but MC significantly increased SOD and decreased fasting and postprandial blood glucose levels.

Keywords: Enzyme, probiotics, acarbose, Fermentation, Diabetesl.

Introduction

Millions of individuals worldwide suffer from the chronic, non-communicable condition known as “Diabetes Mellitus (DM)”; also it is now the leading cause of death. By 2021, Diabetes Atlas predicts that 537 million people will have type 1 diabetes globally (1). Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) are the two main kinds of DM. When pancreatic cells are destroyed, this may be due to acquired or epigenetic factors, type 1 diabetes manifests as a total shortage in insulin synthesis. Only 10% of diabetes cases are T1DM; while it may afflict people of any age, children under the age of five accounts for the bulk of cases. T2DM, the most common form of diabetes, accounts for 90% of all cases, and is similarly characterized by chronic hyperglycemia, a sustained rise in blood sugar levels caused by a problem with either insulin production or insulin action (2). The gut microbiota is a complex microbial ecology that inhabits the human gastrointestinal tract (GIT). Beneficial GIT bacteria perform various tasks, including synthesizing many nutrients, preventing pathogen infections, and controlling immune reactions (3). The manufacturing of probiotic

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foods is the segment of functional foods that is expanding the quickest. In many different food matrices, probiotic microorganisms are effectively used. Probiotics have been delivered via various foods, including dairy, meats, drinks, cereals, vegetables, and fruit (4). The term "probiotics" refers to a class of living microorganisms that have positive effects on both people and animals, mostly through restoring balance to the gut flora. Probiotics have been linked to several therapeutic effects, including immune regulation, lowering serum cholesterol, products that are antimutagenic and anticarcinogenic, relief from the signs of lactose intolerance, strengthening of defense mechanisms, improved intestinal barrier function, and increased nutritional value(5). A successful therapy must be discovered due to managing diabetes has become a major worldwide problem. The medicinal treatments for diabetes that include unfavorable side effects, such as those caused by insulin injections and oral hypoglycemic drugs, include liver issues, lactic acidosis, and digestive issues (6). Several treatments have been employed to minimize the risk of diabetes, including the frequent use of anti-diabetic drugs, food alteration, lifestyle changes, and regular exercise, all to maintain acceptable blood glucose levels (7). Vegetables and fruits may be fermented to boost their vitamin, amino acid, bioactive peptide, or phytochemicals content. Fermentation is the primary and easiest method besides heat or chemicals for ensuring the safety of vegetable products (8). T2DM typically affects more than 90% of people with diabetes. Due to metformin's substantial hypoglycemic impact, it is frequently advised that patients with T2DM supplement diet and lifestyle recommendations with metformin use (9). The generation of a few essential proteins in the insulin signaling pathway significantly impacts the processes that change blood glucose levels and enhance glucose storage (10). Studies on animals show that citrus chemicals tend to have health benefits that are more likely to be chronic than acute. Citrus phenol metabolites have some discernible effects on processes that occur after absorption, such as modifying hepatic glucose metabolism and target tissues' sensitivity to insulin, however, these effects are more subdued regarding digestion and gut sugar absorption (11). Study (12) aims to determine potential causes and treatments for T2DM. Comprehensive investigation of the brain transcriptome, plasma metabolome, along with gut microbiota in mice models of cognitive decline was conducted on the perspective of the brain-gut axis. Researchers looked at a new drug termed bitter melon juice (BMJ)'s effectiveness and related mechanisms against pancreatic cancer cells in both culture along with nude mice (13). Several animal studies, along with clinical trials have shown the extraordinary effects of bitter melon on diabetes. According to study (14), bitter melon can improve insulin sensitivity, restore islet cells destroyed in the pancreas, and increase insulin output. The current research analyses the clinical evidence about M. charantia's putative anti-diabetic properties and recommends further well-planned clinical studies to clarify potential therapeutic effects (15). This research aims to compare and evaluate the safety, hypoglycemia, and antiatherogenic effects of two different dosages of bitter melon in combination with glibenclamide (16). The study (17) was to learn more about the plants that are used in Ghana to treat diabetes mellitus, collect that provide the best available evidence to support these claims, explain how these plants work, and suggest topics for further study. Research (18) is a source of current data for more scientific along with clinical studies into

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herbal treatment for type 2 diabetes viewpoints that are now forming regarding type 2 diabetes treatment methods. In the present study, auditory electrophysiological testing was used to evaluate the effectiveness of type 1 and type 2 therapy diabetic mice models (19).

Materials and Methods

The process for making bitter melon juice

From the Hortimart plantation, they purchased a fresh, unripe, bitter melon. Having a typical weight of 250 g, bitter melon was harvested 40 days after it was planted. The fruit was cleaned and divided to get rid of the seeds. To get the seeds out, the fruit was washed and split. Juice was made from the excised meat without the need of water. For five minutes, the juice was pasteurized at 70°C.

The treatment of animals

The procedures of Abdellatief were used for the treatment of the animals. For the investigation, 24 male Sprague-Dawley rats weighing between 171-230g at eight weeks old were employed. Environmental elements, including a 12-hour light/dark cycle, were used in the experiment to alleviate stress, steady 25°C, normal humidity, and hygienic conditions were preserved. Separate stainless steel cages were used to house the rats. They receive a regular chow diet and freedom to use water. The rats had a seven-day acclimatization phase before the trial started (20). Animal welfare, animal rights, and ethical issues are only a few of the many facets of animal care. While it pertains to the way animals are treated, various countries and cultures have different viewpoints and customs. Others believe that treating animals with care and respect is the correct thing to do, in contrast to those who just regard them as resources to fulfill their own desires.

Creating Type 2 Diabetes

One injection of STZ, freshly dissolved in 0.1 M citrate buffer (pH 4.5), at a concentration of 60 mg/kg, was administered intraperitoneally to overnight fasting rats (21). In comparison, 15 minutes before the infusion of STZ, nicotinamide dissolved in regular saline was delivered to the rats with a dosage of 130 mg/kg. Fasting blood glucose values more than 220 mg/dL was utilized to diagnose type 2 diabetes three days following induction.

Designs for Experiments

Rats were divided into four groups (n = 6) 72 hours after STZ injection. The diabetes mellitus acarbose collection (DM-Ac) of rats consumed 40 mg/100 g of food. Rats in the bitter melon group received 20 mg/kg BW of bitter melon juice orally. Rats in the fermented bitter melon group were orally administered 10 mg/kg of fermented bitter melon juice containing 108 probiotics. The only thing that the rats obtained in the situational control group was distilled water. Regarding a total of 28 days, the rats received treatment every day.

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Obtaining Examples

At the beginning and end of the test period, deep anaesthesia was administered to blood samples taken from vena sinus orbitalis from during the night rats were collected (22). The serum was then spun at 3000 rpm for 15 minutes to get glucose and SOD estimates.

A review of postprandial and fasting blood glucose levels

Using commercial kits that were bought from DiaSys, both postprandial along with fasting blood glucose levels were measured. The term "fasting blood glucose (FBG)" describes the amount of glucose in the blood following at least an 8-hour overnight fast. Usually, it is taken in the morning, just before breakfast. FBG is often used to identify diabetes and keep tabs on blood sugar management. Good fasting blood sugar levels typically range from 70 to 99 mg/dL (4.9 to 5.9 mmol/L). Prediabetes could be indicated by values among 100 and 135 mg/dL (5.6 to 6.9 mmol/L), whereas diabetes is indicated by levels of 146 mg/dL or above on two different occasions.

A measurement of serum oxidative stress

Using commercial kits obtained from BioVision, SOD activity was evaluated. Several indicators that indicate the body's equilibrium between oxidant production and antioxidant defense may be used to quantify serum oxidative stress.

Examination of the Data

The findings were provided by mean, standard deviation (n=6), as well as sampling was carried out before to (pretest) and subsequent to (posttest) treatments. Since all data was normally distributed, the pair test was performed to determine if differences between before and after treatments were statistically significant.

Results

The ranges for fasting along with postprandial blood sugar in each group were 62.3 to 67.6 mg/dl and 71.6 to 72.8 mg/dl, respectively, with no obvious difference between them. The (Figure 1) and (Table 1) displays the baseline fasting and postmeal blood sugar levels.

Table (1): Rapid glucose measurement for blood sugar levels after a meal

Intervention	Normal fasting glucose	Normal postprandial glucose
DM-Ac	65	70
DM-MC	70	75
DM-PMC	63	78
DM-Ctrl	62	68

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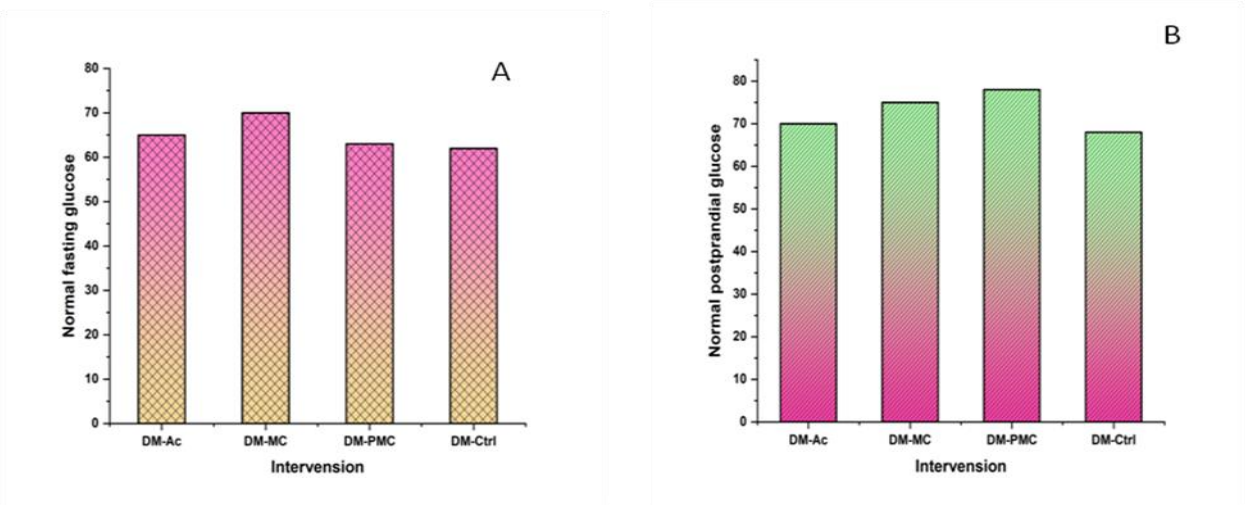

Figure (1): Baseline-acceptable blood sugar **[A]:** rapid glucose measurement, **[B]:** blood sugar levels after a meal

Diabetes and Fermented Bitter Melon Juice

Blood glucose levels significantly dropped after receiving both fermented and unfermented bitter melon juice shows in (Figures 2 and 3) also in (Table 2 and 3). After receiving therapy containing both fermented and unfermented bitter melon juices (DM-MC and DM-PMC), blood glucose levels considerably (p 0.06) dropped in contrast to the diabetic control group (DM-Ctrl). Insufficient insulin synthesis with ineffective insulin production can contribute to high blood sugar levels usage by the body characterizing diabetes, as a chronic illness. Although there is no known cure for diabetes, problems may be avoided with good treatment of the illness.

Table (2): Ahead of therapy and after receiving therapy for fasting glucose in diabetics

Intervention	Fasting diabetic glucose before treatment	Fasting diabetic glucose after treatment
DM-Ac	250	95
DM-MC	252	150
DM-PMC	250	120
DM-Ctrl	255	250

Momordica charantia, usually referred as bitter melon is a vegetable that frequently used in ancient medicine, especially in Asian nations, to help control diabetes. It is thought that bitter melon may possess certain qualities that might assist in controlling blood sugar levels.

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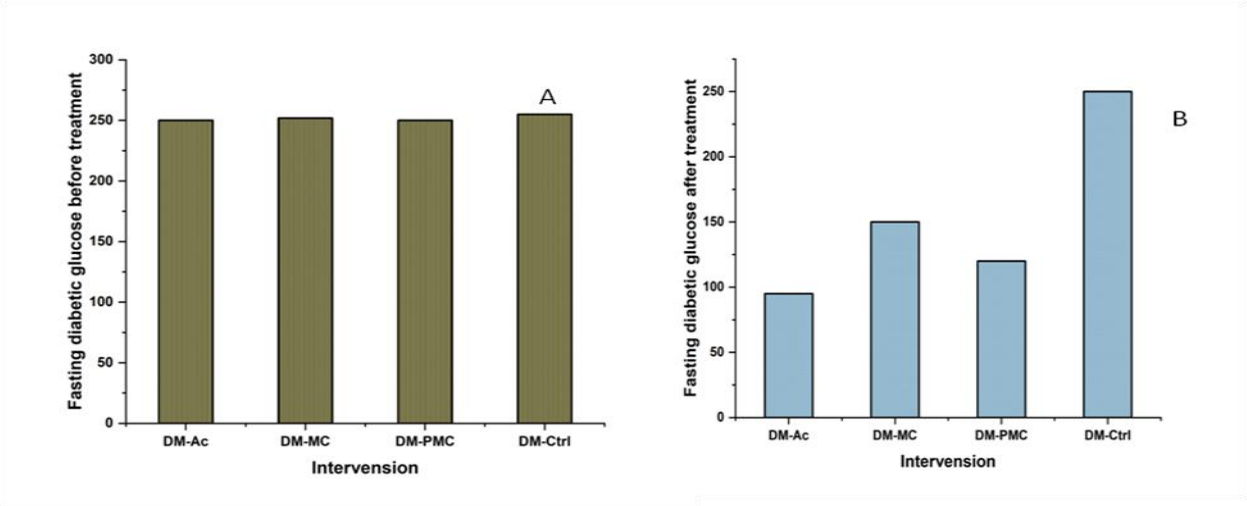

Figure (2): Fasting glucose in diabetics **[A]:** ahead of therapy, **[B]:** after receiving therapy

Table (3): Postmeal diabetes blood sugar for before and after therapy

Intervention	Postprandial diabetic glucose before treatment	Postprandial diabetic glucose after treatment
DM-Ac	270	100
DM-MC	275	170
DM-PMC	278	110
DM-Ctrl	276	275

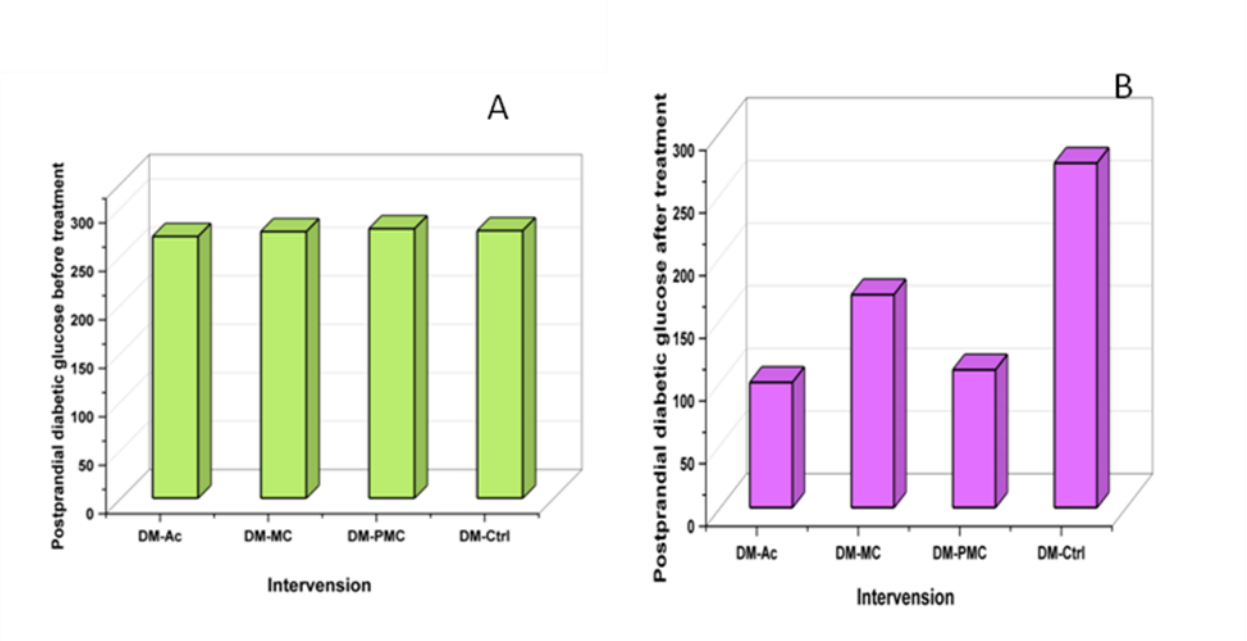

Figure (3): postmeal diabetes blood sugar **[A]:** prior to therapy, **[B]:** the post-treatment

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Impact on the oxidative status

In the concentrations of SOD, there were noticeable variations (Figure 4). SOD concentrations increased significantly (p 0.05) in contrast to diabetes control (DMCtrl) after the intake of nonfermented in addition to fermented sour melon juice. DM-PMC performed superior than DM-MC in accordance with the hypoglycemic effect. The balance among the creation of reactive oxygen species (ROS) and body's antioxidant defense systems is referred to as the oxidative state. The production of ROS is a typical consequence of cellular metabolism, and they play a role in a number of physiological processes. Still, oxidative stress, which is linked to cellular damage and the emergence of several illnesses, may result from ROS generation that surpasses the body's antioxidant capacity.

Blood Glucose and SOD Correlation

The association among SOD and fasting blood glucose was $r = 0.969$, $p = 0.002$, whereas the correlation among SOD and postprandial blood glucose was $r = 0.968$, $p = 0.001$. SOD, along with blood glucose levels are both distinct components of human health. Blood glucose represents the amount of glucose (sugar) in the blood, while SOD is an antioxidant enzyme that aids in preventing cell deterioration brought on ROS. It's crucial to keep blood sugar levels within a healthy range by suitable lifestyle changes or medical treatment to lower the risk of oxidative stress and the issues it might cause.

Discussion

Considering the teams had similar initial fasting, postprandial glucose levels, and SOD levels, comparing the posttest findings, and evaluating the effectiveness of bitter melon and fermented bitter melon is successful. Fasting along with postprandial blood glucose levels had been lowered with diabetic rats when fermented MC juice was given in place of nonfermented (Figures 2 and 3), (Table 2 and 3) , although the reduction was not as significant as with acarbose. According to this research, MC juice had undergone fermentation showed a stronger antihyperglycemic impact than MC juice (23). The improved anti-diabetic potential of fermented MC juice may be related to its higher antioxidant content, since acarbose had a bigger impact than MC and fermented MC juice. Charantin, triterpenoid, and p-polypeptides, which are the bitter melon's active ingredients, regulate blood sugar levels. As an inhibitor of -glucosidase, charantin works. Triterpenoids and p-polypeptides have the ability to improve cellular glucose absorption and reduce insulin resistance by acting as insulin sensitizers and insulin mimics, respectively. The administration of MC juice in terms of glucose levels trailed the fermented one despite a substantial drop (24). Charantin, an antioxidant and -glucosidase inhibitor, is linked to lower glucose levels in the fermented group. Using Lactobacillus fermentum (LLB3) to ferment bitter melon juice enhanced antioxidant activity by 15% and boosted -glucosidase inhibitory activity in vitro. Fermented MC has a greater effect on decreasing blood sugar levels due to more -glucosidase generated by lactic acid bacteria in food. The enzyme -glucosidase produced by lactic acid bacteria increases the inhibition of -glucosidase activity by hydrolyzing charantin to sitosteryl glucoside along with glucoside stigmasteryl. A reduction in glucose absorption results from

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inhibition of -glucosidase, which also inhibits postprandial surge. Reduced fasting and postprandial glucose levels are mostly due to the probiotic bacteria present in fermented bitter melon juice. Glucose absorption via PI3-K pathways is aided by probiotic peptides generated by bacteria (25). Additionally, probiotic bacteria create Short Chain Fatty Acids (SCFAs), which improves the epithelial function of the host cell. SOD levels have been demonstrated to rise after receiving MC juice, both fermented and unfermented (Figure 4 and Table 4). Compared to those who got nonfermented juice, those who received fermented MC juice had considerably greater levels of SOD. Even though the impact was smaller than acarbose's, the therapy with fermented MC significantly improved the antioxidant state. The production of free radicals is induced by high glucose levels. SOD levels rose as a consequence of MC juice's antioxidants and the fermented MC juice's ability to scavenge free radicals created by the oxidation of glucose (26). Probiotic bacteria generate bacteriocins, bile salt hydrolase (BSH), SCFAs, and peptides in the intestines (27). The improvement in the subject's antioxidant level is consistent with the presence of probiotics SCFA functions as an antioxidant. Through the PI3-K pathway, peptides lower blood sugar levels (28). Significantly lower levels of SOD were seen in postprandial blood glucose along with fasting blood glucose (Figure 5), it has to do with how SOD works to catalyze the transformation of superoxide anion ($_{O2}$) into hydrogen peroxide along with molecule oxygen. Antioxidants were present in MC juice, and fermentation made them more active (29). Consuming antioxidants may reduce oxidative stress; reduce ROS that increase antioxidant enzymes, all of these have been linked to a lower risk of developing diabetes. Due to the bitter melon's bioactive components, which promote insulin sensitivity and -glucosidase inhibitors, bitter melon may regulate blood sugar levels (30). Although it still lacks acarbose efficacy, fermented MC juice provides a greater antihyperglycemic impact when compared to nonfermented MC juice. This effect is due to the increased antioxidant content in fermented MC juice (31). It is unrelated to the way SOD catalyzes the conversion of superoxide anion ($_{O2}$) into hydrogen peroxide along with oxygen molecules.

Table (4): Before and after therapy for level of SOD

Intervention	Blood SOD before treatment	Blood SOD after treatment
DM-Ac	25	85
DM-MC	23	58
DM-PMC	25	65
DM-Ctrl	22	20

Although it still lacks acarbose efficacy, fermented MC juice provides a greater antihyperglycemic impact when compared to nonfermented MC juice. This effect is due to the increased antioxidant content in fermented MC juice (31). It is unrelated to the way SOD catalyzes the conversion of superoxide anion (O2) into hydrogen peroxide along with oxygen molecules.

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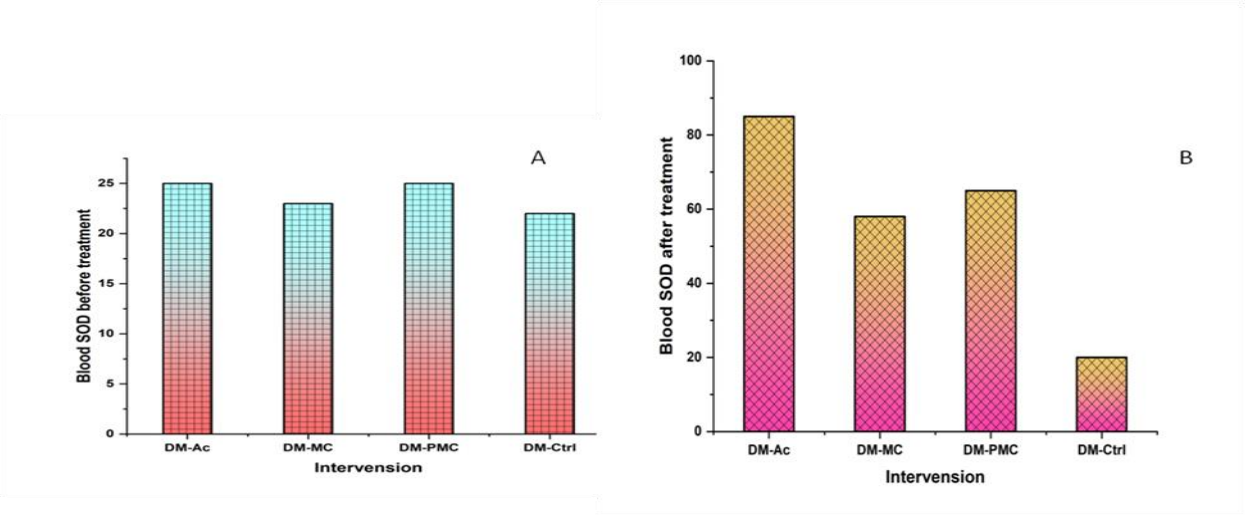

Figure (4): Level of SOD **[A]:** preceding therapy, **[B]:** After therapy

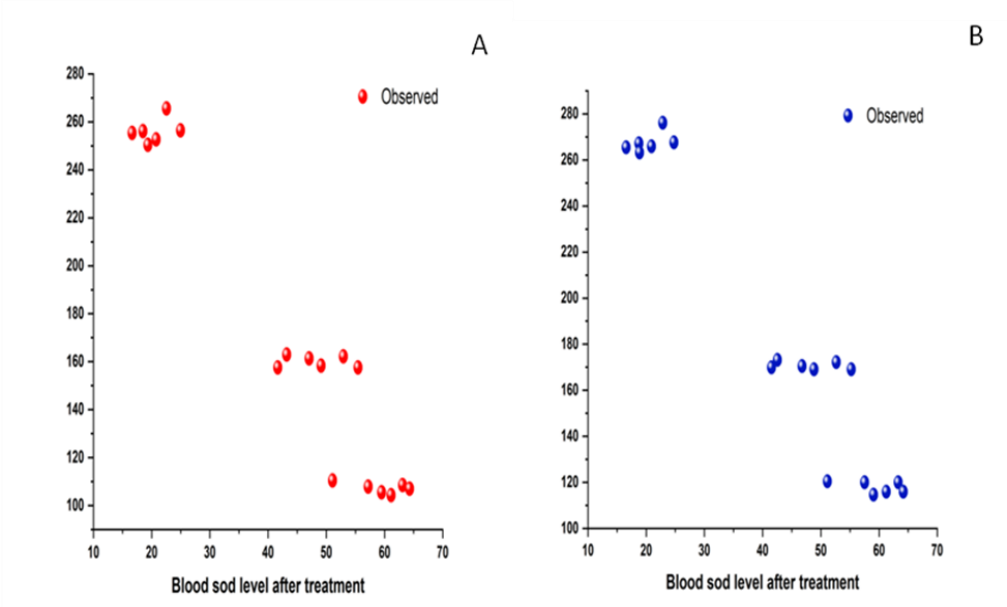

Figure (5): Intermingling of **[A]:** Fasting diabetes glucose vs. SOD, **[B]:** SOD vs. diabetic glucose after meals.

Conclusion

The current study showed that the Trimetion is caused different pathological lesions in male testicular and epididymal tissues of adult rabbits which appeared microscopically sever necrosis and degenerations and complete suppression of spermatogenesis with vaculation of spermatogonia. However, moreover experiments are required to identify other toxicopathological changes in other body tissues.

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