RESEARCH ARTICLE

Effect of Coadministration of Hydroalcoholic Extracts of *Aegle marmelos* Leaves and *Tamarindus indica* Seeds on Streptozocin-Nicotinamide Induced Type- 2 Diabetic Rat Model

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ABSTRACT

It is difficult for doctors to control hyperglycemia in type 2 diabetes patients without having an effect that is too detrimental to the patient, despite the advancements that have been made in diabetes medications. Because of their adaptability and capacity to lower blood sugar with very few unintended side effects, herbal preparations are quickly gaining in popularity worldwide. In this study, hydroalcoholic extracts of *Tamarindus indica* seeds and *Aegle marmelos* leaves were tested to determine what kind of effect they had on a model of diabetes carried out on rats.

For the purpose of this investigation, 54 male Sprague Dawley (SD) rats were used as participants. Group I comprised of six people who did not have diabetes and were considered to be in good health. Intraperitoneal injections of streptozocin (55 mg/kg) and nicotinamide (NAD) (120 mg/kg) were used to induce diabetes in the remaining animals (n = 48). Following that, we divided them up into a total of eight distinct categories. Animals in groups IV and V were given AM at doses of 25 and 50 mg/kg/day, animals in groups VI and VII were given TI at doses of 25 and 50 mg/kg/day, animals in groups VIII and IX were given a combination of the two extracts (AM25+TI25 & AM50+TI50), while diabetic animals were used as controls in group II. A positive control consisting of metformin given at a dose of 200 mg/kg per day was utilized in group III.

When compared to the diabetic control group, the fasting blood glucose levels and the area under the curve (AUC) of the oral glucose tolerance test were lower in all treatment groups. When compared to the metformin groups, the performance of the extract combination groups was considerably superior (p 0.01). Additionally, as compared to the diabetic control group, all of the treatment groups had significantly reduced levels of MDA and hepatic G-6-Pase activity (p 0.001).

In terms of effectiveness, the combination group performed much better than the metformin group despite the fact that all pharmacological groups showed significant increases in body weight, relative liver weight, HDL level, and liver glycogen content in comparison to the diabetic controls. ($p < 0.05$). When compared to the diabetic control group, all therapy groups showed an increase in G-6PD activity (p 0.001), catalase levels (p 0.001), and glutathione (GSH) levels (p 0.05).

In STZ-induced type 2 diabetic rats, a combination of extracts (AM50+T150) demonstrated the best improvement after 30 days of therapy when compared to metformin and hydroalcoholic extracts (AM and TI) alone. This was the case even when both treatments were administered separately. There was no difference in the circumstances no matter which extracts were utilized.

Keywords*:* Antioxidant, Area under the curve, Lipid profile, Liver enzymes.

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INTRODUCTION

Alterations in the metabolism of carbohydrates, lipids, and proteins have all been related to diabetes mellitus.[1] Despite the fact that a decreased sensitivity to insulin is not the major cause of this condition, it is a contributing factor. The fundamental reason is the malfunction of beta cells in the pancreas, which leads to insufficient insulin production.[2] A reduction in sensitivity to insulin produces an increase in the amount of glucose produced by the liver, as well as an increase in gluconeogenesis.[3] This rises in direct proportion to a person's body mass index (BMI), which is recognized as one of the primary contributors to the development of type 2 diabetes.[4,5] Mild insulin resistance with concomitant hyperglycemia and dyslipidemia are two of the advanced aberrant metabolic symptoms of obesity-dependent diabetes. Increased insulin resistance and the inability of beta cells to properly compensate for the situation both contribute to the development of type 2 diabetes in humans. The imitating of these effects in animal models helps us to understand the pathology as well as develop a way to manage or treat the illness with novel medications or plant extracts that have the potential to combat diabetes."

Many medications have been developed to treat diabetes mellitus type 2. However, this comes at the price of the treatments' often severe side effects.[6] So, scientists have shifted their attention to plants in pursuit of a cure for type 2 diabetes. Hydroalcoholic extracts of *Aegle marmelos* leaves and *Tamarindus indica* seeds were used in this study to control the onset and progression of diabetes in Sprague Dawley rats induced by streptozocin-nicotinamide (STZ-NAD). Streptozocin and nicotinamide were the culprits in the development of this diabetes.[7]

A. marmelos, often known as AM, has a history of usage as a herbal treatment in the conventional medical systems of India, Bangladesh, and Sri Lanka.[8] It has been asserted that the plant possesses analgesic, antidiabetic, anti-inflammatory, antipyretic, and anticancer qualities.[9-11] It contains flavonoids, oleic acid, retinoic acid, linolenic acid, polyphenols (rutin and gallic acid), and other related phytoconstituents.[8] It boosts insulin production and encourages damaged beta cell repair.[12]

T. indica (TI) is a fundamental component of traditional Indian cooking, and it is also regarded as a medicinal herb due to its powerful anti-inflammatory effects.[13] This tropical plant has the potential to be utilized as a treatment for a wide range of ailments and illnesses. Because different portions of the plant contain different phytochemicals, such as phenols, triterpenoids, alkaloids, flavonoids, and saponins, this tree has a wide range of applications.[14] It has been documented that the seeds of the *T. indica* plant contain both hypoglycemia and hypolipidemic properties.[15]

The purpose of this research was to examine the effects of streptozocin-nicotinamide (STZ-NAD) on decreasing hyperglycemia and hyperlipidemia in a rat model of diabetes by administering hydroalcoholic extracts of AM leaves and TI seeds (separately and together).

MATERIAL AND METHODS

Plant Extract

T. indica (family Caesalpiniacae) The seeds and fully developed leaves of the Rutaceae family plant *A. marmelos* were collected from the Udupi district of Karnataka and then thoroughly cleaned. Dr. K Gopalkrishnan Bhat, a botanist who had previously worked at Mahatma Gandhi College in Udupi, was the one who authenticated the plants. After being dried out, the plant components were then pulverized. Maceration was used to get the hydroalcoholic extract in a separate process. Maceration was performed on 100 grams of powdered *A. marmelos* leaves and 100 grams of powdered *T. indica* seeds for seven days at room temperature using a mixture of 500 mL of ethanol and 500 mL of distilled water at a ratio of 50:50. Following the filtration of the extracts, the solvent was removed using a vacuum evaporator, then a water bath was used to dry the concoction. The results of this process were *A. marmelos* leaf paste and *T. indica* seed powder.[16,17]

The experiment required the use of a total of 54 adult male Sprague Dawley rats. All of these rats were of the sexe Sprague Dawley. The experiment was carried out in Manipal, India, at the Central Animal Research Facility, in accordance with the Committee for the Control and Supervision of Studies on Animals (CCSEA), which is an organization that governs how studies with animals are carried out[18] guidelines for selection. The Kasturba Medical College Institutional Animal Ethics Committee in Manipal approved the study (IAEC/KMC/44/2015). The rats were provided with a typical rat food from VRK Nutritional Solution in Pune, India, and free access to water, as well as a temperature range of 22 to 24°C, a light-dark cycle lasting 12 hours, humidity levels ranging from 40 to 60%, and clean, comfortable housing.

Induction of Diabetes Mellitus

There was a control group (n = 6) of male Sprague Dawley (SD) rats used in the study that did not develop diabetes. There were a total of 54 rats in the control group. Glucose oxidase-peroxidase reactive strips (made by Roche Diagnostics in Germany and available under the brand name Accu-chek Active) were used to measure the concentrations of glucose in the fasting blood of the rats. Streptozocin (STZ) at a 55 mg/kg dose was injected intraperitoneally (IP) with the solution maintained cold pH 4.5 cold sodium citrate buffer with 0.1 M streptozocin is recommended for dissolving the drug,[7] 15 minutes after an intraperitoneal injection of 120 mg/kg of nicotinamide diluted in saline[7] were administered in order to produce a diabetes model suitable for research purposes. After 10 days of receiving IP injections of STZ and nicotinamide, the patient had a measurement of their fasting blood glucose level obtained. For the purposes of this study, diabetic animals were classified as having a blood glucose level that was more than or equal to 200 mg/dl. This was done so that the results of the study could be more accurately interpreted.

Following the induction of diabetes by artificial means in all 48 rats, they were arbitrarily separated into 8 groups (Group II–IX). Each of the three groups contained a total of six unique creatures. Group I, which served as the standard control, was given 1% sodium carboxy methyl cellulose (also known as sodium carboxy methyl cellulose) at a rate of 2 mL per kg on a daily basis. The diabetics in group II were given 1% Na-CMC at 2 mL per kg daily. Metformin was administered at a dosage of 200 mg/kg/day to the participants in group III, which served as the positive control group.[19] The amoxicillin dosage for group IV was 25 mg/kg/day, whereas for group V it was 50 mg/kg/day. Tetracycline was administered at the rates of 25 mg/kg/day to group VI and 50 mg/kg/day to Group VII. Amoxicillin and tetracycline were given 25 mg per kg daily to group VIII and AM50+TI50 for group IX (AM50 mg/kg/day with TI50 mg/kg/day). As a carrier for the administration of the conventional medication and the extracts, sodium carboxy methyl cellulose 1% (also known as Na-CMC) was utilized. The rats in each group were provided with the regular rat diet as well as water on an unlimited basis. Day 1 was regarded to have begun when the medication and hydroalcoholic extract were first administered. Every treatment group received their medication in the form of oral therapy for a duration of 30 days in a row.

Estimation of fasting blood glucose levels and oral glucose tolerance test

After abstaining from food for a full 24 hours, the rats were given their last oral dosage of glucose, which was calculated to be 2 gm per kg of body weight. In order to get blood samples at 0 minutes (immediately before to the glucose injection), 15 minutes (right after the injection), 30 minutes (right after the injection), 60 minutes (right after the injection), 90 minutes, and 120 minutes (right after the injection), a very small incision was made near the base of the tail. In order to monitor the amounts of glucose and peroxidase in the blood, Roche Diagnostics in Germany produced glucose oxidase-peroxidase reactive strips. On day 0 (before intraperitoneal injection of STZ-nicotinamide), on day 10 (when the model was established and subjects were randomly assigned to treatment groups), on day 25 (after administration of the standard drug and hydroalcoholic extract), and on day 40 (after completion of treatment for 30 days), experiments were carried out.

Estimation of lipid profile

A blood sample was taken using retro-orbital puncture 24 hours after the completion of the most recent treatment. The serum was separated from the blood through centrifugation at a speed of 4400 rpm for 15 minutes at a temperature of 40°C. The biochemical analysis was carried out while the serum was kept at a temperature of -20°C. This study comprised the measurement of triglycerides, total cholesterol, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) cholesterol. Following the standard methods that were contained in the reagent kits that were purchased from Aspen Laboratories in New Delhi, India, a semi-automated analyzer was used to determine the levels of triglycerides, cholesterol, HDL cholesterol, VLDL cholesterol, and LDL cholesterol that were present in the subject's blood."

Liver homogenates preparation

After the rats' blood was drawn and collected for the purposes of the biochemical investigation, the rats were then murdered. It was decided to remove the liver from the body. A cold solution of 50 mM potassium phosphate buffer with a pH of 7.4 was processed with a Remi homogenizer in order to obtain liver homogenates with a concentration of 10% weight-per-volume. Using a Remi C-24 cold centrifuge, intact cells and cell debris were separated from one another. The process of centrifugation lasted for 30 minutes and involved 10,000 rpm. The supernatant that was produced as a consequence was frozen at a temperature of -20°C so that it could be used in the following estimation.[20]

We were able to evaluate the levels of liver glycogen, glucose 6-phosphate dehydrogenase (G6PD), glucose 6-phosphatase (G-6-Pase), catalase (CAT) activity, malondialdehyde (MDA), and reduced glutathione (GSH) by using kits that Aspen Laboratories developed in New Delhi, India.

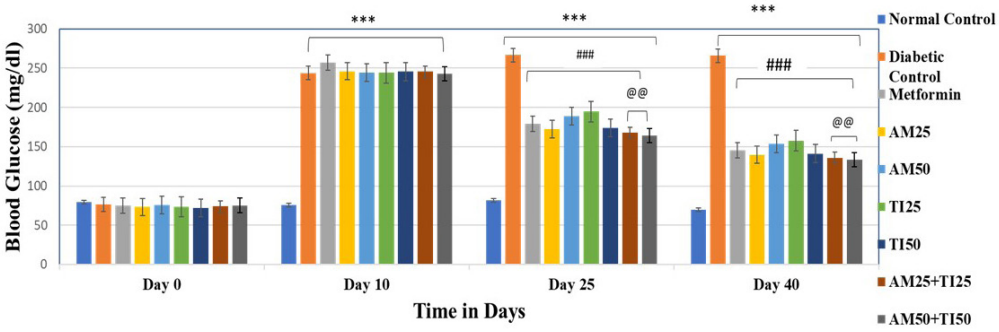

Figure 1: Induction of diabetes mellitus and effect of treatment with metformin(standard drug) and hydroalcoholic extracts (AM, TI and their combination) on fasting blood glucose level on Day 0(before induction of diabetes),10 (induction of diabetes),25(15 days after the treatment) and 40 (30 days after the treatment) in STZ-NAD induced diabetic male SD rats among different groups (Data was analysed by one way ANOVA followed by post hock Bonferroni); p value < 0.05 was considered statistically significant; (mean values and error bars of +1- 2SE); (*** p < 0.001 (Normal control vs Diabetic control, Metformin, AM25,AM50, TI25,T150, AM25+TI25, AM50+T150); (### p < 0.001 (Diabetic Control vs Metformin, AM25,AM50, TI25,T150 , AM25+TI25, AM50+T150); (@@ p<0.01 Metformin vs AM25+TI25, AM50+T150).

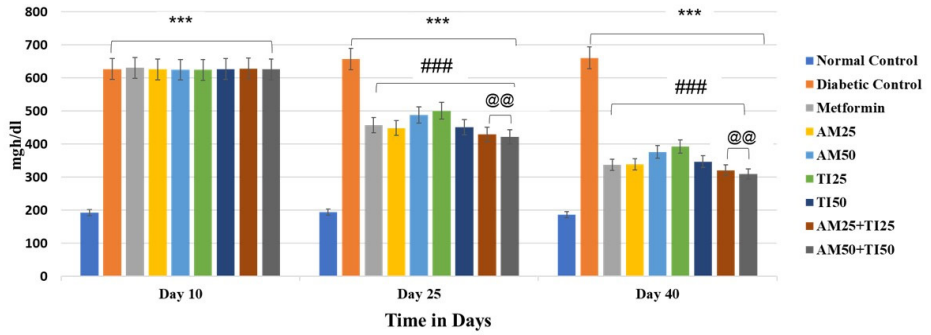

Figure 2: Oral Glucose Tolerance Test(OGTT) results represented as Area Under the curve (AUC) day 10, 25 &40 (10 days after in STZ-nicotinamide IP injection and 15(day 25) & 30(day40) days after the treatment standard and hydroalcoholic extracts) in STZ —nicotinamide induced diabetic rats among different groups. (Data was analysed by one way ANOVA); p value < 0.05 was considered statistically significant; (mean values and error bars of -1-1- 2SE)(*** p < 0.001 (Normal control vs Diabetic control, Metformin, AM25,AM50, TI25,T150, ANI25-FT125, AM50+T150; (### p < 0.001 (Diabetic Control vs Metformin, AM25,ANI50, TI25,TI50 , AM25+TI25, AM50+TI50) ;(@@ p<0.01 Metformin vs AIv125+T125, ANI50-1-TI50)

Statistical Analysis

In order to carry out the study, a copy of the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, USA) was used, specifically version 20.0. The data are first placed through a one-way analysis of variance (ANOVA), and then they are put through a post hoc Bonferroni test once the ANOVA has been completed. The results in question have a normally distributed mean and standard error. The findings are shown with both the mean value and the mean standard error. In order for a *p-value* to be considered statistically significant, it must be lower than 0.05.

RESULT

Induction of Diabetes Mellitus and Effect of Hydroalcoholic Extracts of AM & TI alone and in Combination in STZ-NAD Induced Diabetic Rats (Figure 1)

Figure 1: In the graph, Day 0 indicates the blood glucose level in male Sprague Dawley rats (SD rats) that was measured when the animals were fasting before the STZ-NAD injection. On day 10, fasting blood glucose levels rose significantly (p 0.001) across all groups compared to the normal control. This was seen in every one of the healthy controls. All treatment groups demonstrated lower levels of fasting blood glucose after 15 days of therapy compared to the diabetes control group (p 0.001); the combination group showed better results in decreasing the fasting blood glucose in comparison to metformin, p 0.01 (Metformin vs AM25+TI25, AM50+TI50). However, the glucose levels in the blood were not returned to normal.

Effect of Hydroalcoholic Extracts of AM & TI alone and in Combination in STZ-NAD Induced Diabetic Rats on Area Under Curve (AUC) of the Oral Glucose Tolerance Test (Figure 2)

Figure 2: Ten days after receiving an intraperitoneal injection of STZ-NAD (STZ 55 mg/kg body weight, NAD 120 mg/kg body weight), the area under the curve (AUC) of the oral glucose tolerance test (OGTT) was significantly elevated in mice compared to the normal control group. There was a significant (p 0.001) increase in AUC in all groups compared to the normal control on day 25 and day 40, which correspond to 15 and 30 days of treatment with standard and hydroalcoholic extracts alone and in combination. We did this by contrasting the AUC with results from days 25 and 40. The AUC was significantly lower (p 0.001) in the diabetes group compared to the control group.The advantages of the combo medication were statistically considerably higher than those of metformin (p 0.001)."

Effect of Hydroalcoholic Extracts of AM & TI alone and in Combination on Body Weight in STZ-nicotinamide Induced Diabetic Rats (Figure 3)

Figure 3: The usual body weight of male SD rats is represented on day 0 of the graph, which was taken before the STZ -NAD IP injection. The line on day 10 of the graph depicts the decrease in total body weight that occurred after diabetes was induced. There was a substantial (p 0.001) decrease in body weight across the board when compared to the normative control group. Therapy with extracts, either alone or in combination, resulted in a significant (p 0.001) improvement in body weight at the end of 30 days of treatment, which was day 40, compared with diabetes management. In terms of gaining weight, the combo group outperformed the metformin group by a statistically significant margin (p 0.01).

Effect of Hydroalcoholic Extracts of AM & TI alone and in Combination on Relative Liver Weight% in STZ-Nicotinamide Induced Diabetic Rats (Figure 4)

Figure 4: There was a significant (p 0.001) decrease in the relative liver weight% between the diabetic rats and the normal control group. Treatment with extracts alone or in combination with metformin significantly increased relative liver weight% compared to diabetes control (p 0.001). The AM50+TI50 group improved the most significantly (p 0.01) compared to the other treatment groups.

Treatment with the common drug metformin in conjunction with hydroalcoholic extracts (AM, TI, and their combination) was studied for its effect on liver glycogen storage. (One-way ANOVA with post hoc Bonferroni correction for multiple

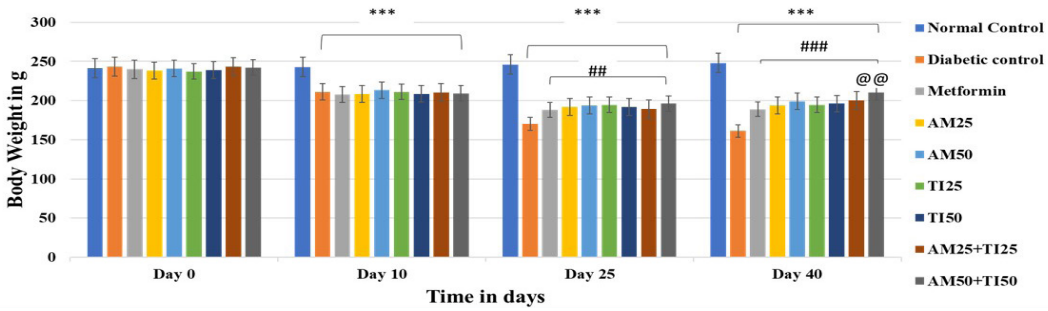

Figure 3: Effect of treatment with metformin(standard drug) and hydroalcoholic extracts (AM, TI and their combination) on body weight in STZ- NAD induced diabetic male SD rats (Data was analyzed by one way ANOVA followed by post hock Bonferroni); p value < 0.05 was considered statistically significant; (mean values and error bars of +1- 2SE) (*** p < 0.001 (Normal control vs Diabetic control, Metformin, AM25,AM50, TI25,T150, AM25+TI25, AM50+T150); ### p < 0.001 (Diabetic Control vs Metformin, AM25,AM50, TI25,T150 , AM25+T125, AM50+T150; ## p < 0.01 (Diabetic Control vs Metformin, AM25,AM50, TI25,T150 , AM25+TI25, AM50+T150 @@p<0.01 Metformin vs AM50+TI50;).

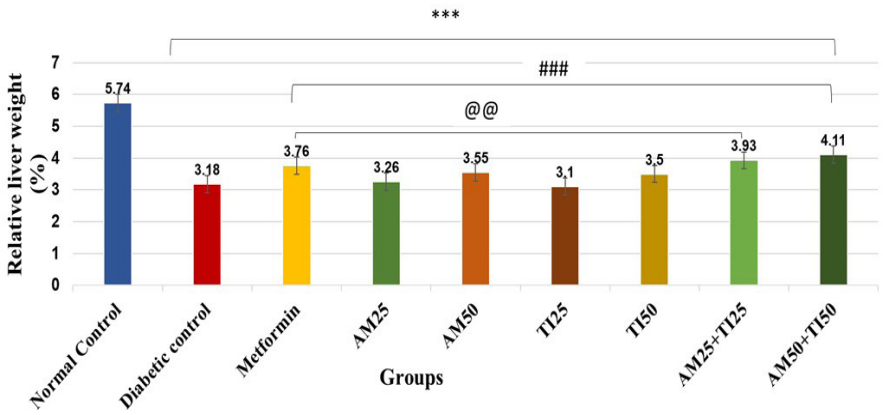

Figure 4: Effect of treatment with metfonnin(standard drug) relative liver weight in STZ-NAD induced diabetes among by post hock Bonferroni); p value < 0.05 was considered stall p < 0.001 (Normal control vs Diabetic control, Metformin, AD (Diabetic Control vs Metformin, AM25,AM50, TI25,T150 AM25,AM50, 1125,T150 , AM25+TI25). an hydroalcoholic extracts (AM, TI and their combination) on ffe nt groups (Data was analyzed by one way ANOVA followed Ily significant; (mean values and error bars of +1- 2SE) (*** 25 AM50, T125,T150, AM25+TI25, AM50+T150); #N# p < 0.001 M2 +T125, AM50+TI50); tittp<0.01 AM50+T150 vs Metformin

comparisons; p 0.05, statistical significance; (mean values and error of +/- 2SE); (Metformin, AM25, AM50, TI25, TI50, AM25+TI25, AM50+TI50); (Glucose-6, ### p 0.001);

As can be shown in Table 1, the hepatic glycogen content and G-6-PD activity of diabetic rats were both considerably reduced, whereas the G-6Pase activity was significantly greater. The experiment was controlled on a group of normal rats. When compared to the diabetic control group, all treatment groups showed considerable enhancement in this regard.

The goal of this research was to compare the effects of metformin (a standard medication) and hydroalcoholic extracts, specifically AM and TI, used alone and in combination to treat STZ-NAD-induced diabetes in different experimental groups on catalase, glutathione (GSH), and malondialdehyde (MDA) levels. Post hoc Bonferroni testing and one-way ANOVA were used to analyze the collected data statistically. A *p-value* of less than 0.05 was considered statistically significant. The error was reported as ± 2 standard errors (SE) and shown with the mean values. Statistical testing revealed substantial differences between the study groups. In particular, when comparing the treatment groups (metformin, AM25, AM50, TI25, TI50, AM50+TI25, and AM50+TI50) to the normal control group and the diabetic control group, a *p-value* of less than 0.001 (***) was identified. In addition, when comparing the treatment groups (metformin, AM25, AM50, TI25, TI50, AM25+TI25, and AM50+TI50) to the diabetic control group, a *p-value* of less than 0.001 (###) was obtained.

Table 2: Treatment with the extracts (alone and in combination) significantly reversed the changes in plasma MDA, catalase and reduced GSH level as compared to the diabetic control group.

Effect of Hydroalcoholic Extracts of AM & TI alone and in Combination on Lipid Profile in STZ-NAD Induced Diabetic Rats Figure 5

Figure 5: There was a significant increase in serum cholesterol, TG, LDL and VLDL levels and Decrease in HDL levels in the diabetic rats group, which was significantly reversed following treatment with extracts.

DISCUSSION

The hydroalcoholic extracts of *A. marmelos* leave and *T. indica* seeds (AM and TI) employed for the therapy in this study were efficient, and various chemicals included in those extracts

Table 1: Effect of hydroalcoholic extracts of AM & TI alone and in combination on liver glycogen content and liver enzymes Glucose-6-phosphate (G-6Pase) and Glucose 6 phosphate dehydrogenase (G-6-PD) in STZ-nicotinamide induced diabetic rats

Groups	*Glycogen (mg/g of tissue)*	*G-6 Pase (units/mg of protein)*	*G-6-PD ($X\ 10^{-4}$ mL U/ mg of protein)*
Normal control	37.33 ± 2.21	0.13 ± 0.08	4.42 ± 0.13
Diabetic control	22.5 ± 1.05 ***	0.24 ± 0.16 ***	2.58 ± 0.13 ***
Metformin	30.66 ± 1.02 ***###	0.14 ± 0.08 ###	3.58 ± 0.12 ###
AM25	25.50 ± 1.50 ***	0.15 ± 0.09 ###	3.44 ± 0.10 ###
AM50	24.50 ± 1.60 ***	0.16 ± 0.07 ###	3.42 ± 0.17 ###
TI25	25.66 ± 1.64 ***	0.14 ± 0.08 ###	3.56 ± 0.08 ###
TI50	26.00 ± 1.39 ***	0.15 ± 0.01 ###	3.49 ± 0.08 ###
AM25 + TI25	31.00 ± 0.68 *** ###	0.15 ± 0.09 ###	3.40 ± 0.12 ###
AM50 + TI50	35.00 ± 1.36 *** ###@@	0.14 ± 0.01 ###	3.64 ± 0.17 ###

were useful in clinical scenarios involving diabetes.[21,22] When compared to the hyperglycemia seen in rats with diabetes caused by STZ alone, hyperglycemia caused by STZ-NAD in rats is more mild.[7, 48] As a result, it would be an improved model for evaluating the anti-hyperglycemic potential of a wide variety of chemicals and extracts. In addition, when compared to other models, this one will be superior for researching the potential for regeneration of pancreatic beta cells due to the fact that beta cells will be destroyed in this model to a lesser extent.[7,23,24] Due to the fact that this model maintains its capacity to have an insulin secretory response to other stimuli, it is utilized in the research of several aspects of beta cell failure. In addition, because this model lacks an intact intracellular antioxidant defense mechanism, it is an excellent research tool for examining how various substances compare to one another in terms of their antioxidant capacities.[25]

In our research, both the extract of AM leaves and the extract of TI seeds showed anti-hyperglycemic action individually. However, coadministration of both extracts resulted in greater glycemic level control than separate extracts, demonstrating the synergistic activities of the two compounds. Compounds

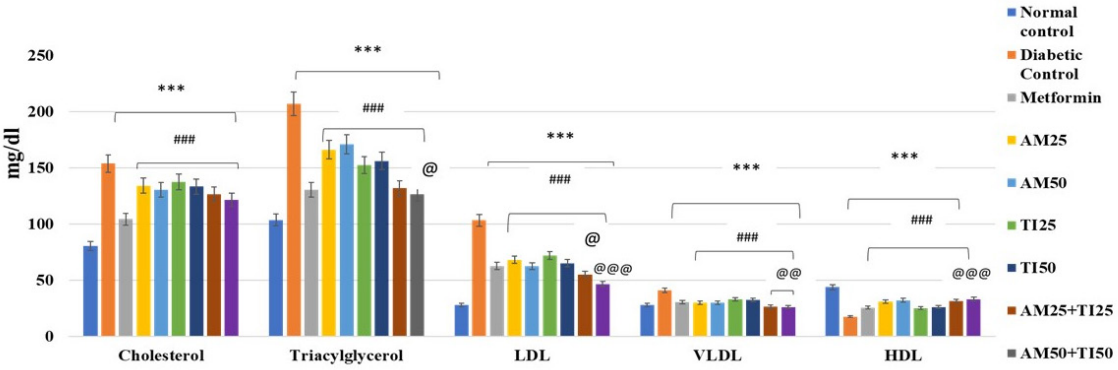

Figure 5: Effect of treatment with metformin(standard serum cholesterol, triacylglycerol, LDL, VLDL and HD 1i was analyzed by one way ANOVA followed by post ho k (mean values and error bars of +1- 2SE); (*** p < 0 01 TI25,T150, AM25+TI25, AM50+T150; p < 0.001 D abe AM50+TI50) , (6 p< 0.05 Metformin vs AM50+TI50, ((Metformin vs AM50+T150);) and hydroalcoholic extracts (AM, TI and their combination) on vets in STZ-NAD induced diabetes among different groups (Data onferroni); p value < 0.05 was considered statistically significant; Normal control vs Diabetic Control , Metformin, AM25,AM50, is Control vs Metformin, AM25, AM50, TI25,T150, AM25+TI25, <0.01 Metformin vs AM25+TI25, AM50+TI50); (6"6@p< 0.001)

Table 2: Effect of hydroalcoholic extracts of AM & TI alone and in combination on catalase, reduced glutathione (GSH) and malondialdehyde (MDA) level in STZ-nicotinamide induced diabetic SD rats

Groups	*Catalase (μmoles of H_2O_2 decomposed/min/mg of protein)*	*GSH (nmol/mg of protein)*	*MDA (nmol/mg of protein)*
Normal control	2.86 ± 0.23	22.66 ± 1.30	55.50 ± 3.05
Diabetic control	1.11 ± 0.04 ***	8.50 ± 0.81 ***	152.83 ± 3.49 ***###
Metformin	2.43 ± 0.11###	18.00 ± 0.68 ***###	86.00 ± 2.35 ***###
AM25	2.58 ± 0.13###	20.00 ± 1.50 ***###	82.33 ± 2.06 ***###
AM50	2.46 ± 0.16###	21.83 ± 1.30 ***###	88.16 ± 1.57 ***###
TI25	2.55 ± 0.10###	19.33 ± 1.20 ***###	87.66 ± 2.60 ***###
TI50	2.57 ± 0.13###	22.00 ± 1.12 ***###	84.83 ± 2.65 ***###
AM25 + TI25	2.67 ± 0.10###	20.66 ± 2.65 ***###	81.83 ± 2.84 ***###
AM50 + TI50	2.33 ± 0.11###	22.66 ± 0.71 ***###	77.33 ± 2.31***###

such as gallic acid, rutin, and other polyphenols may be found in AM extract.[26] TI seed extract, on the other hand, contains chemicals such as glycosides, alkaloids, anthraquinones, saponin, catechin, epicatechin, and so on.[27] It is possible that the simultaneous administration of extracts influenced numerous processes involved in STZ-NAD's induction of hyperglycemia and hyperlipidemia.

In diabetes, free radicals are produced by glucose oxidation, protein glycation, and the oxidative degradation of glycated proteins. When the body's antioxidant defenses are overwhelmed by free radicals, cell damage, lipid peroxidation, and insulin resistance result.[28] Increased amounts of malondialdehyde (MDA), a marker of lipid peroxidation, were also observed. The reduction in catalase and glutathione peroxidase levels demonstrates that the antioxidants were unable to shield cells from the cytotoxic effects of STZ-NAD. However, when both extracts were present, either alone or together, antioxidant levels rose and lipid peroxidation was reduced. Because it contains polyphenols and phytonutrients like gallic acid and rutin, AM leaf extract is regarded as a powerful antioxidant. This is due to the fact that it comprises these substances.[26-28] The bioactive metabolites found in AM extracts can decrease oxidized intermediates by donating electrons, which results in free radical scavenging action. The polyphenols included in the extract scavenge free radicals and prevent their own production.[27,28] This makes the harm that oxidative stress causes and the maintenance of the integrity and function of cells more difficult.

According to certain studies, the polyphenols that are included in TI seed extract can scavenge free radicals. Furthermore, these polyphenols can lower blood glucose levels by blocking gluconeogenesis and adrenergic-mediated glucose absorption.[28-30] TI seed extracts include catechin and epicatechin, both of which exhibit free radical scavenging action as well as antioxidant activity. This activity is achieved by lowering pro-oxidant, inducing antioxidant enzymes, and synthesizing phase II detoxifying enzymes.[31] Therefore, the combined administration of the two extracts resulted in a lower level of oxidative stress compared to the effects of each extract alone.

It has been shown that the active ingredients in AM extract can help regulate blood sugar by increasing insulin production by the pancreatic beta cells. Polyphenols included in AM extracts have an action that inhibits alpha-amylase. This activity slows the pace of carbohydrate digestion and decreases the glucose absorption rate, leading to lower glucose levels after meals.[32] In addition to this, it has been demonstrated that polyphenols inhibit glucose export from hepatocytes and promote glucose absorption in liver cells through modulating the cell signaling system.[34,35] Both gallic acid and rutin, included in AM extract, have been shown to block glucosidase, reducing the amount of carbohydrates absorbed in the intestines. Additional polyphenols included in AM extract have been proven to trigger the insulin signaling cascade, which stimulates GLUT 2 and promotes glucose transport into the cell.[36]

It has been claimed that the saponin found in TI seed extract can block the breakdown of disaccharides into monosaccharides, which can also reduce the amount of glucose in the blood.[16,33] When the activity of pancreatic beta cells is taken into consideration, the intracellular flow of ionic calcium is of significant physiological importance. When there is a higher quantity of glucose within the cell, the beta-cell membrane becomes more depolarized, which in turn causes an inflow of calcium from the surrounding extracellular space. When entering the beta cells, calcium sets off a cascade of events that leads to the release of insulin-rich secretory granules.[37] The TI seed extract was discovered to enhance the amount of ionic calcium found within the cells of the pancreatic islets, which favored the release of insulin. The fact that TI seed extract contains polyphenols such as catechin and epicatechin suggests that these compounds were involved in the modulation of intracellular calcium ions. The flavonoids in the TI seed extract preserve the neuropeptides involved in calcium entrance into the beta cells of the pancreas, which further promotes insulin release.[30]

Sterol regulatory element binding proteins, also known as SREBPs-1c, are a family of transcription factors that control the production of cholesterol as well as fatty acids. Specifically, they promote the transcription of acetyl CoA carboxylase and cytosolic HMG-CoA synthase, which are required for the endogenous formation of cholesterol, fatty acid and TG and phospholipids. They are regarded as master regulators of cholesterol synthesis and lipogenesis. It is well established that the activity of these transcription factors in liver may be increased by TI seed extract.[30] Diabetes is related to the comorbid condition known as hyperlipidemia, which is marked by anomalies in lipoproteins and elevated levels of triglycerides, phospholipids, and cholesterol.[38,39] The management of hyperlipidemia is of the highest relevance for the prevention of diabetes-related complications such as diabetic microangiopathy, diabetic macroangiopathy, and cerebrovascular disease. In this light, the extracts used in this experiment appear to be beneficial in controlling both hyperglycemia and hyperlipidemia.

In the current investigation, both extracts, whether given individually or in combination, demonstrated considerable control over cholesterol and triglyceride levels. Every abnormality in lipid profile that was produced in STZ-NAD models was noticeably controlled. The combination of extracts [AM50+T150] has exhibited superior regulation than any of the other extracts that were tested. It's possible that this is because of the synergistic effect they have. Certain components, such as polyphenols, gallic acid, and rutin, which were found in AM extract, and saponin, catechin, epicatechin, and flavonoids, which were present in TI seed extract, were able to modulate glycemia. Other chemicals such as coumarins-umbelliferone, a benzopyrone esculetin, and a hydroxycumarin found in AM extract and beta-sitosterols, taxifolin, epigenin, and luteolin found in TI seed extract independently controlled lipids.[40-44] Rats that had been treated with STZ-NAD had a lower overall body weight, as was found. It's possible that this is related to

an increased rate of lipolysis. When extracts or metformin are present, there is an improvement in the body weight.

A medication's ability to reduce hyperglycemia can be judged by its effect on liver glycogen levels.[45] A considerable increase in glucose-6 phosphatase and a notable reduction in liver glycogen were seen in diabetic rats throughout this experiment. Previously published results are in agreement with these new findings.[46-48]

The results of this study show that the synergistic effects of AM extract and TI seed extract greatly reduced the severity of hyperglycemia and alleviated lipid abnormalities in the STZ-NAD model of diabetes. A synergistic effect of several polyphenols and phytonutrients made this feasible.

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