



Effect of Agmatine on Non-Alcoholic Fatty Liver Disease Induced by Type 2 Diabetes in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author SFM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAAASA and AE revised the article critically for important intellectual content. All authors contributed to and have approved and the final manuscript.

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ABSTRACT

Aim: To determine the potential hepatoprotective effect of Agmatine (AGM) on NAFLD-induced by Type 2 diabetes mellitus (T2DM) in rats.

Study design: Forty male Wistar rats weighing from (200 -250 g) were distributed at random into five groups (8 rats per group): group 1 as control; group 2 as untreated-T2DM; groups 3 & 4 as T2DM cotreated with AGM (40 & 80 mg/kg/d), while group 5 T2DM cotreated with Silymarin (100 mg/kg/d).

Place and duration of study: Department of Pharmacology, Faculty of Medicine, King Abdul-Aziz University; between October 2020 and January 2021.

Methodology: A rat model of T2DM with NAFLD complication was established by feeding rats with 10% fructose in drinking water and intraperitoneally injecting them with a single low dose of streptozotocin (STZ) (45mg/kg). The fasting blood glucose was detected, serum levels of hepatic biomarkers were all assessed. Moreover, histopathological examination was performed by hematoxylin and eosin (H&E) staining.

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Results: STZ induced T2DM in rats causes a significant ($p < 0.05$, $n = 8$) rise in serum levels of FBG, ALT, AST, TB, TC, TG, and LDL in comparison with the corresponding control group. Co-treatment with AGM (40 & 80 mg/kg) and silymarin significantly alleviated hyperglycemia and amended hepatic biomarkers that was reflected on improved histopathological changes.

Conclusion: The current data suggest that oral AGM co-treatment could have a hepatoprotective effect against T2DM associated with NAFLD in rats. Further investigations are recommended to elucidate molecular mechanisms accountable for the useful effects of AGM on hepatocytes.

Keywords: NAFLD and T2DM disease; AGM; FBG; STZ; hepatocytes.

1. INTRODUCTION

The liver is one of the primary organs that control the metabolic homeostasis. Metabolic diseases such as IR, T2DM, dyslipidemia, and NAFLD are linked through molecular-biochemical, and complex immune mechanism [1]. Both NAFLD and diabetes are chronic diseases that usually delineate nonalarming changes that can lead to disorder and many other metabolic complications [2]. They are all independently mortality and morbidity risk enhancers, and overall global financial consumer disorders [3]. There is a strong association between NAFLD and T2DM risk [4]. The mechanism of T2DM aggravating NAFLD progress may include insulin resistance, inflammatory response and chemokine mediated oxidative stress [5]. Therefore, for the pathophysiology of the disease, NAFLD can be treated by reducing the accumulation of liver fat, the production of metabolic stress products, the advancement of blood sugar level and IR. As well as, by inhibiting the related injuries caused by oxidative stress and inflammation [4]. NAFLD is a highly popular disorder with limited treatments [1]. Hence, finding an effective treatment is necessary [6]. Since most drugs may increase liver toxicity, it is important to select the appropriate drug therapy that able to cure NAFLD and other hepatic diseases [6]. An endogenous aminoguanidine compound - Agmatine (AGM) - is a decarboxylated product of L-arginine made from arginine-by-arginine decarboxylase [7]. Animal studies by Molderings et al. [8] revealed that exogenous agmatine sulfate, the commonly used salt form of agmatine, is absorbed in the gastrointestinal tract (GI) and then rapidly (within minutes) distributed throughout body, including the brain [8]. As a therapy, agmatine treatment is considered relatively safe [9]. In humans, a randomized double-blind placebo-controlled trial demonstrated the safety and efficacy of oral agmatine sulfate (1,335–3,560 mg/day for up to 3 weeks) in lumbar disc-associated radiculopathy [9]. Furthermore, a human case study reported no adverse reactions of oral agmatine sulfate at

a high daily dose of 2,670 mg (approximately 36–46 mg/kg) for a period of 5 years [10]. Other studies indicated the safety of acute oral administration of agmatine sulfate up to 480 mg/kg and sub-chronic administration via drinking water at an estimated daily dose of 100 mg/kg in rats for 95 days [11]. Moreover, a preclinical study by Bergin DH et al. [9] showed that oral administration of agmatine sulfate via gavage at doses up to 900 mg/kg (once daily for 7 days) or at 300 mg/kg (once daily for 105 days) is safe for adult male mice [9]. Agmatine possesses neuroprotective, antidiabetic, antioxidant and anti-inflammatory properties that has been a growing interest in clinical applications [7,12,13]. Also, it has shown an obvious positive effect on multiple disorders in animal models, such as cognitive decline and spinal cord injury [14]. A study also reported that AGM have anti-diabetic characteristic, which made this drug useful for treating diabetic animals [15]. As well as, it has been shown an insulin-like action by reduced advanced glycation end products that control different diabetic complications [16]. In addition, AGM enhanced the production of insulin from pancreatic islet B-cells and regulated fructose-induced IR. Other studies showed that administration of AGM has partially attenuated oxidative damage in liver injury after Chlorpromazine (CPZ) hepatotoxicity of the Wistar rat's model [17]. Furthermore, it effectively inhibited nuclear factor kappa-B (NF- κ B) the cardinal transcription factor, which leads to suppressing the mediator of inflammation such as tumor necrosis factor- α (TNF- α) [18]. This data confirmed its role as a potent anti-inflammatory drug [7]. Thus, AGM may have various promising molecular and metabolic effects in treating various illnesses. While the impacts of AGM to treat diabetes & liver dysfunction have been stated separately. The potential medicinal effect of AGM on both T2DM and NAFLD in rats has not yet been evaluated. Accordingly, this work aimed to investigate the possible protective effect of AGM against NAFLD associated with T2DM in rats.

2. MATERIALS AND METHODS

2.1 Reagents

Fructose, Streptozotocin, Glucose, and Agmatine were obtained from (Merck®, U.S.A.).

2.2 Animals

Male rats their weights as follows 200-250 g, were retained, and housed under conditioning area ($23 \pm 1^\circ\text{C}$) with a 12-hr (light- dark cycle) and were supplied *ad libitum* with water and rodent chow through the experiment.

2.3 Experimental Study Design

Forty male Wistar rats weighing from (200 -250 g) were distributed at random into five groups (8 rats in each cage). Group one served as control and was given a vehicle as Sodium carboxymethylcellulose (0.5% Na-CMC) by oral gavage five times per week. Group two served as diabetic rats were administered single-low-dose-STZ (45 mg/kg, i.p.) which was liquified in a citrate buffer, the pH (4.4) [19]. As well as rats were fed with 10% fructose in drinking water. Rats with fasting serum glucose level >200 mg/dl were classified as T2DM. Group three and four were fed with 10% fructose along with AGM Sulfate (40 & 80 mg/kg/d dissolved in saline: respectively) five times per week orally. Group five were fed 10% fructose along with the positive standard Silymarin as a reference at (100 mg/kg/d) five times per week. Fasting blood glucose (FBG) was assessed weekly. After twenty-four hours from the last dose of treatment, rats were anesthetized. Then, blood was gathered from the retro-orbital plexus to isolate serum. Afterward, rats were euthanized by cervical dislocation and liver tissues were dissected out and rinsed promptly. For histopathological hematoxylin and eosin (H&E) staining, representative liver tissues were preserved in 10% neutral buffered formalin. The remaining liver tissue specimens were kept at -80°C .

2.4 Fasting Blood Glucose

Accu-Chek Aviva meter was applied for Fast blood sugar detection. Blood samples of the fasting animals were collected from the tail vein. The blood was obtained from the rats by introducing the rat into a closed plastic cabinet, only the tail of the rat is outside. An alcohol swab

was used to clean the tail. The tip of the tail was punctured with a small needle. Thus, about 2 drops of blood were taken on the strip attached with a glucometer. The rats were considered diabetic when blood glucose levels were above 250 mg/dl [20]. FBG was re-measured weekly.

2.5 Assessment of Liver Function

Activities of *high* density-lipoproteins (HDL), low density-lipoproteins (LDL), alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), triglycerides (TG), total-cholesterol (TC), and serum levels of total bilirubin (TB), were examined colorimetrically and commercial kits purchased from (Bioengineer, U.S.).

2.6 Histopathological Assessment

Liver tissues (n =three) were fixed in (10% neutral buffered formalin) within a period of 48 hours and embedded in paraffin. Then, serial sections (4 μm -thick) were cut using a microtome [Model RM2245, Germany]. Subsequently, sections were stained with H&E. In the last step, the specimens were investigated underneath a light microscope (Olympus BX-50; Japan).

3. RESULTS

3.1 Effect of Tested Drugs on Fasting Blood Glucose

Fig. 1 indicates FBG levels in different groups during 12 weeks of the experiment. FBG was detected on week 0 before starting the experiment and on weeks 4, 8, &12 after fructose feeding and induction of T2DM by STZ (45 mg/kg, ip). At week 0, no significant changes were detected among all groups, as shown in (Fig. 1A). On weeks 4 and 8, FBG of all groups were significantly elevated in comparison to the corresponding control group, as shown in (Figs. 1B, 1C). Interestingly, co-treatment with AGM (40 & 80 mg/kg) and silymarin significantly decreased FBG levels by about 16%, 28%, 26% on week four and about 33%,36%,40% on week eight, respectively, compared to the corresponding diabetic group (Figs. 1B, 1C). At the last week of the experiment (week 12), the hypoglycemic effect was more distinct, where co-treatment with AGM (40 & 80 mg/kg) and silymarin significantly decreased FBG levels by about 47%, 50%, 53% respectively, compared to the diabetic group (Fig. 1D).

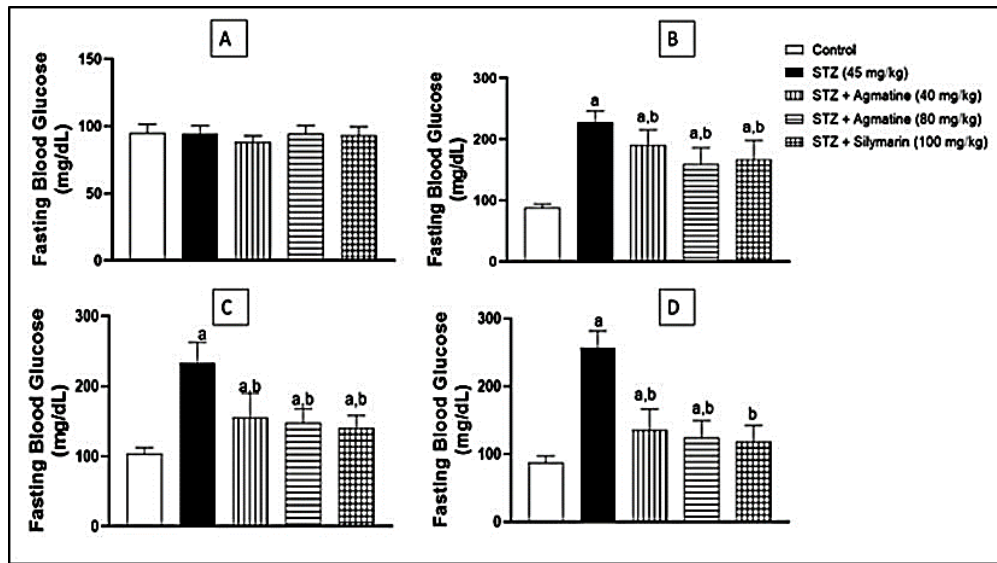


Fig. 1. Effect of different doses of AGM (40, 80 mg/kg) on fasting blood glucose levels at different time intervals in T2DM-induced NAFLD in rats

[A] before starting the experiment, [B] on week 4, [C] on week 8 and [D] on week 12.

(a) Statistically significant from the corresponding control, $P < 0.05$. (b) Statistically significant from the corresponding STZ-challenged group, $P < 0.05$. Data are shown as Mean \pm S.E.M, $n=8$. (a) $P < 0.05$ versus control group. (b) $P < 0.05$ versus STZ-challenged group. STZ: Streptozotocin

3.2 Effect on Liver Function and Lipid Profile

Streptozotocin (STZ)-induced T2DM disease in rats causes a significant ($P < 0.05$, $n=8$) rise in serum levels of ALT, AST, TB, TC, TG, and LDL, in comparison with the corresponding control group, as illustrated in Tables 1 & 2. However, HDL concentration in the diabetic group were

significantly diminished in contrast with the control group by about 38%. In contrast, AGM groups (40 & 80 mg/kg) were significantly decreased ALT, AST, TC, TB, TG, and LDL serum levels as compared to the corresponding diabetic group. Conversely, silymarin co-treatment alleviated all biomarkers to almost normal values, so that no statistical distinct from the corresponding control group was detected.

Table 1. Effect of AGM on liver function biomarkers in T2DM-induced NAFLD in rats

Group (n = 6)	ALT (U/L)	AST (U/L)	TB (mg/dL)
Control	14.27 \pm 2.23	18.30 \pm 3.12	0.69 \pm 0.25
STZ (45 mg/kg)	69.67 ^a \pm 12.0	105.5 ^a \pm 8.83	1.45 ^a \pm 0.29
STZ + AGM (40 mg/kg)	33.17 ^{a,b} \pm 8.75	37.03 ^{a,b} \pm 4.46	0.65 ^b \pm 0.23
STZ + AGM (80 mg/kg)	20.48 ^{b,c} \pm 1.20	28.53 ^{a,b} \pm 3.14	0.64 ^b \pm 0.12
STZ + Silymarin (100 mg/kg)	17.5 ^{b,c} \pm 1.18	20.37 ^{b,c} \pm 3.41	0.59 ^b \pm 0.19

Data are presented as mean \pm SD ($n = 6$). Statistical analysis was carried out by one-way ANOVA followed by Tukey's post hoc test.

a) Statistically significant from the corresponding control at $P < 0.05$.

b) Statistically significant from the corresponding STZ-challenged group at $P < 0.05$.

c) Statistically significant from the corresponding (STZ + AGM 40 mg/kg) group at $P < 0.05$.

STZ: streptozotocin, AGM: agmatine, ALT: alanine- aminotransferase, AST: aspartate-aminotransferase, TB: total bilirubin.

Table 2. Effect of AGM on lipid profile in T2DM-induced NAFLD in rats

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	122.67 ± 7.00	72.5 ± 2.43	41.83 ± 1.33	78.67 ± 9.93
STZ	256 ^a ± 10.33	180 ^a ± 10.56	25.67 ^a ± 5.09	238 ^a ± 31.01
(45 mg/kg)				
STZ + AGM (40 mg/kg)	139.5 ^b ± 26.18	75.83 ^b ± 6.85	40.5 ^b ± 1.05	115 ^{a,b} ± 18.15
(80 mg/kg)				
STZ + AGM (80 mg/kg)	116 ^{b,c} ± 8.88	74.67 ^b ± 3.98	43.00 ^b ± 2.97	75.83 ^{b,c} ± 4.75
STZ + Silymarin (100 mg/kg)	125 ^{b,c} ± 7.79	73.17 ^{b,c} ± 3.13	46.5 ^{b,c} ± 3.51	72.83 ^{b,c} ± 9.02

Data are presented as mean ± SD (n = 6). Statistical analysis was carried out by one-way ANOVA followed by Tukey's post hoc test.

a) Statistically significant from the corresponding control at $P < 0.05$.

b) Statistically significant from the corresponding STZ-challenged group at $P < 0.05$.

c) Statistically significant from the corresponding (STZ + AGM 40 mg/kg) group at $P < 0.05$

STZ: streptozotocin, AGM: agmatine, TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, VLDL: very low-density lipoprotein, LDL: low density lipoprotein

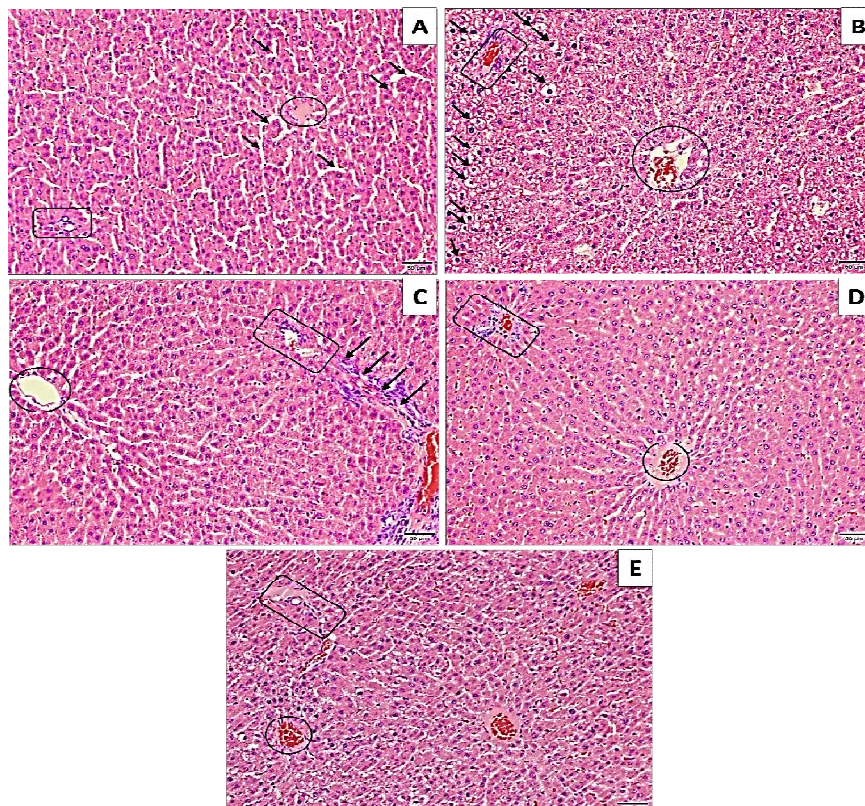


Fig. 2. Representative photomicrographs of liver sections stained by H&E (×100)

(A) Control group showing with normal hepatocellular structure of the central vein (circle), surrounding sinusoids (arrows) and portal triade (square), (B): T2DM-challenged group with congested and dilated central veins (circle) with intense cellular vacuolization (arrows) and congested portal triade (square) (C): Co-treatment with AGM (40 mg/kg) showing partial improvement of hepatic architecture, central and portal changes with perivascular infiltration (arrows), (D): Co-treatment with AGM (80 mg/kg) showing normal hepatocytes, central vein (circle) and portal triade (square) with minimal congestion. (E): Co-treatment with silymarin (100 mg/kg) showing normal hepatocytes, central vein (circle) and portal triade (square) with minimal congestion

3.3 Histopathological Assessment

Liver sections from the control group presented normal liver histology of the central vein and hepatic cells, as shown in (Fig. 2A). In STZ-induced T2DM group (Fig. 2B), obvious congested and dilated central veins with perivascular infiltration of mononuclear cells and early manifestation of collagen deposition have been detected. Furthermore, hepatocytes with cytoplasm rich of lipid droplets and the perivascular cells apparently showed more and larger vacuolation than the centrilobular cells. On the other hand, AGM low dose treatment group (40 mg/kg) displayed minimal protective effect with perivascular infiltration of mononuclear cells, as shown in Fig. 2C. In contrast, the high dose AGM-treated group (80 mg/kg) demonstrated minor degenerative changes with some congestion (Fig. 2D). While silymarin showed observable protection with minimal congestion, as shown in (Fig. 2E).

4. DISCUSSION

Hepatic steatosis under condition of type 2 diabetes is a result of imbalance in the uptake, synthesis, export, and oxidation of free fatty acids [21]. Current treatment strictly focuses on the management of glycaemia without reduction of associated diabetic complications, including NAFLD [22]. Therefore, the purpose of this study was to determine the protective effect of AGM against NAFLD associated with T2DM in rats. Streptozotocin (STZ)-induced T2DM in rats impaired glucose tolerance, elevated FBG levels, worsened lipid profile, and led to pathological injury of liver tissue [23]. A rat model of type 2 DM designed to mimic the pathogenesis of human disease by 10 % of high-fructose diet feeding along with i.p. STZ injection as previously reported. The results of this study suggest that treatment with AGM showed a significant reduction in FBG levels in diabetic rats. The current findings agree with the results reported by Sharawy et al. [16] that AGM has an insulin-like action. In this regard, AGM was found to diminish advanced glycation end products that are correlated to the complications of diabetes. In addition, it can regulate fructose-induced IR [16]. Furthermore, a previous published work of Bila et al. [24] has illustrated that AGM exerts hypoglycemic effects [24,25]. The present study demonstrated that treatment with AGM (40 and 80 mg/kg) or silymarin significantly decrease FBG levels. In diabetic animals, the abnormal insulin metabolism lead to the existence of

NAFLD [25]. Hepatic enzymes which include aminotransferases (AST and ALT) as well as lipid profile (TCH, TG, and LDL) parameters are the first line of markers used to determine hepatic injury [25]. Elevated serum transaminases are commonly observed in diabetics due to the functional disturbances of hepatocyte membranes [6]. Upon oral administration of AGM in T2DM rats, the serum levels of these biomarkers were reduced significantly compared to the diabetic untreated group. This was confirmed by other studies [26,27], revealing that AGM treatment could improve the lipid profile. The major contributor to IR in hepatic steatosis is TG accumulation [27], which was confirmed in the present study by histopathological examination, as the dose of AGM (80 mg/kg) mitigated hepatic injury. While silymarin showed observable protection of the hepatocytes. These findings were consistent with previous studies [16,25]. Thus, AGM is considering a promising agent that could be produce a hepatoprotective effect in diabetic rats.

5. CONCLUSION

The current data suggest that oral AGM co-treatment could have a hepatoprotective effect against T2DM-induced NAFLD in rats. Further investigations are recommended to elucidate molecular mechanisms accountable for the useful effects of AGM on liver cells.

CONSENT

Not applicable

ETHICAL APPROVAL

All experiments have been examined and approved by Ministry of Education King Abdulaziz University, Faculty of Pharmacy, Research Ethics Committee (Reference No. PH-1442-45).

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COMPETING INTERESTS

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.

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