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Co-treatment with saponin and metformin improves the biochemical status of type 2 diabetes mellitus rats

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ABSTRACT

Saponins (Sap) are effective bioactive compounds for ameliorating various diseases complications. This study addressed the effects of co-treatment with Sap and metformin (Met) on the biochemical status of type 2 diabetes mellitus (T2-DM) rats. Forty male Sprague-Dawley rats were divided into 5 groups (n = 8) as follows: group 1 (Gp1) was the negative control group. Gp2 was fed on a high fat diet (HFD) for 8 weeks (wks) and received a single intraperitoneal (i.p) injection of 30 mg/kg streptozotocin (STZ) after 8 wks to induce T2-DM. Gp3 was treated as Gp2, after 8 wks rats received 250 mg/kg Met by oral gavage daily for 4 weeks. Gp4 was treated as Gp2, and after 8 wks rats received 300 mg/kg Sap by oral gavage daily for 4 weeks. Gp5 was treated as Gp2, after the 8 wks rats received it, and then co-treated with Met as in Gp3 and Sap as in Gp4. The percentage of body weight (% b.wt) changes, hematological, and biochemical parameters were determined. The results showed that co-treatment with Met and Sap led to significant improvement in the % b.wt of T2-DM rats. Co-treatment of T2-DM rats with Met and Sap led to synergistic effects in the reduction of serum glucose levels and an increase in the C-peptide levels. Co-treatment of T2-DM rats with Met and Sap led to significant improvements in the hepato-renal functions and antioxidant status. Sap could play an important role in regulating T2-DM and can be developed as a promising natural material for diabetes management.

Keywords:

Type 2 diabetes mellitus**,** Saponin, Metformin, Antidiabetic, Biochemical status

1. Introduction

A chronic condition known as diabetes mellitus (DM) is characterized by hyperglycemia brought on by insufficient insulin production or insulin resistance (Shi *et al*., 2018). Type 1 diabetes mellitus (T1-DM) and type 2 diabetes mellitus (T2-DM) are the two main classifications for diabetes (Nuckols *et al*., 2018). Obesity or having a greater body fat percentage, primarily in the abdominal area, are the main characteristics of T2-DM patients. Symptoms of T2-DM tend to develop slowly over the time including weight loss (Yang *et al*., 2016). Microvascular consequences from T2DM include retinopathy, neuropathy, and nephropathy, whereas macrovascular issues include cardiovascular, cerebrovascular, and peripheral vascular disease (Harding *et al*., 2019).

One of the commonly given drugs in the US and Europe is metformin (Met), a member of the biguanide family. Metformin is still the first-line treatment for T2-DM management throughout the entire world. It has been shown that Met mainly inhibits the increased basal endogenous glucose production in people with T2-DM by lowering hepatic gluconeogenesis. (Hundal *et al*., 2000).

Met prevents the activation of AMP-activated protein kinase (AMPK) and the mitochondrial respiratory chain complex 1, which has an impact on energy metabolism (Vial *et al*., 2019).

Traditional treatments for T2-DM have largely relied on herbal remedies. According to a previous article, saponins (Sap) are responsible for the antihyperglycemic effects of medicinal herbs used to treat diabetes. They are made up of sugar molecules attached to sapogenin, a hydrophobic aglycone that may also be a triterpenoid or a steroid (Francis *et al*., 2002).

It According to reports, using Sap as a treatment induced the release of insulin, reduced oxidative stress, boosted glucose-6-phosphate activity, and increased the expression of glucose transporters (GLUT-4) (Marie *et al*., 2016; Bhavsar *et al*., 2019). Furthermore, after the pancreas released insulin, the injection of Sap to diabetic rats drastically decreased the plasma glucose levels. (Alli Smith and Adanlawo, 2012). This study evaluated the effects of co-treatment with Sap and Met on the biochemical status of T2-DM rats.

2. Materials and Methods

Chemicals

Streptozotocin (STZ) was purchased from MP biomedicals company (Illkirch, France). Metformin (Met) was purchased from Alfa Aesar company (Haverhill, Massachusetts, USA). Saponin (Sap) from Quillaja Saponaria Molina was purchased from Acros Organics company (Fisher Scientific AG, USA). Sap was diluted by PBS and the concentration was adjusted to 300 mg/kg b. wt. in 300 µl for oral administration. All biochemical kits were purchased from Bio diagnostic Company in Egypt.

Animals and experimental design

One dose of STZ was intraperitoneally (i.p.) injected into rats (30 mg/kg) to induce diabetes in rats (Guo *et al*., 2011). Forty male Sprague-Dawley rats were divided into 5 groups (n = 8) as follows: group 1 (Gp1) was the negative control group. Gp2 was fed on a high fat diet (HFD) for 8 weeks (wks) and received STZ (30 mg/kg) after the 8 wks to induce T2-DM. Gp3 was treated as Gp2, after 8 wks rats received Met (250 mg/kg) by oral gavage daily for 4 weeks. Gp4 was treated as Gp2, after 8 wks rats received Sap (300 mg/kg) by oral gavage daily for 4 wks. Gp5 was treated as Gp2, after 8 wks rats received, then co-treated with Met as in Gp3 and Sap as in Gp4. At the end of the experiment all groups were euthanized, the percentage of body weight (% b.wt) changes, glucose level, hematological, and biochemical parameters were determined.

Determination of the percentage of total body weight changes

Rats were weighed at the beginning (initial b.wt) and the end of the experiment (final b.wt). The percentage of body weight changes (% b.wt).

Determination of biochemical parameters

Blood and sera samples were collected for hematological and biochemical analysis. Serum glucose and C-peptide were determined according to Tietz, (1995) and Jones and Hattersley (2013). Serum AST and ALT activities were determined as described by Thomas (1998) and Rei (1984). Serum total protein levels were determined (Tietz, 1995). Kidney functions (urea and creatinine) were determined as described by Thomas (1998). . Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels were determined (Nishikimi *et al*., 1972; Aebi, 1984; Li and Chow, (1994), respectively.

Statistical analysis

Using one-way ANOVA, comparisons between groups were made. Tukey post hoc comparisons among different groups were performed. For all statistical tests, P values $\leq$ 0.05 were statistically significant.

3. Results

Effect of the treatment with Met or/and Sap on the percentages of body weight changes

The results showed that in the T2-DM rats' group, there was a significant reduction in % b.wt to 31.94% when compared with the normal control. Treatment with Met or/and Sap improved body weight to 71.64%, and 68.19%, respectively. Co-treatment of T2-DM rats with Met/Sap showed a significant increase in the kinetic body weight and the % b.wt to 80.15% (Fig. 1).

Effects of the treatment with Met or/and Sap on glucose and C-peptide levels in the different groups under the study

The obtained results showed a significant increase in serum glucose in T2-DM rats (287.23 ± 4.95 mg/dL) compared to the control group. On the other hand, administration of T2-DM rats with Met or Sap led to significant reductions in the serum glucose level (141.63 ± 3.12 mg/dL) and (152.29 ± 3.32 mg/dL), respectively. Although, treatment with Met/sap showed improvement in glucose level (116.40 ± 2.77 mg/dL) compared to T2-DM rats. T2-DM rats have a significant decrease in C-peptide level (0.03 ± 0.015 ng/mL). Treatment with Met or Sap in T2-DM rats resulted in a significant increase in their C- peptide levels (0.09 ± 0.013 and 0.07 ± 0.009 ng/mL), respectively. Also, co-Treatment with Met/sap in T2-DM rats resulted in a significant increase in their C- peptide level (0.10 ± 0.011 ng/mL) (Table 1).

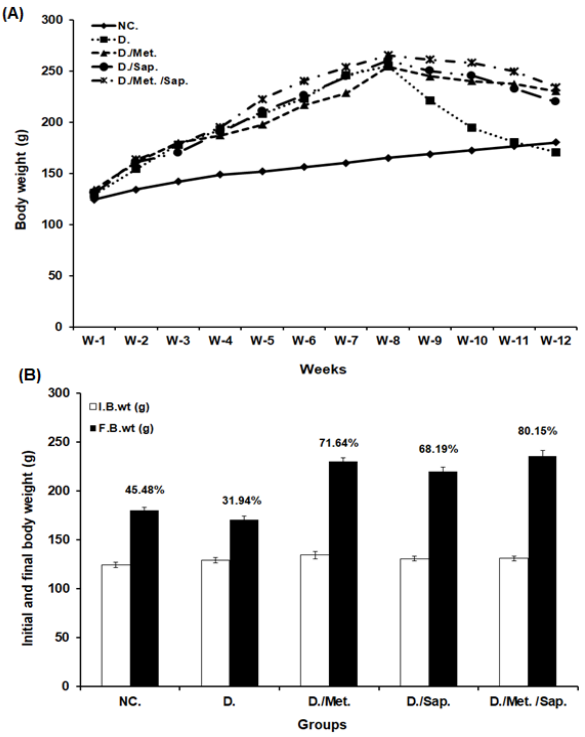

Fig. 1. The percentages of change in the different groups under the study. The values represented mean ± SD; I.B.wt: Initial body weight; F.B.wt: Final body weight; N.C.: Normal control group; D.: T2-DM rats; Met: Metformin; Sap.: Saponin. *P*-value < 0.05 was statistically significant.

Table 1. Serum glucose and C-peptide levels in the different groups

Groups	Glucose (mg/dL)	C-peptide (ng/mL)
NC.	83.67 ± 2.43 ^{e}	0.13 ± 0.010 ^{b,d}
D.	287.23 ± 4.95 ^{a}	0.03 ± 0.015 ^{a}
D./Met.	141.63 ± 3.12 ^{b}	0.09 ± 0.013 ^{d}
D./Sap.	152.29 ± 3.32 ^{b}	0.07 ± 0.009 ^{c,d}
D./Met. /Sap.	116.40 ± 2.77 ^{c}	0.10 ± 0.011 ^{d}

The values represented mean ± SD. N.C.: Normal control group; D.: T2-DM rats; Met: Metformin; Sap.: Saponin; *P*-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

3.3. Effects of the treatment with Met or/and saponin on the liver transaminases, and the total protein levels in the different groups under the study

The levels of ALT and AST enzymes were increased in T2-DM rats. Treatment of T2-DM rats with Met or sap led to significant decreases in the activities of ALT and AST enzymes to 44.65 ± 3.41 and 201.75 ± 9.65 U/L, respectively when compared with T2-DM rats ($P < 0.05$). Treatment of rats with Met and Sap led to significant improvement in liver functions, as evidenced by a significant decrease in serum ALT and AST activities. The T2-DM rats showed a change in serum total protein levels (8.11 ± 0.15 g/dL). Treating T2-DM rats with met or sap led to a significant increase in total protein levels to 8.38 ± 0.28, and 8.42 ± 0.20 g/dL, respectively. When co-treated with met/sap there was a significant improvement in total protein levels (8.49 ± 0.21 g/dL) (Table 2).

Effects of the treatment with Met or/and saponin on urea and creatinine levels in the different groups under the study

The results showed that serum urea and creatinine levels were significantly increased in STZ-induced T2-DM group when compared to the normal control group ($P < 0.05$). Co-treatment of T2-DM rats with Met and Sap led to a significant decrease in urea and creatinine levels when compared to single treated T2-DM groups (40.47 ± 4.01 and 0.52 ± 0.05 mg/dL), respectively when compared to T2-DM rats (Fig. 2).

Effects of the treatment with Met or/and saponin on the oxidative stress biomarkers in the different groups

The hepatic MDA level was significantly increased due to T2-DM induction in rats (79.77 ± 3.87 nmol/mg protein) ($P < 0.05$). The level of MDA in the T2-DM rats decreased after the treatment with Met or Sap. Moreover, T2-DM rats receiving combination of Met/Sap showed a synergistic effect on the lipid peroxidation improvement and reduction of MDA level (50.56 ± 3.44 nmol/mg protein) when compared to single treatments (Table 3). The results showed that there was decrease in the activities of antioxidant enzymes (SOD and CAT) in the T2-DM rats (5.85 ± 0.47 and 55.18 ± 3.48 U/mg protein, respectively) when compared to the normal (Table 3). Moreover, the treatment of T2-DM rats with Met or/and Sap led to significant increase in their SOD and CAT levels ($P < 0.05$) (Table 3).

Table 2. Serum ALT, AST, and total proteins levels in the different groups

Groups	ALT (U/L)	AST (U/L)	Total protein (g/dL)
NC.	36.24 ± 2.32 ^{b}	187.21 ± 7.41 ^{b,c}	8.78 ± 0.19 ^{a,b,c}
D.	56.72 ± 2.79 ^{a}	227.14 ± 8.93 ^{a}	8.11 ± 0.15 ^{a}
D./Met.	44.65 ± 3.41 ^{a,b}	208.23 ± 10.44 ^{b,c}	8.38 ± 0.28 ^{c}
D./Sap.	42.52 ± 2.21 ^{a,b}	201.75 ± 9.65 ^{b,c}	8.42 ± 0.20 ^{b,c}
D./Met. /Sap.	39.60 ± 1.96 ^{b}	195.15 ± 8.76 ^{b,c}	8.49 ± 0.21 ^{b,c}

The values represented mean ± SD. N.C.: Normal: control group; D.: T2-DM rats; Met: Metformin; Sap: Saponin; N: Naïve; D: Diabetic; ALT: Alanine transaminase; AST: Aspartate transaminase. *P*-value < 0.05 was statistically significant

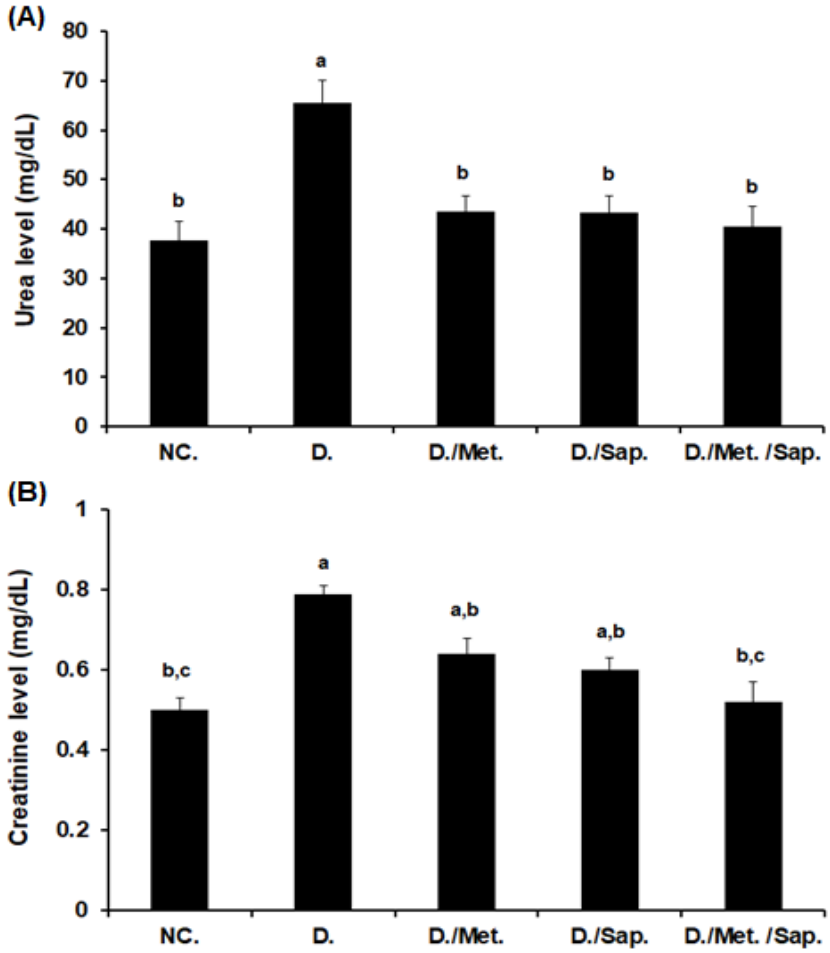

Fig. 2. Serum urea **(A)** and creatinine **(B)** levels in the different groups under the study. The values represented mean ± SD. N.C.: Normal control group; D.: T2-DM rats; Met: Metformin; Sap: Saponin; N: Naïve; D: Diabetic. *P*-value < 0.05 was statistically significant.

Table 3. Hepatic MDA, SOD, and CAT levels in the different groups.

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
NC.	41.76 ± 3.12 ^{d}	14.92 ± 1.04 ^{a,b}	90.69 ± 3.76 ^{b}
D.	79.77 ± 3.87 ^{a}	5.85 ± 0.47 ^{d}	55.18 ± 3.48 ^{e}
D./Met.	60.17 ± 2.91 ^{b}	8.86 ± 0.63 ^{c,d}	64.85 ± 3.74 ^{d}
D./Sap.	55.34 ± 2.78 ^{b,d}	9.88 ± 0.75 ^{b,c}	70.37 ± 3.15 ^{c,d}
D./Met. /Sap.	50.56 ± 3.44 ^{c,d}	11.79 ± 0.94 ^{b,c}	79.44 ± 3.90 ^{b,c}

The values represented mean ± SD. N.C.: Normal control group; D.: T2-DM rats; Met: Metformin; Sap: Saponin; MDA: Malondialdehyde; SOD: superoxide dismutase; CAT: Catalase. *P*-value < 0.05 was statistically significant.

4. Discussion

Diabetes mellitus (DM) has emerged as one of the most common chronic diseases causing life threatening (Tonioloa *et al*., 2019). DM disease is characterized by raised blood glucose levels and considered one of the top ten leading causes of death worldwide (Li *et al*., 2021). The current study was conducted to address the effects of the co-treatment with Sap and Met on the biochemical status of T2-DM rats. Unintentional weight loss is referred to as unexplained weight loss, and it can be an

indication that you may have diabetes. In diabetics, a lack of insulin hinders the body from delivering blood glucose to the body's cells for use as fuel. The body begins utilizing fat and muscle for energy, which results in a loss of total body weight. The results of the current investigation revealed that the percentage of the total body weight change in T2-DM rats was significantly decreased. Despite diabetic rats receiving metformin or/and saponin treatment, their percentage of total body weight increased. Rahmani et al. (2023), who reported that diabetes control rats displayed a drop in body weight when compared to non-diabetic rats, concurred with these findings.

The findings of the present study demonstrated that the glucose levels in the diabetes group were significantly higher than those in the normal control group. However, T2-DM rats receiving Met or/and Sap therapy have significantly lower glucose levels than T2-DM rats. These findings agreed with those of Al-Saud (2020), who found that the T2-DM rats' glucose level increase in comparison to the normal control group. In contrast to the T2-DM rats, the glucose level reduced during curcumin administration.

The results of C-peptide analysis can be used to evaluate, monitor, and/or treat diseases like hypoglycemia and diabetes that are related to how well your body produces insulin. A marker of endogenous insulin production is serum C-peptide (Yang and Kang, 2018). The study reported that the C- peptide level in the T2-DM group was significantly lower than that of the normal control group. In addition, the T2-DM rats receiving Met or/and Sap have significantly higher C-peptide levels than the T2-DM rats. These results were in consistence with a previous study by Yang and Kang (2018), who found that the diabetic rats treated with natural constitutes showed an increase in C-peptide levels.

The metabolism of carbohydrates and the regulation of blood glucose levels are both significantly influenced by the liver. Due to conditions like insulin resistance, glucose intolerance, and diabetes, the metabolic balance of glucose is compromised in the presence of hepatic illness. Elevated liver enzyme (ALT and AST) activities (Han *et al*., 2016).

This study showed that there was significant increase in the serum level of ALT and AST in T2-DM rats, however, treatment of T2-DM rats with Met or/and Sap led to significant improvement in the ALT and AST activities. A previous study of Zhu (2021) reported that the serum ALT and AST activities were increased in the diabetic rat model, and co-treatment with Met and natural products led to significant decrease in ALT and AST activities.

The findings of the current investigation indicated that there was no significant difference between the total protein levels in the T2-DM rats and the normal control group. However, there has been a significant increase in the T2-DM rats that had Met or/and Sap treatment. These results are similar to those reported by Sunmonu and Afolayan, (2013), who reported a decrease in the total protein content in T2-DM rats. However, giving diabetic rats an aqueous extract of *Artemisia* significantly decreased the amount of proteolysis brought on by the insulin shortage, raising the level of plasma proteins to close to normal.

Progressive nephron function will gradually decline at the stage of chronic kidney disease brought on by diabetes complications, which is indicated by high serum urea and creatinine levels. The study showed that T2-DM rats had a significant increase in urea and creatinine levels when compared to the normal control. Although the T2-DM rats' urea and creatinine levels significantly decreased after receiving therapy with Met or/and Sap, Similar findings were presented by Hussein et al. (2018), who showed that treatment of lycopene to diabetic rats dramatically decreased their increased serum levels of urea and creatinine compared to the normal control group of rats with diabetic nephropathy.

Oxidative stress is one of the causes of T2-DM development, which is caused by an imbalance between the cellular antioxidant system and ROS generation under hyperglycemic circumstances (Bhatti *et al*., 2022). Our findings showed that MDA levels in the T2-DM rats were significantly increased than those in

the normal control group. T2-DM rats that received Met or/and Sap treatment had significantly decreased MDA levels. The findings showed that SOD and CAT activities were significantly lower in the T2-DM rats than in the normal control group. However, after receiving T2-DM rats Met or/and Sap therapy, SOD and CAT activities were enhanced. These findings were in accordance with Sadri *et al*. (2017) who reported that T2-DM rats had significantly increased MDA levels than normal control rats. Despite, there was a significant reduction in the activities of SOD and CAT in diabetic rats was reported. However, treatment with natural products increased the activities of enzymatic antioxidants.

5. Conclusion

Co-treatment of T2-DM rats with Met and Sap led to significant improvements in the hepato-renal functions and antioxidant status. Sap could play an important role in T2-DM management via improving biochemical alterations and can be developed as a promising natural agent for T2-DM control.

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