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Abstract

The current study is designed to investigate the in vivo action of ginger- thyme combined crude extract on hyperglycemia and obesity related measurements in sucrose induced chronic hyperglycemia experimental model in albino male rats. Twenty-five male albino rats were divided into five groups (N=5 for each); First group was regarded as control which consists of rats feed with normal diet and water ad libitum, while second group was the model which consists of rats fed with high sucrose fed (HSF) (50%sucrose). and other three groups were rats which treated with three different doses of ginger-thyme combination extract (125mg/kg, 250 mg/kg and 500 mg/kg). Animals were received one dose/day using oral stomach gavage for eight weeks. Blood samples were collected for quantitative determination of glucose levels, Oral Glucose Tolerance Tests (OGTTs), serum insulin, Glycohaemoglobin (HbA1c) and various other laboratory analyses such as lipid profiles, in addition to water intake and body weight throughout the study. Significant reduction of blood glucose levels were occurred in all plant combination extract doses compared to Sucrose diet rat model group, especially the medium dose 250mg/kg which found to be the best one. Serum insulin was reduced in the model rats compared to control and treatment groups, while it was increased in all extract treating groups compared to the model group.

Keywords

Hyperglycemia, Ginger, thyme. Rats

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RESEARCH ARTICLE

Hypoglycemic and Hypolipidemic Potential of Ginger-Thyme Combined Extract in Sucrose-Induced Hyperglycemic Rates

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ABSTRACT

The current study was designed to investigate the *in vivo* action of ginger-thyme combined crude extract on hyperglycemia and obesity-related measurements in sucrose-induced chronic hyperglycemia experimental model in albino male rats. Twenty-five male albino rats were divided into five groups (n = 5 for each); the first group was regarded as control, while the second group was the model which consists of high sucrose fed (50% sucrose in water) and other three groups were rats which treated with three different doses of ginger-thyme combination extract (125 mg/kg, 250 mg/kg, and 500 mg/kg). Animals were received one dose/day using oral stomach gavage for 8 weeks. Blood samples were collected for quantitative determination of glucose levels, oral glucose tolerance tests, serum insulin, glycohemoglobin (hemoglobin A1c), and various other laboratory analyses such as lipid profiles, in addition to water intake and body weight throughout the study. Significant reduction of blood glucose levels was occurred in all plant combination extract doses compared to sucrose diet rat model group, especially the medium dose 250 mg/kg which found to be the best one. Serum insulin was reduced in the model group compared to control and treatment groups, while it was increased in all extract treating groups compared to the model group.

Keywords: Hyperglycemia; Ginger; Thyme; Rats

INTRODUCTION

Diabetes mellitus (DM) is one of the ancient and chronic illnesses. It is a metabolic disorder, usually characterized by hyperglycemia (Gharravi et al., 2018). Pancreatic beta-cells (β-cells) produce the insulin hormone which facilitates the glucose entering into the cells to provide energy for a variety functions (Gharravi et al., 2018). Hence, the main conditions for presenting this metabolic disease are insulin secretion or insulin action (Viswanath et al., 2017). Insulin treatment, drugs with antidiabetic properties, suitable diet, and exercise are the main methodology in the medication of this disease. Although, existing medical treatments have toxic side effects, and sometimes, prolonged use results in reduced response (Hollander, 2007). A study, that investigated the Medical Expenditure Panel Survey, found that hyperglycemic individuals were 1.6 times extra probable to use complementary alternative medicine in the comparison of overall individuals (Egede et al., 2002). Plants and their medicinal functions have been recognized for millennia (Chatterji and Fogel, 2018).

Ginger has a long history of use as herbal medicine in the treatment of the many indicators containing vomiting, pain, and cold symptoms, and it has been shown to have anti-inflammatory, antidiabetic, anticlotting, and analgesic properties (Rahmani, 2014). It has a wide history of the treatment as an herbal medication for a lot of diseases comprising vomiting, pain, indigestion, and cold-induced syndromes. In 2012, it was described to have anti-cancer, anticlotting, anti-inflammatory, and analgesic activities (Li et al., 2012). Latest studies have presented that ginger itself and its diverse compounds have anti-inflammatory, antimicrobial, and antioxidant potency, also immune modulatory and cytoprotective/regenerative actions on unsymmetrical organ systems with a very minor degree of poisonousness (Cakir et al., 2018).

Thyme is used by traditional clinicians and doctors, as an antitussive, expectorant, antimicrobial, and antispasmodic causative substance agent (Basch et al., 2004). As well, thyme owns antimicrobial, antifungal, antioxidative, and antiviral activities (Soliman and Badeaa, 2002). Thymus shows many biological actions containing antioxidant and free radical scavenging properties (Hanna et al., 2014). Patel et al., found that a combinations of several medicinal plants reveal many mechanisms of action such as acting

directly on the pancreas and stimulating insulin levels in blood or changing the activities of regulatory enzymes (Patel et al., 2012).

In the past years, several animal and human studies have discovered the significance of ginger intake on metabolic status, hyperglycemia, blood lipids, and blood pressure. Most of these studies suggested that ginger can improve a number of parameters of lipid profile, especially in diabetic patients (Arablou et al., 2014).

The aim of this study is to determine the antidiabetic and antihyperlipidemic potential of the ginger-thyme combination plants in equal amount for the treatment of hyperglycemia-induced male rat models.

MATERIALS AND METHODS

Plant Extract Preparation

Fresh drying fine powder of ginger and thyme leaves were prepared by grinding dried leaves (purchased from an herbal store in the Mosul-Iraq) using blender. The powders were stored separately at dry place until extract preparation.

The aqueous plant extract was prepared by modified description of Cakir et al., 2018. The raw extract was prepared by dissolving 20 g of powder for each plant material within 150 ml of hot distilled water for 2 days at room temperature with shaking at 150 rpm to prepare 500 mg/ml as a stock solution, the stoke solution was diluted to make solutions of 250 mg/ml and 125 mg/ml. The extracts were purified by filtration through filter paper. The mixture was filtered; the crude extract was stored in a refrigerator until use. The concentration was considered to have 500, 250, and 125 mg/ml based on the weight of the starting material.

Animals and Study Design

Twenty-five healthy adult male Wistar albino rats aged about 12 weeks old, weighing approximate body weight (BW) 200 g were used. The rats were obtained from the Animal House of College of Education – Salahaddin University (Erbil, Iraq) and housed in Animal House of College of Science – Salahaddin University (Erbil, Iraq). All animals were kept under an environmental condition, adapted for 3 weeks before experimentation. The rats were housed at normal atmospheric temperature (23 ± 2°C) and relative humidity of 50–60% on a 12 h light/dark cycle with good ventilation. All the animals received a supply of water and a standard diet.

The rats were randomly divided into five groups as below: First group: Control group which consists of rats feed with normal diet and water *ad libitum*.

Second group: Considered as the model, in this group, the rats were fed with high sucrose fed (HSF) (50% sucrose).

The other three groups were fed with high sucrose diet and treated with 125, 250, and 500 mg/kg BW of ginger-thyme combination in equal amounts. All of the animals were received 1 dose/day using oral stomach gavage for 8 weeks. Water intake was calculated for each group and recorded weekly.

Laboratory Estimations

Rat BWs were calculated for each group and recorded before study initiation, middle, and in the end of the study thereafter and the changes were measured.

The blood glucose levels were determined by the glucose monitoring meter and test strips using a blood glucose monitoring system (ACCU-CHEK Glucometer, Roche Diabetes Care, Germany) (Florence et al., 2007). That blood glucose concentration of each rat was measured; the tail vein was pricked with a sterilized needle to measure levels at 0 times as a fasting state and recorded, then oral glucose tolerance tests were performed by glucose powder loading (2 g/kg of BW) to each rat by gavage method. After glucose loading, blood samples were taken from the tail vein at 30, 60, 90, and 120 min.

All rats were sacrificed under xylazine (40 mg)/ketamine (10 mg) ampules anesthesia, whole blood was taken from each group by cardiac puncture of all animals. An amount of blood was collected in EDTA tubes to assay hemoglobin A1c (HbA1c). The remaining blood samples were collected in clean vacuum tubes allowed to clot, and the serum separated to determine the serum insulin and lipid profile.

RESULTS AND DISCUSSION

The results of the study showed very high significant differences (P = ***) among the treatments regarding blood glucose levels. Hyperglycemia animals (model group) showed a very high significant (P = ***) comparing to the control rats. During fasting, the sucrose administration led to increasing of glucose (106 ± 5.228) comparing to control rats (68.2 ± 3.215). Hyperglycemia animals showed high level of glucose in 30 min (190 ± 4.847) as compared to normal control animals (117.8 ± 7.519). Furthermore, in 60 min, blood glucose levels showed increase in the model rats (156.6 ± 6.712) when compared with the control group (116.4 ± 8.686), while in 90 min, the glucose levels in the hyperglycemia rats indicated (150.2 ± 4.509) comparing to the control group (101.4 ± 5.249). In addition at 120 min, blood glucose levels showed in the model rats showed 140.8 ± 2.634 when compared with the control group (95.8 ± 6.537).

Treatment of HSF rats with all doses of the combined plant extracts of (ginger-thyme) showed a very high significant reduce (P = ***) in the comparison with model rats. All doses of extracts are led to restoring of glucose to be non-significant with control rats but the best dose that restored the level of glucose non-significant to the control group is 250 mg/kg which is the medium dose. During fasting, the 250 mg/kg of extract led to declining of glucose levels (75.2 ± 4.381) comparing to model rats (106 ± 5.228). After 30 min, it also showed decrease of glucose level (119.4 ± 12.637) as compared to model animals (190 ± 4.847). In 60 min, blood glucose levels showed 110.2 ± 1.568 in the comparison with model group (156.6 ± 6.712). Furthermore at 90 min, the glucose levels in the 250 mg/kg are 103.4 ± 4.779 comparing to the model group (150.2 ± 4.509). In addition in 2 h, blood glucose levels in the same dose showed 98 ± 4.433 when compared with the model group (140.8 ± 2.634), as shown in Figure 1.

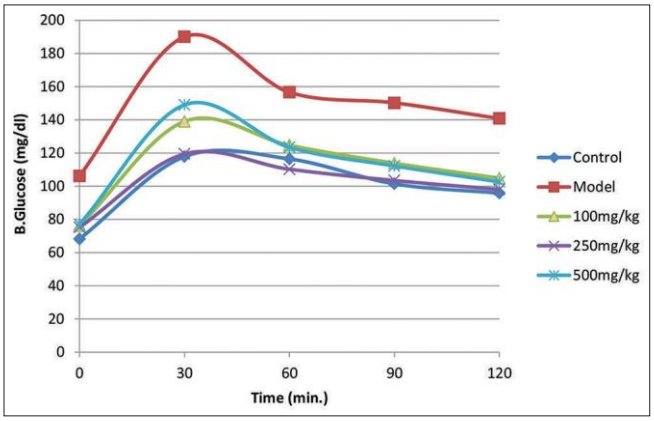

Figure 1: Oral glucose tolerance test curve in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract (*P* value * = very high significant)**

The current results demonstrated very high significant differences (P = ***) among the treatments regarding insulin levels.

Hyperglycemia animals showed a very high significant comparing to the control rats (P = ***). The sucrose administration led to declining of insulin significantly (5.240 ± 0.199) comparing to control rats (9.580 ± 0.159). Treatment of HSF rats with all doses showed a very high significant reduce in the comparison with model rats (P = ***). All doses of extracts are led to restoring of glucose to be non-significant with control rats and the best dose of extracts was 250 mg/kg as it led to restoring of insulin (8.760 ± 0.283) to be non-significant with control rats (9.580 ± 0.159), as shown in Figure 2.

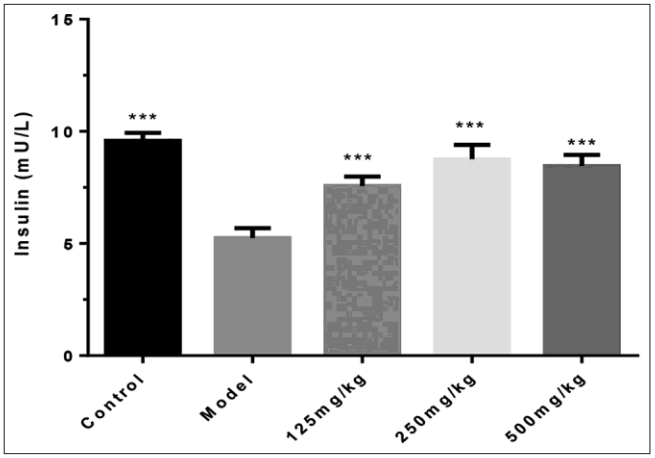

Figure 2: Serum insulin level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract. (*P* value * = very high significant)**

The weight gain in all groups exhibited a very high significant improvement differences (P = ***) in all groups that increased in control, model and all dosages except 250 mg/kg and the apparent digestibility of this group showed a very high significant decrease compared to the BW in model group (P = ***).

The highest excess BW was seen in the model group that is 405 mg by the ratio 53.8% but the lowest increase in BW is in the 125 mg/kg that is 100 mg by the ratio 8.19%. In contrast, diabetic rats treated with 500 mg/kg the excess 395 by the ratio 40.3% and control group is excess 405 mg by the ratio 38.2% in opposite there are decrease in weight is shown in 250 mg/kg extract group by 175 mg in the ratio 15.6%. Figure 3 displays the BW in all groups.

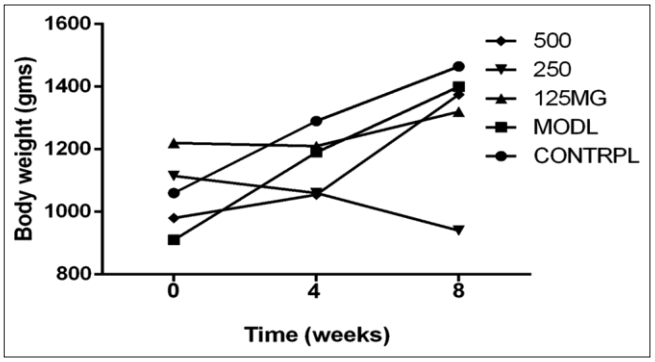

Figure 3: Body weight level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract. (*P* value * = very high significant)**

Water intake was measured every week during the experiment for all groups and showed a very high significant improvement differences (P = ***) between all groups.

The water intake was approximately fixed in the control group with very limited deviation (23 ± 2 ml), while a clear polydipsia was appeared in HSF rats with high water intake about 40 ml. The administration of plant extracts exerts its therapeutic action through reduction of water intake,

however, the highest dose leads to decreasing water intake below the normal range (about 16 ml as average), as shown in Figure 4.

The data analysis showed a significant improvement differences (*P* value = *) among the treatments regarding cholesterol level. Hyperglycemia rats showed non-significant (53.00 ± 6.419) comparing to control rats (51.60 ± 6.266), while the treatment of rats with all doses of combined plant extracts is non-significant compared with the model rats, as depicted in Figure 5.

The antihyperglycemic effect of the combine plants was confirmed by the results of triglyceride level by significant differences among the treatments (*P* value = *). Diabetic rats exhibited a non-significant (49.20 ± 6.851) comparing to control rats (39.80 ± 3.917), while the treatment of rats with 500 mg/kg dose of combined plant extracts is non-significant (34.75 ± 1.933) compared to model but the doses 125 mg/kg (31.40 ± 3.641) and 250 mg/kg (29.60 ± 4.007) are significant (*P* = *) with the comparison with model rats (49.20 ± 6.851), as represented in Figure 6.

The current results demonstrated significant differences (*P* = *) among the treatments regarding high-density lipoprotein (HDL) level. The sucrose administration of HDL level is non-significant (16.29 ± 2.476) comparing to control rats (16.22 ± 2.735), while the treatment of rats with all doses of combined plant extracts is non-significant increased compared to the model group, as shown in Figure 7.

The current results demonstrated high significant differences (*P* = **) among the treatments regarding low-density lipoprotein (LDL) level. The sucrose administration of cholesterol is non-significant (26.80 ± 2.922) comparing to control rats (27.60 ± 3.059). The doses of 500 mg/kg and 125 mg/kg (39.25 ± 2.332 and 39.00 ± 1.924), respectively, are significant (*P* = *) with model (26.80 ± 2.922), as shown in Figure 8.

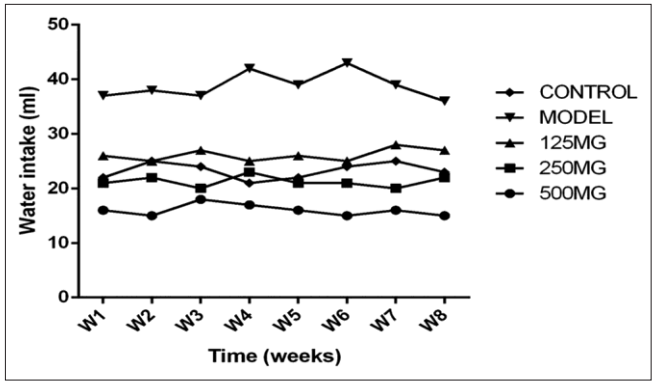

Figure 4: Water intake level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract. (*P* value * = very high significant)**

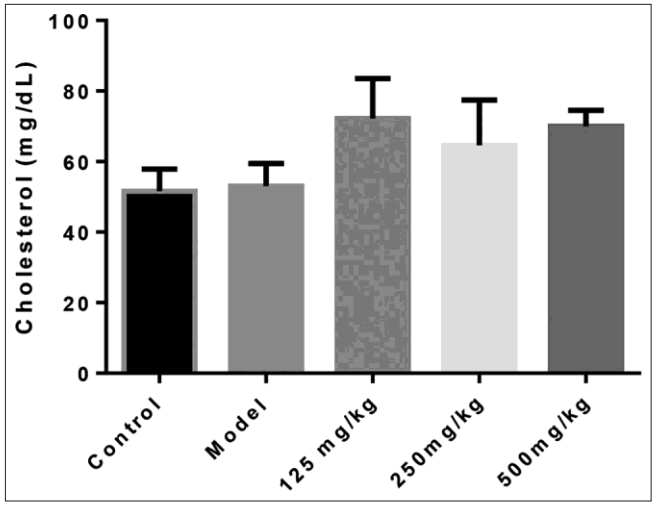

Figure 5: Serum cholesterol level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract

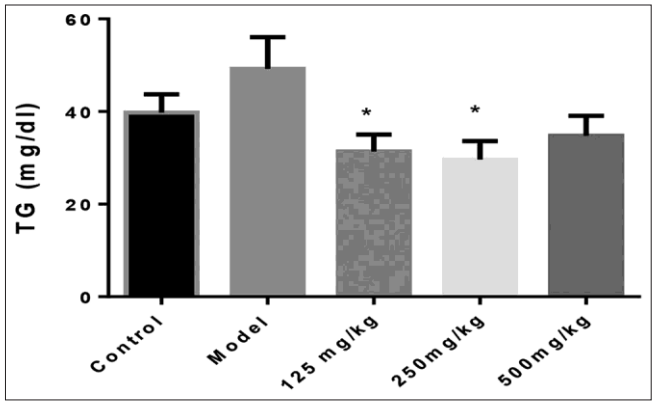

Figure 6: Serum triglyceride level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract. (*P* value * = significant)

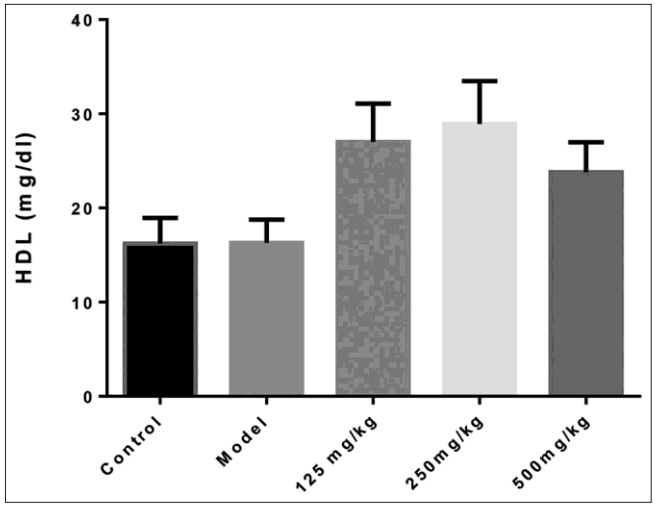

Figure 7: Serum high-density lipoprotein level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract

The current results demonstrated significant differences (P = *) among the treatments regarding HbA1c% level. The sucrose administration of HbA1c level is non-significant (3.460 ± 0.02449) comparing to control rats (3.600 ± 0.03162), while the treatment of rats with all doses of combined plant extracts i also non-significantly compared to model, as shown in Figure 9.

DISCUSSION

Diabetes has become one of the largest global health and economic problems, with its increased prevalence and high complication ratio. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines, for the prevention and administration of diabetes and related complications (Bi et al., 2017).

The present study is directed to evaluate the hypoglycemic and hypolipidemic properties of thyme-ginger combine extract on HSF rats. In addition, unpublished, preliminary screening data, of these combine plants, showed a very highly

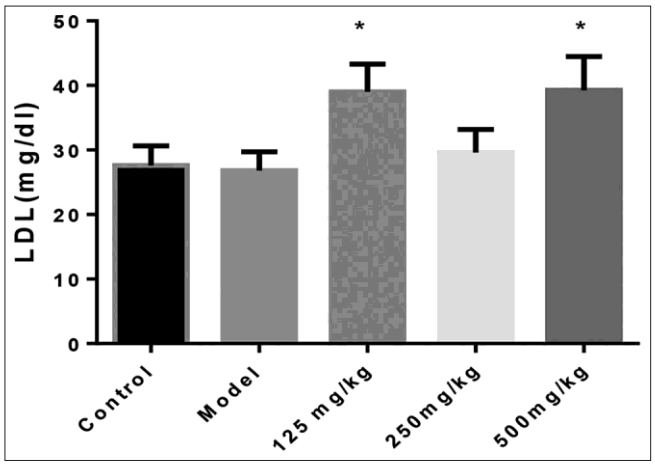

Figure 8: Serum low-density lipoprotein level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract. (*P* value * = significant)

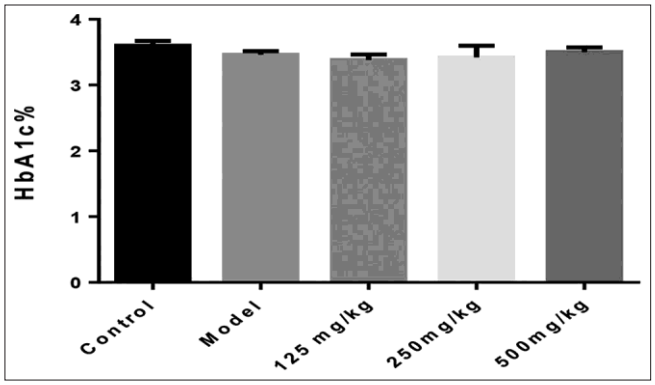

Figure 9: Hemoglobin A1c % level in hyperglycemic rats treated with various doses (125, 250, and 500 mg/kg. BW) of ginger-thyme combined extract

significant improvement in glucose levels and serum insulin, so it was preferred to use this plants combine for diabetics.

The current study showed reduction in serum glucose and restoring the impaired glucose tolerance in all doses compared to model rats, especially the 250 mg/kg group rats that are restore control and this is agreed with the result of several studies have been reported the hypoglycemic effect of different forms of ginger in both animals and human subjects. Among the fairly recent reports are: Arablou et al. used ginger powder in Type 2 diabetic patients (Arablou et al., 2014); Son et al. used 6-gingerol isolated from ginger in obese diabetic mice (Son et al., 2015); Abdulrazaq et al. used aqueous ginger extract STZ-induced diabetic rats (Abdulrazaq et al., 2012); while Jafri et al. used aqueous extract in alloxan-induced diabetic rats (Jafri et al., 2011). Because hypoglycemic effect of ginger may be attributed to the bioactive and pharmacological compounds that they may help in suppressing the free radical in diabetes, this will ultimately lead to decreased levels of blood glucose (Shanmugam et al., 2011).

The fact that ginger-thymes combination can reduce hyperglycemia and normalize glucose tolerance in rats feeding high sucrose diet could have implications for the prevention of type 2 diabetes mellitus (T2DM).

Furthermore, our results revealed a very highly significant improvement in insulin levels in all dose treatments when compared with untreated HSF group and this consequence agree with the result of the previous study has been reported by Chakraborty et al. who revealed that ginger has been shown to modulate insulin release in rat pancreatic β-cells, thus enhanced serum insulin levels in conjunction with lowered blood glucose, this may be due to 6-gingerol, which is active component in ginger, which showed a protective effect on pancreatic β-cells and restored the plasma insulin level (Chakraborty et al., 2012).

An improvement was reported in combine plant extracts concerning to lipid profiles (total cholesterol, triglyceride, LDL cholesterol [LDL-C], and HDL cholesterol [HDL-C]) because the current results in the present study demonstrated significant differences among the results regarding to lipid profiles and sucrose administration of lipid profile levels is non-significant comparing to control rats. Our results are disagree with another study done by Zar et al. that they found that LDL-C, triglycerides, and total cholesterol were significantly reduced in comparison with model group, but HDL-C was significantly increased in diabetic rat treatment by the administration of ginger (Zar et al., 2016).

The combined plant extract reduced water intake and decreased the weight gain. Our finding showed significant

results in the administration of ginger-thyme combine that BW significantly increased in control, model, and in small ratio in 125 mg/kg and 500 mg/kg doses except 250 mg/kg that's significantly decreased in comparison with model and control groups. The apparent digestibility of 250 mg/kg group showed significantly decreased compared to that in all groups and this result agrees with the result of the previous study has been reported by Khattab et al. when diabetic rats treated with ginger showed significantly improvement and ameliorated reduction in final BW, when compared with untreated HSF rats (Khattab et al., 2013). That's leading to a very highly significant improvement also in water intake that's approximately fixed in control group with very limited deviation (23 ± 2 ml), while a clear polydipsia was appeared in HSF rats with high water intake about 40 ml. The administration of plant extracts exerts its therapeutic action through the reduction of water intake, however, dose leads to decreasing water intake below the normal range.

Moreover, our finding showed that significant differences among HbA1c levels in all groups but these results are disagree with the result of the previous study have been reported by Islam and Choi, whose their clinical finding showed decreasing in HbA1c levels using ginger for the management of T2DM in rats (Islam and Choi, 2008).

CONCLUSION

According to the results of the present study, it was preferred to use ginger and thyme in combination for improving of diabetics patients, because it caused a gradual reduction in serum glucose in all doses compared to model rats, especially the 250 mg/kg group rats which restored them to be similar to control rats.

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