Sumer Journal for Pure Science (ISSN: 2790-7031)
2nd International Scientific Conference on Pure and Medical Sciences/University of Sumer 2024

Impact of Natural Antioxidant on Liver Function

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Abstract

Objective: This study was designed to investigate the antidiabetic activity of natural antioxidants (kaempferol, apigenin, and alpha-alanine) on liver enzymes in experimentally diabetic rats (Rattus norvegicus).Methods: The rats were divided into five groups (each group contains six male rats); the first group (negative control) injected intraperitoneally with 0.1 ml of normal saline; the second group (positive control) injected intraperitoneally with 100 mg/kg of alloxan, for once only to induce diabetes, after infecting the other groups with D.M. treated daily for 28 days as: the third group treated with 25 mg/kg Keampferol (KF), the fourth group treated with 25 mg/kg Apignine (AP), and the fifth group treated with 25 mg/kg al-alanine (AA).Results: The results indicate that the treatment of experimentally diabetic rats with natural antioxidants caused a significant increase in TG concentration in the third and fourth groups compared with the first and second groups, a significant decrease in the fifth group compared to other groups, and a significant decrease in LDL, HDL, and TC in the third, fourth, and fifth groups compared with the first and second groups. Also, the results showed a significant decrease in ALT and AST in the third, fourth, and fifth groups and in ALP (except for the fourth group) compared with the second group. The results revealed a significant increase in TBIL in the third and fourth groups and a significant decrease in the fifth group compared with the second group, as well as a significant decrease in ALB in the third, fourth, and fifth groups compared with the second group. Conclusion: The present study indicates that there are positive effects of kaempferol, apigenin, and alpha-alanine on the liver function of experimentally diabetic rats.

Keywords: kaempferol,; apigenin , al alanine ,diabetic

تأثير مضادات الاكسدة الطبيعية على وظائف الكبد

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الخلاصة

الهدف: هدفت الدراسة الى بحث تأثير مضادات الاكسدة الطبيعية (الكمبيفيرول، الابيجينين ، الالانين) على وظائف الكبد ونسيجه في الجرذان المختبرية المصابة بالسكري تجريبيا.طريقة العمل: قسمت الجرذان المختبرية الى خمسة مجموعات (كل مجموعة تتكون من ستة جرذان ذكور) ، المجموعة الأولى هي مجموعة السيطرة السلبية ، حقنت تحت الغشاء البريتوني بـ 0.1 مل من المحلول الفسيولوجي اما المجموعة الثانية فهي مجموعة السيطرة الإيجابية أصيبت بالسكري من خلال حقنها تحت الغشاء البريتوني بـ 100ملغم/كغم من وزن الجسم بالالوكسان. بعد إصابة باقي المجاميع بالسكري عولجت لمدة 28 يوم : المجموعة الثالثة عولجت بـ 25ملغم /كغم من وزن الجسم من الكمبيفيرول ، المجموعة الرابعة عولجت بـ 25 ملغم /وزن الجسم من الابيجينين اما المجموعة الخامسة فقد عولجت بـ 25 ملغم /كغم من الالانين, أيضا أوضحت النتائج انخفاضا معنويا في انزيمات الكبد ALT, AST في المجاميع الثالثة والرابعة والخامسة وكذلك في مستوى ALP (باستثناء المجموعة الرابعة) مقارنة مع المجموعة الثانية . النتائج: اشارت النتائج الى ان معالجة الجرذان المصابة بالسكري بمضادات الاكسدة سبب ارتفاعا معنويا في تركيز الدهون الثلاثية في المجموعتين الثالثة والرابعة مقارنة مع المجموعة الأولى والمجموعة الثانية وانخفاضا معنويا في المجموعة الخامسة مقارنة مع باقي المجاميع وانخفاضا معنويا في تركيز البروتين الدهني منخفض الكثافة والبروتين الدهني عالي الكثافةوالكولسترول في المجاميع الثالثة والرابعة والخامسة مقارنة مع المجموعتين الأولى والثانية. اشارت النتائج الى ارتفاع معنوي تركيز البيليروبين في المجموعتين الثالثة والرابعة وانخفاضا معنويا في المجموعة الخامسة مقارنة مع المجموعة الثانية كذلك انخفاضا معنويا في تركيز الالبومين في المجاميع الثالثة والرابعة والخامسة مقارنة مع المجموعة الثانية. اشارت نتائج الدراسة الحالية الى تاثيرات إيجابية للمعالجة بمضادات الاكسدة الطبيعية على وظائف الكبد.

Introduction

Diabetes is one of the most tired and expensive chronic diseases, caused by inherited or acquired deficiency in the production of insulin by the pancreas (type 1) or by the ineffectiveness of insulin (type 2) [1]. Oxidative stress plays an effective role in developing diabetes and its complications, such as atherosclerosis and heart disease [2]. Different physiological functions in the body stop these oxidation reactions and protect the body from harmful chain reactions [3].

Kaempferol (KF) is a natural flavonoid in many plants and foods derived from plants like Aloe vera, Coccinia grandis, Moringa, tea, and tomatoes. It has anti-oxidative stress, hypoglycemic, and hypolipidemic effects [4], regulates lipid metabolism, and improves lipotoxicity. Also, it improves insulin signaling, restores the balance between glucose utilization and production, and restores the imbalance in apoptosis to protect B cells. The antidiabetic mechanism of kaempferol is to comprehensively prevent the progression [5].
Apigenin is abundant in medicinal plants, fruits, and vegetables. This compound has an antioxidant effect and decreases inflammation and diabetes complications [6]. It is an anti-diabetic agent as it can suppress the activity of α-glycosidase, stimulate insulin secretion, and manage reactive oxygen species, which can manage diabetic complications [7].

Branched-chain amino acids (BCAAs), namely valine, leucine, and alanine, are essential amino acids that make up muscle proteins. Many studies have shown that insulin resistance is key to the underlying mechanism linked with increased circulating concentrations of BCAAs [8].

Objective: This study was designed to investigate the antidiabetic activity of natural antioxidants (kaempferol, apigenin, and alpha-alanine) on liver functions in experimentally diabetic rats (Rattus norvegicus).

1. Materials and Methods

2.1 Experimental induction of diabetes

The study was conducted on male white rats, the type of Norwegian rattus, with ages 13–16 weeks. Diabetes was induced by intraperitoneal injection of a single dose of alloxan monohydrate in normal saline water in a volume of about 3 mL at a dose level of 100 mg/kg body weight. After this process, the rats become diabetic.

2.2 experimental design

The rats were divided into five groups (each group contains six male rats); the first group injected intraperitoneally with 0.1 ml of normal saline; the second group (D.M.) was injected intraperitoneally with 100 mg/kg of alloxan. For once only to induce diabetes, after infecting the other groups with D.M. treated daily or for 28 days: The third group was treated with 25 mg/kg of kaempferol (KF), and the fourth group was treated with 25 mg/kg of apigenin (AP). The five groups were treated with 25 mg/kg of al-alanine (AA) daily for 28 days.

2.3 Statistical Analysis

The Statistical Analysis System (SAS) (2018) program was used to detect the effect of different factors on study parameters. The least significant difference (LSD) and Duncan multiple range test (Analysis of Variation) (ANOVA) were used to significantly compare the means in this study.

2. Result and Discussion

3.1 Physiological study

3.1.1 Effect of the natural antioxidant on the lipid concentration of diabetic rats.

The results of the current study showed a significant increase ($p \leq 0.01$) in the TG in all groups (G2, G3, G4, and G5) when compared with the G1 (negative control), a significant increase in the third and fourth groups, and a significant decrease in the fifth group when compared with the G2 (positive control). Also, the result revealed a significant increase in the G3 and G4 groups when compared with the fifth group.

On the other hand, there is a significant increase ($p \leq 0.01$) in the concentration of LDL in G2 (the positive control), a significant decrease in G3, G4, and G5 when compared with G1 (the negative control), a significant decrease in G3, G4, and G5 when compared with G2 (the positive control), and a significant increase in G3 and G5 when compared with the fourth group.

In the results of the present study about the HDL concentration, there appears to be a significant decrease ($p \leq 0.01$) in the G3, G4, and G5 groups when compared with the G1 (negative control) and G2 (positive control), and a significant decrease in the G3 and G5 groups when compared with the G4, and a significant decrease in the G3 group when compared with the fifth group.

Also, the results reveal a significant decrease ($p \leq 0.01$) in the TC concentration in the G3, G4, and G5 and a significant increase in the G2 (positive control) when compared with the G1 (negative control) and a significant decrease in the G3, G4, and G5 when compared with the second group, as well as a significant increase in the G4 compared with the third and fifth groups.

Table 1- Effect of the natural antioxidant on lipid concentration of diabetic Rats

Mean ± SE				
Lipid profile / Groups	**TG (mg/dl)**	**LDL (mg/dl)**	**HDL (mg/dl)**	**TC (mg/dl)**
G1 Neg. Cont. (0.1 N.S.)	49.70 ±0.51 d	45.52 ±0.33 b	61.92 ±1.19 a	83.12 ±1.42 b
G2 Pos. Con (DM -100 Mg/Kg)	106.27 ±0.49 b	54.47 ±0.33 a	70.70 ±0.30 a	96.22 ±0.31 a
G3 KF (25mg/Kg)	126.37 ±0.48 a	25.57 ±0.33 c	2.32 ±0.04 d	59.37 ±1.10 d
G4 AP (25mg/Kg)	126.73 ±1.72 a	24.13 ±0.86 d	28.33 ±1.38 b	71.13 ±0.73 c
G5 AAL (25mg/Kg)	98.06 ±0.65 c	26.55 ±0.31 c	14.23 ±0.20 c	61.50 ±1.67 d
LSD	**2.359 ****	**1.301 ****	**2.303 ****	**3.675 ****

the different of letters refer to different significance .** ($P \leq 0.01$).

The result of the present study indicates a significant increase in triglyceride concentrations (TG, LDL, and TC) in diabetic rats experiential groups when compared with the negative control. This result is in agreement with the results of the studies of Rahimi-Madiseh et al.(2017) and – Samuel et al.(2019) [9] and [10], which revealed that alloxan causes dyslipidemia (including increases in triglyceride, LDL, HDL, and cholesterol), which enhances the risk of acquiring cardiovascular disease [11]. The reason for the high concentration of LDL in diabetic rats is to increase oxidative stress due to free radicals, as well as to increase levels of lipid peroxidation that are stimulated by high glucose. As a result, this leads to damage or deficiency of LDL and VLDL receptors in the cell membrane, which increase in their concentration in the blood [12].

The present study showed a significant decrease in the concentration of both HDL, LDL, and TC and an increase in TG concentration. In the diabetic rats which treated with natural antioxidants Keampferol (G3), the diabetic rats which treated with apigenin (G4) and a significant decrease in therats which treated with amino acid (Al–alanine)(G5).The effect of kaempferol and apigenin on high triglycerides indicates a physiological condition in the liver called cholestasis, which leads as a result of the accumulation of fat in the liver and blood circulation to damage to liver cells, or cirrhosis. This result agrees with the results of the studies of [13] and [14]. While a decrease in the concentration of TC, LDL, and HDL in the diabetic rats treated with natural antioxidant kaempferol and treated with apigenine compared to the positive control group, this result agrees with the result of the study of [15], which revealed that Kaempferol reverses the abnormalities that occur in TG, LDL, and HDL concentrations that might be due to improvements in glucose control. It may also be that kaempferol helps to decrease the low-density cholesterol concentration due to its strong effectiveness as an antioxidant, and it improves lipoprotein synthesis and metabolism. may cause an increase in TG in the treated natural antioxidant groups due to an increase in the mobilization of free fatty acids from peripheral fat depots since insulin inhibits the hormone-sensitive lipase [16]. Regarding the effect of apigenin, this result agrees with the result of the study of [17], where he indicated that apigenin extract could improve the disturbance of the lipid profile of diabetic rats, hence the decreased risk of cardiovascular disease that can occur as a result of reducing blood glucose concentration. Also, this result agrees with the result of the study of [18], where they confirmed that apigenine has the ability to overcome dyslipidemia by decreasing cholesterol and free fatty acid ratios. It can decrease cholesterol by encouraging hepatic LDL bad cholesterol absorption and enhancing bile acid, and thus apigenin can mend disorders in the lipid profile [19]. About the effect of amino acid (ALalanin) on a decrease in fat concentration, this may be due to the effectiveness of the amino acid to repair TG, LDL, HDL, and TC receptors in the cell by lowering blood sugar, eliminating free radicals, and reducing oxidative stress.

3.1.2 Effect of natural antioxidant on Liver enzyme function of diabetic Rats .

The results of the present study explain a significant increase ($p \leq 0.01$) of the Alanine Transaminase (ALT) level in the G2 and G4 and a significant decrease in the G5 when compared with the G1 (negative control) and a significant decrease in the G3, G4, and G5 when compared with the G2 (positive control), as well as a significant decrease in the G3 and G5 when compared with the fourth group and a significant decrease in the G5 when compared with the third group. Also, the results show a significant increase in AST level ($p \leq 0.01$) in the G2, G3, and G4 when compared with the G1 (negative control) and a

significant decrease in the G3, G4, and G5 when compared with the G2 (positive control), a significant decrease in the G4 and G5 when compared with the G3, and a significant decrease in the G5 when compared with the fourth group. The results of the present study explain a significant increase ($p \leq 0.01$) in the level of ALP in G2, G3, G4, and G5 when compared with the G1 (negative control), a significant decrease in the G3 and increase in the G4 and G5 when compared with the G2 (positive control), a significant decrease in the G3 and G5 when compared with the fourth group, and a significant decrease in the G3 and G5 when compared with the fourth group.

Table 2- Effect of the natural antioxidant on Liver enzyme level of diabetic Rats

	Mean ± SE		
Liver enzyme / Groups	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**
G1	24.82 ±0.17 c	142.84 ±0.78 d	247.94 ±1.41 e
Neg. Cont. (0.1 N.S.)			
G2	67.85 ±1.26 a	279.40 ±1.32 a	330.23 ±0.32 c
Pos. Con (DM -100 Mg/Kg)			
G3	22.94 ±0.74 c	251.24 ±0.83 b	289.65 ±1.41d
KF (25mg/Kg)			
G4	37.10 ±1.31 b	210.02 ±7.07 c	672.32 ±12.72 a
AP (25mg/Kg)			
G5	12.65 ±0.21 d	137.13 ±0.57 d	393.03 ±7.22 b
AAL (25mg/Kg)			
LSD	2.678 **	9.852 **	19.92 **

the different of letters refer to different significance. ** ($P \leq 0.01$)..

The results of the present study in Table 2 show a significant increase in all liver enzyme levels in rats infected with diabetic rats (positive control) compared with healthy rats (negative control). This may be due to the alloxan effect on liver metabolism. This result agrees with the result of the study of [20], which states that the increase of liver enzymes is considered an indicator of the ability of some compounds to stimulate oxidative stress that affects the liver, and the release of large amounts of free radicals causes an effect on liver function. Liver injury, characterized by a loss of hepatocyte function in patients, results from mitochondrial oxidative stress, endoplasmic reticulum stress, inflammation, and autophagy in hepatocytes [21].

The results of the present study also indicated that there was a significant decrease in the levels of liver enzymes (AST, ALT, and ALP) in the blood serum of diabetic rats stimulated with alloxan and treated with the antioxidant kaempferol (G3) when compared with the

second group (diabetic rats). This result agrees with the result of the studies [22], which revealed that one of the signs of high oxidative stress and fat oxidation is the high levels of AST and ALT in response to cellular damage, and increased levels of these enzymes can lead to liver damage. As the researcher [23]confirmed, kaempferol works to reduce free radicals through its ability to prevent CYP2E1 from expression and activity, which thus leads to a decrease in liver enzyme concentration because kaempferol can inhibit apoptosis in hepatocytes by reducing apoptosis-related protein expressions [24].

Also, the results of this study show a significant decrease in liver enzyme levels (AST, ALT) and an increase in ALP in the serum blood of diabetic rats treated with the antioxidant apigenin (G4) when compared with G2 (the negative control). This result agrees with the result of the study of [5], which indicated that natural antioxidants, when used as a treatment for diabetics, are able to lower the liver enzyme concentration in patients due to their ability to reduce the amount of free radicals and oxidative stress in the body.

On the other hand, the results show that diabetic rats treated with the amino acid alanine had a significant decrease in liver enzyme levels when compared with the second group due to its high ability as an antioxidant to eliminate free radicals. Amino acids are involved in various cellular metabolisms, the synthesis of lipids and nucleotides, as well as detoxification reactions. In the liver, there are abundant non-essential amino acids, such as alanine, aspartate, glutamate, glycine, and serine, and essential amino acids, such as histidine and threonine. The role and effects of amino acids protecting the liver when attacked can be promising for the prevention and treatment of liver disease [25].

3.1.3 Effect of the natural antioxidant on the albumin and bilirubin concentrations of diabetic rats.

The results of the present study showed a significant increase ($p \leq 0.01$) in total bilirubin (TBIL) in the blood in the G2, G3, and G4 groups, a significant decrease in the G5 group when compared with the first group, and a significant increase in the G3 and G4 groups when compared with the fifth group. There is also a significant increase in albumin concentration in the G2 (positive control) group when compared with the G1, G3, G4, and G5 groups, and a significant decrease in the concentration of the G4 and G5 groups when compared with the first and third groups.

Table 3- Effect of the natural antioxidant on albumin and bilirubin concentration of diabetic rats.

Mean ± SE		
parameters / **Groups**	TBIL (mg/dl)	ALB (g/L)
G1	0.225±0.008 bc	36.77 ±0.27 b
Neg. Cont. (0.1 N.S.)		
G2	0.345 ±0.008	42.22 ±0.89
Pos. Con (DM -100 Mg/Kg)	b	a
G3	1.17 ±0.08 a	36.42 ±1.24 b
KF. (25mg/Kg)		
G4	1.36 ±0.13 a	30.95 ±0.29 c
AP. (25mg/Kg)		
G5	0.022 ±0.003 c	30.46 ±0.81 c
AAL. (25mg/Kg)		
LSD	0.209 **	2.398 **
the different of letters refer to different significance.		**** (P≤0.01)..**

The results of the current study showed an increase in the concentration of total bilirubin and albumin in the experimentally diabetic rats (G2). This result of the present study is similar to the result of [26], Oxidative stress plays a central role in diabetes-induced complications. Fibrosis, glycogen deposition, cirrhosis, and increased hepatic enzyme activities are some of the liver abnormalities associated with diabetes [27].

While the groups treated with natural antioxidants showed a significant increase in total bilirubin, except for the group treated with alpha-alanine, which showed a decrease in total bilirubin when compared with the healthy group (negative control), While all groups treated with natural antioxidants and amino acids showed a clear decrease in albumin concentration, this result agrees with the result of the study of [28], which indicates a role for antioxidants in plasma. The conversion of heme to bilirubin involves the fact that the bilirubin produced is sparingly soluble in water at physiological pH. Regarding the effect of alanine on total albumin, this result is consistent with [28].

4. Conclusion

The present study indicates that there are positive effects of kaempferol, apigenin, and alpha-alanine on the liver function of experimentally diabetic rats.

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