Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Effects of Betaine and Kaempferol on some Criteria Related to Glucose Homeostasis in Methionine over Load Rats -Part 2

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DOI: https://doi.org/ 10.31185/wjps.258

Received 12 October 2023; Accepted 25 November 2023; Available online 30 December 2023

Abstract: This study was designed to evaluate the role of Kaempferol (KPF) and Betaine (BET) in attenuating deleterious effects of methionine overload on some parameters related to diabetes in rats. Thirty (30) adult rats were randomly selected and divided equally into five experimental groups and treated for 45 days as the follows: G1:Rats in this group served as control, Rats in all other groups (G2,G3,G4 and G5 were received 100mg/kg B.W of D-L methionine in drinking water, and received orally as follows: Rats in group G3 were given 150 mg/kg B.W. of KPF, rats in group G4 were given 250 mg/kg B.W. BET, rats in group G5 were given 150 mg/kg B.W. of KPF , 250 mg/kg B.W. BET and 100 mg/kg B.W of D-L methionine in drinking water. Fasting blood sample were collected by cardiac puncture technique at the end of the experiments and serum were collected for estimation glycemic indices, C-peptide and B12 concentration. The results of the experiment that showed given D-L methionine for 45 days (G2 group) caused disturbance in glycemic indices as detected by elevation in serum glucose and decrease in serum insulin hormone concentration and decrease in serum C-peptide and B12 concentration. While oral intubation of KPF and BET or their combination caused correction of all recorded parameters. In conclusions from the results obtained and discussed in this study, we can conclude that exposure to methionine over load in water effects many criteria related to metabolic syndrome, especially those related to carbohydrate and lipid homeostasis as well as oxidative stress.

Key words: Kaempferol, Betaine, B12, C-peptide, DL-Methionine

1. INTRODUCTION

Homocysteine (Hcy) is a naturally occurring derivative of methionine, which plays an important role in numerous biochemical reactions throughout the body [1]. Chronically induced hypermethioninemia (methionine over load) was associated with Hyperhomocysteinemia (HHcy) caused severe organ damage such as renal [2], cardiac [3] and reproductive [4] dysfunction. Oxidative

stress plays an important role in the pathophysiology of HHcy and its complications [5,6]. A number of studies have shown that elevated plasma total-Hcy level is associated with Alzheimer's disease, atherogenesis, neurodegeneration and cancer [7]. Therefore, the plasma total-Hcy level has been used as a metabolic disorder parameter in clinic [8, 9, 10]. Clinical significance of serum Hcy as a biomarker for early diagnosis of diabetic nephropathy (DN) in type 2 diabetes mellitus (T2DM) [11]. HHcy also associated with chronic kidney disease [12]. BET, a methyl derivative of glycine, is abundant in many common foods, such as sugar beet, shellfish, spinach, and wheat [13,14,15]. BET supplementation to broiler chicken's diet improved their productive performance [16]. Additionally, as an amino acid, BET is a neutral zwitterion compound found in most organisms [17]. It possesses many important physiological functions such as methyl group donor and osmoprotectant. BET involved in one-carbon metabolism are essential for the methylation and synthesis of DNA [18]. As an osmotic agent, it regulates the cell volume and maintains cell membrane integrity under hyperosmotic pressure [19]. As a methyl donor, BET participates in the methionine-Homocysteine cycle, which affects DNA and RNA methylation [20]. Several studies have revealed that BET deficiency increases the severity of NAFLD [21,22]. Studies have focused on BET as a treatment due to its classification as a lipotrope, i.e., an agent that reduces or prevents the accumulation of fat in the liver [23], in addition to its hepatoproductive effect against acrylamide toxicity in rat [24]. KPF is a yellow-colored dietary flavonoid, present in numerous fruits and vegetables including apples, beans, broccoli, carrot, gooseberry, strawberries, saffron, tea and honey [25,26]. KPF, was the main flavonoids in Eruca sativa [27], Corn silk [28] and Aspleniun species cultivated in north of Iraq [29]. The pharmacological actions of KPF, including anti-inflammatory, cardiovascular, antioxidant, and anti-diabetic effects, have been reported previously [30,31,32,33,34,35]. This study aimed to investigate the effect of two natural antioxidants (BET and KPF) on some criteria related to glucose homeostasis in methionine over load rats.

2. MATERIALS AND METHODS:

Thirty (30) adult male rats were randomly selected and divided equally into five experimental groups and treated for 45 days as the follows: Group G1: Control group were received tap water, Group G2: Rats in this group were given 100mg/kg B.W of D-L methionine in tap water, Group G3: Rats in this group were given 150 mg/kg B.W. of KPF orally and 100 mg/kg B.W of D-L methionine in drinking water, Group G4 : Rats in this group were given 250 mg/kg B.W. BET orally and 100 mg/kg B.W of D-L methionine in drinking water, Group G5: Rats in this group were given 150 mg/kg B.W. of KPF, 250 mg/kg B.W. BET orally and 100 mg/kg B.W of D-L methionine in drinking water. Fasting blood (for 8-12 hrs) samples were collected at the end of the experiment (45day). Blood was drawn by cardiac puncture technique from anesthetized rats [intramuscular injection of Ketamine (90

mg/Kg B.W.) and xylazine (40 mg/kg B.W.) [35], using the disposable syringe. Then blood samples were kept in nonheparinized tubes and let for 10 minutes for standing, Serum were obtained by centrifugation for 15 minutes at 3000 rpm and kept tightly stopper tubes frozen at – 20°C for mastering the following: Serum glucose concentration, serum C-peptide concentration, serum Vitamin B12 concentration (Bio system, Spain) and Serum Insulin hormone concentration (DRG, Germany).

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant [35].

3. RESULT

The effect of oral administration KA or BET on serum glucose, insulin and c-peptide concentration were clarified in figure (1,2 and 3). The results showed a significant decrease in the serum glucose concentration after oral intubation of KPF, BET or their combination compering to the value in the (G2) group (Figure 1).

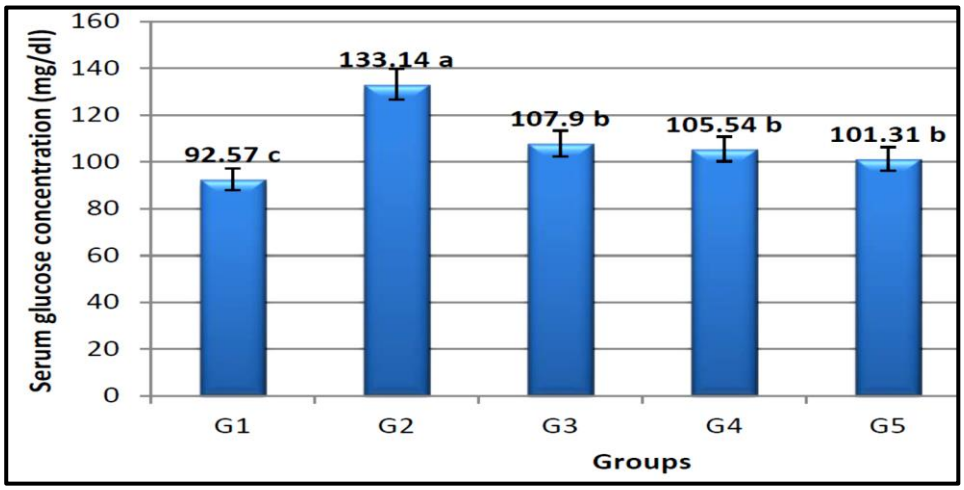

Figure 1. Effect of oral intubation of Kaempferol, Betaine / or their combination on serum glucose concentration (mg/dl) in methionine over load adult male rats.

Besides, significant elevation was observed in serum insulin hormone concentration after oral intubation of KPF, BET or their combination groups (G3 to G5) compering to the value in the (G2) group. The result also showed that the combination of KPF and BET group G5 caused significant elevation compering to the other treated groups except control (figure 2).

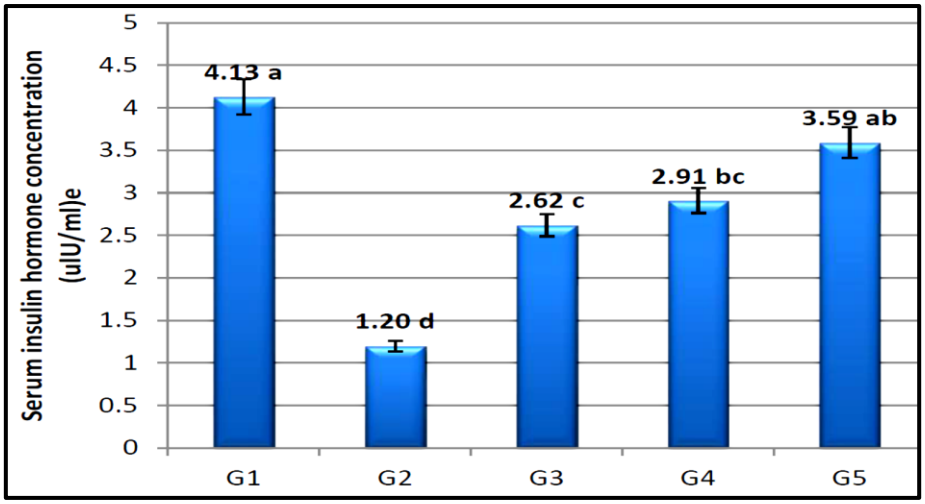

Figure 2. Effect of oral intubation of Kaempferol, Betaine or their combination on serum insulin hormone concentration (ulU/ml) in methionine over load adult male rats

Serum C-peptide concentration elevated significantly in the group G5 compering to the other groups and value was near to the control. Significant elevation was also observed in groups G3 and G4 compering to the value in G2 group (Figure 3).

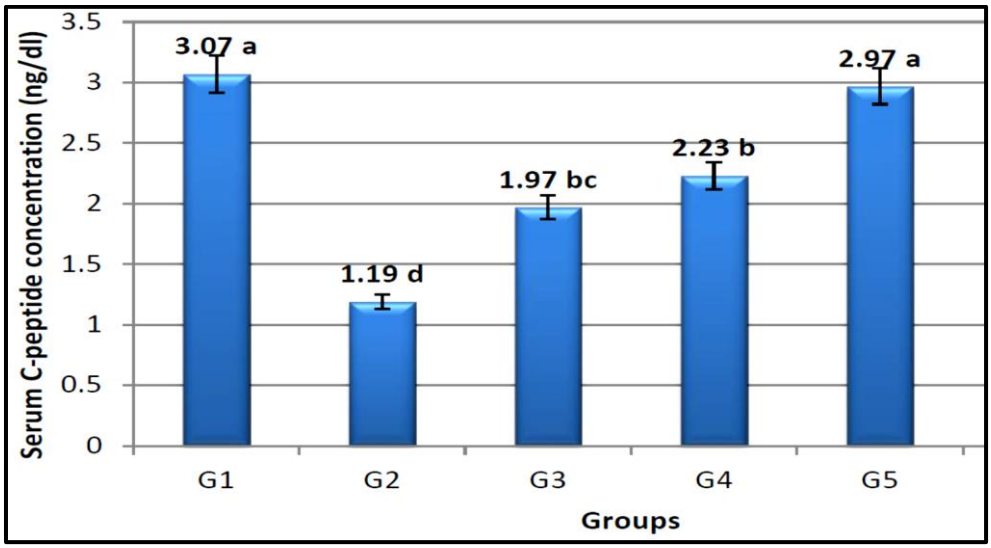

Figure 3. Effect of oral intubation of Kaempferol, Betaine / or their combination on serum C-peptide concentration (ng/dl) in methionine over load adult male rats.

The result also showed that oral intubation of BET or KPF alone caused significant elevation in serum B12 concentration compering to methionine over load treated (G2) group (figure 4), combination of KPF, BET in G5 treated group showed significant elevation in this parameter compering to the value in other treated groups (except control).

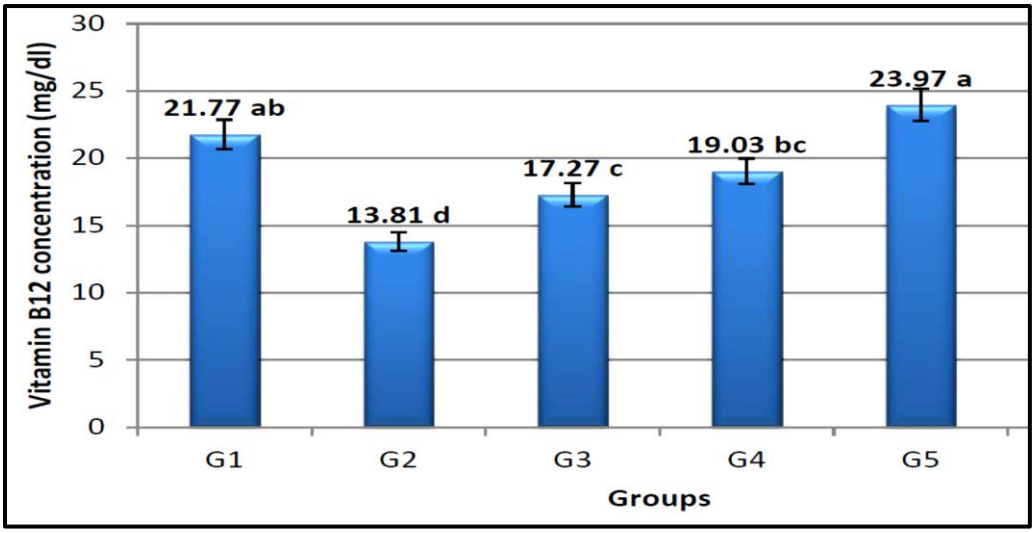

Figure 4. Effect of oral intubation of Kaempferol, Betaine/ or their combination on serum B12 concentration (ng/dl) in methionine over load adult male rats.

4. DISCUSSION

The current changes in glucose, insulin, C-peptide and B12 concentration in G2 group could be attributed to methionine over load induced- HHcy, where 100mg/kg B.W. of Dl-methionine in drinking water or in diet caused HHcy as recorded by [**36,37,38**]. Disturbance glycemic indices could indicate susceptibility to diabetes duo to HHcy which was going in line with [**39,40**]. HHcy could be induced in diabetic patient with elevation in glucose , lipid profile with decrease in HDL-c and insulin [**41**].Several observational studies have discovered HHcy as a risk factor for T2DM and related complications [**42,43,44**].As for the underlying mechanisms, it can be speculated that HHcy might cause oxidative stress, and the increased reactive oxygen species act as functional signaling molecules to activate various stress-sensitive signaling pathways, eventually lead to insulin resistance causing hyperglycemia.[**45,46**].The generation of ROS by Hcy in the redox-cycling reaction leads to a decrease in the viability of insulin-secreting cells, which in turn results in diminished glucokinase phosphorylation, weakened insulin-secreting response and cell death [**47]**, which are essential components of the pathogenesis of T2DM [**48,49**]. Eventually the hyperglycemic effect of Hcy could be duo to B-cell dysfunction and insulin resistance via oxidation and inflammation mediated pathway **[50,51]**. Moreover, such predicted HHcy after methionine overload may decrease the ability of the cells to detoxify H2O2 and other lipid peroxide and reduction in the activity of intracellular antioxidant enzyme **[52]** that could lead to dysfunction of pancreatic B cell with depletion in insulin and c-peptide secretion **.** The result also reported significant decrease in B12 concentration in G2 group which may be attributed to HHcy induced after methionine over load. HHcy is associated with

vitamin B12 deficiency, where the measurements of metabolites, such as methylmalonic acid (MMA) and Hcy, are more sensitive in the diagnosis than the measurement of serum B12 levels alone, with elevated serum MMA levels, and with elevated serum Hcy levels in B12 deficiency cases [**53,54]**. A recent study reported that vitamin B12 deficiency that may be associated with HHcy is associated with altered lipid profile and is predictive of metabolic risk [**55**]. Furthermore, vitamin status(B6 & B12) which are necessary cofactors for enzymes involved in Hcy metabolism and deficiency of folic acid can also cause HHcy [**56,57**], which is considered to be toxic to cells [**58**]. Likewise, it has been found that using of folic acid, vitamin B6 and B12 typically reduces Hcy in a way that suggest synergistic effects **[59,60,61]**.

The result also showed that oral intubation of KPF and BET or their combination caused correction of studied criteria in groups (G3 to G5). Notably, dietary intervention is considered an effective and safe way to regulate metabolic pathways and to lower the risk of T2DM [**62,63,64**]. KPF could ameliorate diabetes in STZ-induced mice by suppressing hepatic pyruvate carboxylase activity and inhibiting gluconeogenesis [**65,66**]. Significant elevation in serum insulin in KPA treated rats may be duo to stimulation of insulin secretion from B cell of pancreas, this will decrease glucose level through increase utilization of glucose by tissue was observed in vitro [**67]**. Besides, improvement to glucose tolerance by KPF could occurred via reduction in serum resistin level [**68**]. An elevation in the AMPK activity could be a mechanism for hypoglycemic effect of KPF [**69**].The activation of AMPK results in an increase in the cellular energy supply [**70**]. AMPK activation was also reported to enhance phosphorylation of insulin receptor substrate and the downstream signalling pathway to increase glucose uptake [**30**]. **Abdel-Hamed and his colleagues (2023)**,illustrated that KPF ameliorate hyperglycemia and elevate insulin concentration and sensitivity , correct lipid profile via elevation of gene expression of AMPK and decrease in PPARy, SREBPI signalling pathway[42].The hypoglycemic effect of BET was documented [**71,72**].BET is used as therapy to lower plasma Hcy, which is tightly associated with insulin resistance and cardiovascular complications, through methylation of Hcy and generation of methionine [**73**].Plasma BET levels are independently and inversely associated with dysglycemia through lowering fasting blood glucose and glycated hemogtbin % [**74**].Eventually, a study based on 4336 participants suggested that BET levels were negatively correlated with TC and TGs, which commonly up-regulated with blood glucose in dysglycemia [**75**].BET supplementation positively affects multiple genes, which expression is dysregulated in diabetes. AMP-activated protein kinase is thought to play a central role in the mechanism underlying the anti-diabetic BET action [**76**].BET also inhibited intramyocellular lipid accumulation and improved insulin resistance in mice fed a high-fat diet [**18]**. C-peptide concentration reflect true insulin secretion from pancreas [**77]**. C-peptide might also act as

antioxidant to limit beta cell dysfunction and loss contributing to diabetes [**78]**. C-peptide significant decrease high glucose and H2O2 induced ROS and prevent Beta cell apoptosis **[79].** KPF and BET as antioxidant [**80,81],** could reserve cellular integrity, restore function of Islet of pancreas ,increase insulin secretion and sensitivity accompanied with release of c-peptide **.** Significant elevation in B12 after BET or KA supplementation could be duo to depression in Hcy level as recorded earlier. BET administration could protect against low-vitamin-B12-induced defects given that low or no vitamin B12 elevates Hcy levels, reduces SAM:SAH ratio and, by modulating SREBF1 and low-density lipoprotein receptor (LDLR) genes, induces cholesterol biosynthesis in human adipocytes [**82**]. The most interesting finding in this study is that the combination of BET and KP exerted a more potent effect in regulating glucose indices and other biomarkers compered to either BET or KP alone, which may be duo to quite different mechanism. AMPK serves as the energy sensor of the body, and disturbance of the AMPK pathway plays important roles in metabolic disorders. It senses cellular stresses such as glucose deprivation, hypoxia, and starvation. Then, it is activated by an increased ratio of AMP/ATP, which is a sensitive signal of falling energy status [**83,84**]. Zhou and his coworker reported that the combined supplementation of two antioxidants (dietary quercetin and resveratrol) exerted synergistic effects on improving glucose and lipid disorders in high-fat diet-fed mice [**85**]. Combination therapy has been demonstrated to be superior to monotherapy in metabolic abnormalities such as T2DM [**86**].

5. CONCLUSIONS

From the results obtained and discussed in this study, we can conclude that the exposure to methionine over load in water effects many criteria related to metabolic syndrome, especially dysglycemia (elevation in serum glucose, depression in insulin, C peptide) and decrease in B 12 concentration.

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