Effects of combination of Betaine and Kaempferol on Induced methionine overload in rats: Lipid profile and oxidative stress

Efectos de la combinación de betaína y kaempferol sobre la sobrecarga de metionina inducida en ratas: perfil lipídico y estrés oxidativo

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Abstract

This experiment was designed to find the role of Kaempferol (KPF) and Betaine (BET) on Metabolic disturbance induced by methionine overload (lipid dysfunction and oxidative stress) in male rats. Thirty (30) adult rats were randomly selected and divided equally into five experimental groups and treated for 45 days as the follows: group G1:Rats in this group served as control, Rats in other groups (G2,G3,G4 and G5 were received 100mg/kg B.W of D-L methionine in tap 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 Fasting blood sample were collected by cardiac puncture technique at the end of the experiments and serum were collected for estimation serum lipid profile and Malondialdehyde(MDA). The results of the experiment showed that oral intubation of D-L methionine for 45 days(G2 group) caused dyslipidemia manifested by significant elevation in serum concentration of total cholesterol (TC), Triacylglycerol (TAG), low density lipoprotein-cholesterol(LDL-C) and very low density lipoprotein-cholesterol (VLDL-C), and significant decrease in high density lipoprotein-cholesterol (HDL-C),also caused oxidative stress manifested by increase in Malondialdehyde (MDA) concentration. while oral intubation of KPF, BET or their combination ameliorate the dyslipidemia and oxidative stress induced by methionine overload.

Key words: kaempferol, betaine, lipid profile, MDA, Dl-Methionine

Resumen

Este experimento fue diseñado para encontrar el papel del Kaempferol (KPF) y la Betaína (BET) en la alteración metabólica inducida por la sobrecarga de metionina (disfunción lipídica y estrés oxidativo) en ratas macho. Se seleccionaron al azar treinta (30) ratas adultas y se dividieron en partes iguales en cinco grupos experimentales y se trataron durante 45 días de la siguiente manera: grupo G1: las ratas de este grupo sirvieron como control, las ratas de otros grupos (G2, G3, G4 y G5 recibieron 100 mg/kg p.c. de metionina D-L en agua del grifo, y se recibieron por vía oral de la siguiente manera: las ratas del grupo G3 recibieron 150 mg/kg p.c. de KPF, las ratas del grupo G4 recibieron 250 mg/kg p.c. de BET, las ratas del grupo G5 recibieron 100 mg/kg p.c. Se recogieron 150 mg/kg de peso corporal de KPF, 250 mg/kg de peso corporal de BET mediante la técnica de punción cardíaca al final de los experimentos y se recogió suero para estimar el perfil de lípidos séricos y malondialdehído (MDA). que la intubación oral de metionina D-L durante 45 días (grupo G2) causó dislipidemia manifestada por una elevación significativa en la concentración sérica de colesterol total (CT), triacilglicerol (TAG), lipoproteínas de baja densidad (LDL-C) y lipoproteínas de muy baja densidad. El colesterol (VLDL-C) y la disminución significativa del colesterol de lipoproteínas de alta densidad (HDL-C) también causaron estrés oxidativo manifestado por un aumento en la concentración de malondialdehído (MDA). mientras que la intubación oral de KPF, BET o su combinación mejora la dislipidemia y el estrés oxidativo inducido por la sobrecarga de metionina.

Palabras clave: kaempferol, betaína, perfil lipídico, MDA, Dl-Metionina

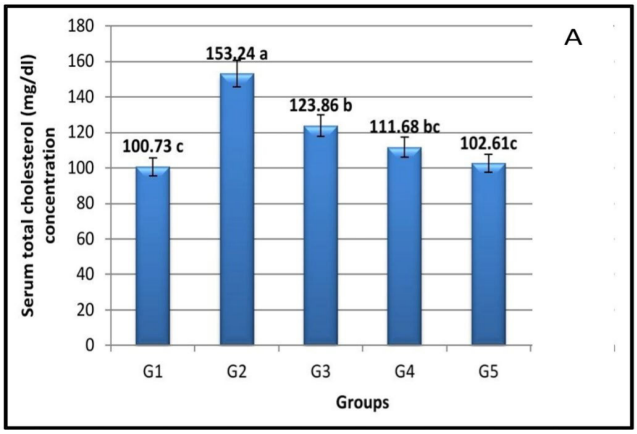
Introduction

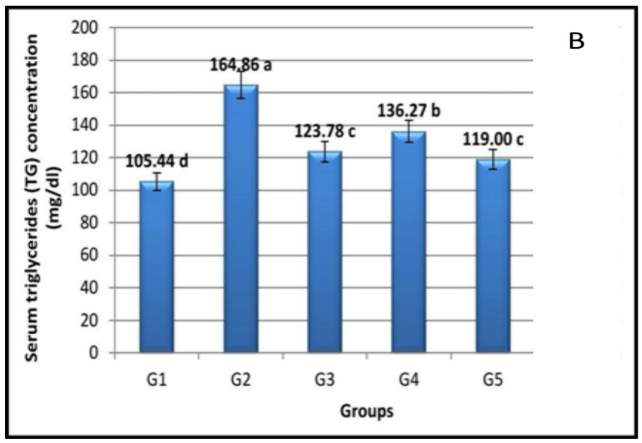
Induced Methionine overload is likely to be involved in dysfunction of several organs such as kidney, heart and reproductive, which may be interrupted to HHcy[1-3]. Homocysteine (Hcy) is a non-protein sulfhydryl-containing amino acid, and intermediate metabolite formed in metabolism of methionine[4]. Elevated level of Hcy , is an important risk factor for arteriosclerosis, Alzheimer's disease, cardiovascular disease, ischemic heart disease, stroke, liver cirrhosis, diabetic retinopathy, and diseases of the central nervous system[5-9].(HHcy) is a methionine metabolism problem that causes a variety of inflammatory illnesses[10].Oxidative stress is among the processes thought to be involved in the pathophysiology of the damage produced by HHcy, medicinal herbs and many food ingredients possess favorable biological properties that contribute to their therapeutic activities. One such natural product is BET, a stable, nontoxic natural substance that is present in animals and plants beets (Beta vulgaris) is a major source of betaine, it is also found at high concentrations in other food sources including wheat bran, wheat germ, and spinach[11], or exogenously consumed through dietary intake[12]. Dietary betaine (trimethylglycine), crucial methyl donor, that restores methionine homeostasis in cells[13]. BET supplementation to broiler chicken's diet improved their productive performance[14]. BET plays a role in preventing/attenuating both alcohol-induced and metabolic-associated liver diseases[13,15]. They also reported that BET has an anti-nociceptive and a sedative role through interactions with opioidergic and -aminobutyric acid (GABA) receptors[16]. KPF is an antioxidant flavonol found in fruits and vegetables including apples, gooseberry, carrot, honey and spinach[17-19]. KPF were recorded to be dominant compound with high concentration in some plants in the north of Iraq such as Adiantum capillus-veneris[20] and Asplenium species[21,22]. KPF and its glycosylated derivatives are found to possess cardio protective[23], neuroprotective[24], anti-inflammatory[25], antidiabetic[26] and antimicrobial activity[27], in addition to its antioxidant effect[28]. KPF, being a polyphenolic neutraceutical compound, exhibits high cytotoxicity, and thus has a promising role in cancer therapy[29]. Researchers have indicated the antioxidant potential of KPF in both in vitro and in vivo models[30]. It causes the scavenging of the free radicals and other ROS molecules, as their generation transforms the normal cells into malignant ones[31]. KPF reduces liver damage in acetaminophen-treated rats through it antioxidant, anti-inflammatory, and anti-apoptotic effects[32]. According to available literature, several studies concerning beneficial effect of the KPF and BET and the damaging effect of the HHcy, However, there are few studies on the beneficial effect of combination of KPF and BET on the harmful effect of hyperhomocysteinemia (HHcy) (overload of Methionine).

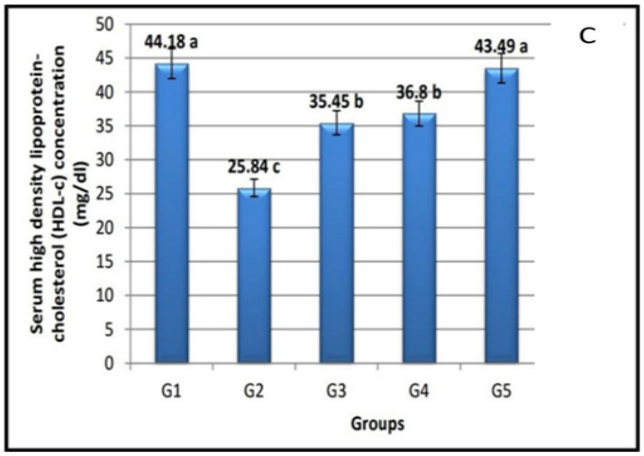
Materials and methods

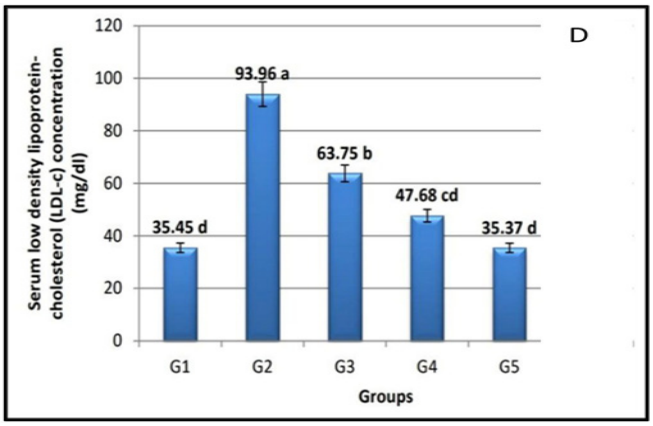
Thirty (30) adult male rats were randomly selected and divided equally into five experimental groups and treated for 45 days as the follows: **Group G1**: Control group were received tap water.; **Group G2:** Rats in this group were given 100mg/kg B.W of D-L methionine in tap water.; **Group G3:** Rats in this group were given 150 mg/kg b.w. of KPF and 100 mg/kg B.W of D-L methionine in tap water; **Group G4**: Rats in this group were given 250 mg/kg b.w. BET and 100mg/kg B.W of D-L methionine in tap water; **Group G5**: Rats in this group were given 150 mg/kg b.w. of KPF, 250 mg/kg B.W. BET and 100mg/kg B.W of D-L methionine in tap water KPF and BET were given orally. 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.) 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 concentrations: total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and Triacylglycerol (TAG) using enzymatic kits (**Bio system, Spain**).While low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to Friedwald formula and Malondialdehyde (MDA) concentrations using enzymatic kits (**Bio system, Spain**).

Serum TC, TAG, LDL-c,VLDL-c, and HDL-c concentrations of different treated groups were summarized in Figure 1 (A,B,C,D,E) respectively. The result showed that rats given methionine overload (G2) caused significant ($p<0.05$) increase in serum TC, TAG, VLDL-c and LDL-c with significant ($p<0.05$) decrease in serum HDL-c concentration comparing to other treated group. While KPF, BET intubation alone or in combination (G3 to G5) showed significant decrease in lipid profile parameters except HDL-C which showed significant elevation ($p<0.05$) compering to other treated groups except control.









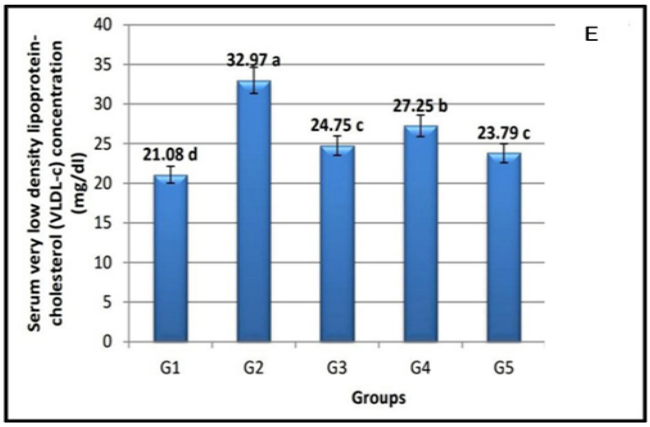


Figure 1: Effect of oral intubation of Kaempferol, Betaine/ or their combination on serum lipid profile concentrations in methionine overload rats. (A) TC, (B) TAG, (C) HDL-c, (D) LDL-c and (E) VLDL-c.

G1: Rats in this group served as control, The other experimental groups from G2 to G5 were given 100mg/kg B.W. of DL-methionine in tap water, in addition Rats in G3 group were given 150mg/kg B.W. of KPF, Rats in G4 were given 250 mg/kg B.W. of BET, Rats in G5 were given combination of 150mg/kg B.W. of KPF and 250 mg/kg B.W. of BET The mean values are presented ± SE, n=6. Significant differences among groups are shown by different small letters ($p<0.05$). Methionine was given in drinking water. experiment was lasted for 45 days KPF and BET were given orally.

At the end of the experiment, the result showed significant elevation ($p<0.05$) in serum Malondialdehyde (MDA) concentrations in (G2) treated group compared with other experimental groups, which showed significant decrease in this parameter (G3 to G5).

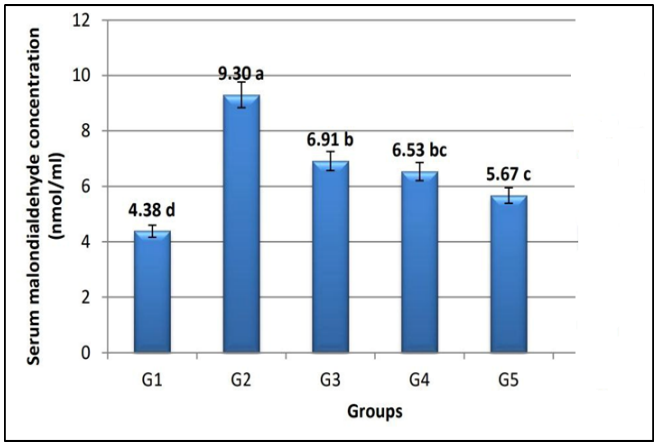


Figure 2: Effect of oral intubation of Kaempferol, Betaine/ or their combination on serum malondialdehyde concentration (nmol/ml) in methionine overload adult male rats.

G1: Rats in this group served as control, The other experimental groups from G2 to G5 were given 100mg/kg B.W. of DL-methionine in tap water, in addition Rats in G3 group were given 150mg/kg B.W. of KPF, Rats in G4 were given 250 mg/kg B.W. of BET, Rats in G5 were given combination of 150mg/kg B.W. of KPF and 250 mg/kg B.W. of BET The mean values are presented ± SE, n=6. Significant differences among groups are shown by different small letters ($p<0.05$). Methionine was given in drinking water. experiment was lasted for 45 days KPF and BET were given orally.

Discussion

This study showed that DL-methionine given to male rats for forty-five (45) days lead to significant changes in lipid profile system. These changes were manifested by increase in TC, TAG, LDL-C, VLDL-C and decrease in serum HDL-C concentrations. These results are in agreement with other studies[33,34]. The mechanism could be duo to D-L methionine overload - induced HHcy. Induction of HHcy after methionine overload in diet or water were agreed with[35-39]. One of the primary biological responses to HHcy includes effects on lipid metabolism such as increased expression of genes involved in de novo lipogenesis, lipolysis and fatty acid oxidation in liver and white adipose tissue[40]. HHcy plays an important role in cholesterol biosynthesis by inducing transcription as well as translation of 3 hydroxy-3- methylglutaryl coenzyme A reductase **(**Limiting enzyme in the cholesterol biosynthesis). It also increased cholesterol synthesis and intestinal absorption and suppressed excretion of cholesterol[41]. Furthermore, Hypercholesterolemia under effect of methionine overload may cause mutation of LDL receptors adaptor protein, leading to defective LDL receptors and consequent reduction in plasma LDL clearance resulting in its accumulation[42].The increase in TAG levels in animals receiving methionine in the present study may be due to increment in plasma VLDL levels (which act as a carrier for the TAG in the plasma) , partial deficiency of lipoprotein lipase , associated with increased put out of lipoproteins from the liver[43]**.**

Current result showed that oral intubation of (KPF), BET or their combination caused correction of dyslipidemia induced by methionine overload indicated their hypolipidemic effect. It has been recently showed that KPF intubation ameliorates glucose and lipid metabolism disorders by enhancing lipid metabolism via Adenosine monophosphate-activated protein kinase (AMPK) activation. KPF down regulates SREBPs and up regulates liver peroxisome proliferator-activated receptor a (PPARa), promoting the expression of propyl CoA oxidase, reducing the accumulation of visceral fat, and improving hyperlipidemia in high-fat diet-fed obese rats[44]. A decrease in TAG could be duo to upregulation the expression of adipose triglyceride (TAG) lipase[45]. Besides, it up regulates liver X receptor (LXR), which regulates lipid transport[46]. KPF attenuates the activity and expression of CYP2E1[47], the resulting falling status of the energy supply, demonstrated by the increased ratio of adenosine monophosphate (AMP) /adenosine triphosphate (ATP), was sensed by AMPK. The activation of AMPK by KPF in turn enhanced lipolysis and inhibited fatty acid synthesis both in vivo and in vitro[48], and caused decrease in level of ALT, LDL, triglycerides, total cholesterol, lipid droplets in liver[49]. Recent studies have shown that AMPK exerted its ability to regulate lipid metabolism by enhancing fatty acid oxidation, while inhibiting the production of cholesterol and fatty acids[50-52]**.** Betaine intubation in herein has been found to be inversely correlated to a case of dyslipidemia and positively correlated to HDL cholesterol concentration indicating its hypolipidemic effect[53,54]. BET increased plasma HDL which may reflect the favorable effects of BET on liver function-related metabolism[55]**.** The positive effects of BET on lipid metabolism have been attributed to the fact that it is an important methyl donor, resulting in a considerable increase in hepatic SAM concentrations[56-59]**.** The increased SAM availability is thought to regulate phosphatidylcholine (PC) synthesis by PEMT and this normalizes VLDL production rates[60]**.** BET has been used for more than 30 years in pyridoxine non-responsive cystathionine beta-synthase (pnrCBS) and cobalamin C (cblC) deficiencies to lower the HHcy[61]. BET may also reduce uptake of triglycerides from circulating lipoproteins by decreasing the mRNA expression of lipoprotein lipase[62-64]**.** BET administration could activates AMPK, which enhances genes encoding proteins involved in fatty acid transport and fatty acid oxidation, while decreasing fatty acid synthesis[65], thereby preventing triglyceride and cholesterol accumulation in the liver[66].

In the current study an elevation in Malondialdehyde (MDA) concentration was observed in G2 group indicating a case of oxidative stress. Such changes may be attributed to HHcy induced after methionine overload[7]. It has been demonstrated that mild hHCY is much more common and is associated with post methionine loading in water[68,69] or in diet[70]. Formation of ROS such as hydrogen peroxide, superoxide anions, and hydroxyl radicals and oxidative stress are among the process that involved in damage produced by HHcy[39,71,72]. The result of the present work was correlated with[72] and[74] who demonstrated significant increase in plasma markers of LPO in rats with HHcy resulting by induced methionine overloading. It has been reported that homocystein (hcy) reduces the antioxidant capacity of the body by inhibiting glutathione production and suppressing the expression of Glutathione peroxidase (GSH-Px) and SOD[75-78]. The mechanism could be strong production of ROSs has been detected at the onset of HHCys when the sulfhydryl HCys group is easily oxidized, producing superoxide anion (O–2) species as a result of both the activation of nicotinamide adenine dinucleotide phosphate oxidase and the inhibition of the expression and function of important antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase[79]. Significant decrease in serum MDA concentration was observed in BET treated group, indicating the antioxidant activity of betaine which was been documented by[12,80,81]. One of the biological and beneficial effects of dietary BET on methyl donor, that restores methionine homeostasis in cells and reduce HHcy that affect oxidative stress. BET can enhance non enzymatic antioxidant defences, attenuate oxidative stress, endoplasmic reticulum stress by regulation of methionine metabolism via removing Hcy and maintaining cellular SAM:SAH ratios[82,13].

BET is a precursor of S-adenosylmethionine (SAM), which contributes to an increase in the supply of substrate needed for the synthesis of GSH[83]**.** Besides, Experimental evidence suggests that SAM has direct antioxidant activity via ROS scavenging and chelation of iron ions used to inhibit hydroxyl radical generation[58]**.** BET exerts its antioxidant properties through its ability to scavenge free radicals**,** or by increasing the activity of antioxidants mainly SOD[84,85]**.** Additionally, the three methyl groups of BET were found to play a key role in its antioxidant activity. The possible reason was that because of the hydrophobicity of the three methyl groups and hydrophilicity of the carboxyl of BET, a tight protective membrane was formed around cells to prevent oxidative stress inducer from inducing ROS generation and cell damage[82]**.**

Significant decrease in serum MDA level was observed after KPF intubation in the current study. Rremarkable antioxidant effects of KPF, namely acting directly in antioxidant enzymes, capable of efficiently inhibit ROS generation and lipid peroxidation, and, finally, preventing the occurrence of cell damages, in a broad-spectrum activity ,KPF is a potent scavenger of superoxide anion, hydroxyl radical, by chelating cuprous or ferrous[87], and peroxynitrite[86], it also inhibits pro-oxidant enzymes, such as xanthine oxidase[88], and activates antioxidant enzymes such as superoxide dismutase, catalase, and heme oxygenase-1[89]. It is important to highlight, is that KPF contains hydroxyl groups at C3, C5, and C4, an oxo group at C4, and a double bond at C2-C3 that might explain its antioxidant activity[86,90]. Additionally, some researchers have revealed the antioxidant ability of KPF in vivo by promoting the expression of Nrf2 and its target genes (SOD1 and GPX3), reducing ER stress and inhibiting apoptosis[91]**.** stimulation of Nrf2 and decreased ROS levels by KPF and H2O2 through the activation of heme oxygenase-1(HO-1) which prevented oxidative damage in vitro[92]**.**

The effect of the BET and KPF combination was obviously more potent than that of KPF alone, suggesting the synergistic effect of these compounds. Combination therapy has been demonstrated to be superior to monotherapy in metabolic abnormalities such as T2DM[93,94]. It can be hypothesized that this synergistic effect might be due to their different mechanisms of action on energy supply pathways. While both KPF and the combination of BET and KPF significantly induced the phosphorylation of AMPK, indicating the upregulation of AMPK activity that effect antioxidant activity, lipid[95] and glucose metabolism[96]. Moreover, the enhancement of catabolic lipid metabolism was due to the activation of AMPK, the sensor of energy status. Hence, BET and KPF combination could regulate lipid metabolism through activation of AMPK as was recorded after combination of Cinnamaldehyde (CA) and KPF[48].

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