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Role of clopidogrel and etoricoxib in obesity complications induced by monosodium glutamate in male rats

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***Abstract*---**This study aimed to estimate therapeutic role of clopidogrel and etoricoxib against cardiovascular changes in rats induced obese by Monosodium Glutamate (MSG). The experiment was performed in 30 rats divided into five groups as C-: negative control group, C+: positive control group (MSG 4mg/g),T1: received clopidogrel at dose 10.97 mg/kg, T2: received etoricoxib at dose 8.48 mg/kg and T3: received combination of clopidogrel and etoricoxib at dose 5.04 and 4.03 mg/kg respectively, then BW, BMI, TG, leptin, adiponectin, troponin-l and histopathology for heart and adipose tissue were measured, depending on results of current study the conclusion that can be drawn is there are a good role of both etoricoxib and clopidogrel alone and in combination therapy by succeeded in protection from obesity complications that induced by MSG.

***Keywords*---**Clopidogrel, Etoricoxib, obesity, mono sodium glutamate.

Introduction

Obesity has turned into a worldwide epidemic. In the last decades, the number of obese patients has been increased considerably. It is especially alarming that in recent years the increase was most pronounced in infants and that it occurs both in developed, but even more, in developing countries [1]. Central obesity is probably the main cause of metabolic syndrome (MetS), which included insulin resistance, type 2 diabetes mellitus, hypertension, obstructive sleep apnea syndrome, non-alcoholic fatty liver disease (NAFLD), and dyslipidemia, all risk factors for cardiovascular disease [2].

Obesity is a multi-factorial disorder, and the roles of both genes and environmental factors are recognized. Although obesity tends to run in families, indicating a hereditary component, the classical Mendelian pattern of inheritance (typical of monogenic disorders) is elusive. Genome-wide association studies (GWAS) have identified several susceptibility loci in the genome which are supposedly associated with obesity. However, by and large, genes are now thought to set only the stage and provide the backdrop, against which the decisive effects are eventually driven by environmental and behavioral factors [3]. The increasing prevalence of obesity throughout life is a global health challenge because of its strong and positive association with significant health problems such as type 2 diabetes, cardiovascular disease, stroke, and some cancers. The complex causes and drivers of obesity include genetic factors, social, ecological, and political influences, food production and supply, and dietary patterns [4].

It has been hypothesized that monosodium glutamate (MSG), a flavor enhancer, is positively associated with weight gain, which influences energy balance through the disruption of the hypothalamic signaling cascade of leptin action [5]. Coronary heart disease (responsible for heart attacks) hypertension (high blood pressure), and atherosclerotic changes on the walls of blood vessels. Atherosclerosis constitutes the basic pathology of hypertension and coronary heart disease, and also a sizeable proportion of cerebrovascular diseases [6].
Etoricoxib also markedly normalized high fat diet–mediated rise of hepatic enzyme activity. Etoricoxib treatment lowered the level of oxidative stress indicators significantly with a parallel augmentation of antioxidant enzyme activities. Furthermore, etoricoxib administration helped in preventing inflammatory cell invasion, collagen accumulation, and fibrotic catastrophe in HF diet–fed rats. As well as, etoricoxib had a role in deterring the metabolic syndrome furthermore, other deleterious pathological changes afflicting the high fat diet fed rats [7]. Clopidogrel considered a most effective drug in reducing the risks of myocardial infarction, ischemic stroke and vascular death, despite the obvious advantages of clopidogrel, many clinical studies have shown that approximately 5–40 % of patients treated with conventional doses of clopidogrel display inadequate antiplatelet responses, which may be lead to serious cardiovascular complications [8]. So, the current study was aimed to estimate clopidogrel and etoricoxib therapeutic role against obesity complications induced by MSG in rats.

Materials and Methods

Experimental animals

This study was done in the animal house vassal to the college of Veterinary medicine/ Baghdad University. Newborn male Wistar rats (weight 65±10) have been utilized in the current study. Throughout the experiment, the animals had fed on the optimal diet pellet, the room temperature was preserved at 25±2 ºC, the circadian rhythm had on a 12-hour light/dark rhythm with lightening on at 07:00 a.m. and off at 07:00 p.m.

Experimental design

The induction of obesity was done by administration of MSG at dose 4 mg/g IP for 4 consecutive days for 30 newborn rats, according to a method described by

Cunha, De Abreu [9]; Hata, Kubota [10]. After 30 days from first injection rats have been divided into equal five groups as follows: The 1st group: treated with Clopidogrel at dose 10.97 mg/kg. The 2nd group: treated with Etoricoxib at dose 8.48 mg/kg. The 3rd group: treated with a combination of Clopidogrel and Etoricoxib at dose 5.04 and 4.03 mg/kg respectively. The 4th group: were obesity induced and treated with distilled water and the 5th group: were lifted without any treatment. All treatments were given orally by stomach tube and the animals were sacrificed on day 30 at the end of experiment.

Blood Sampling

Fasting blood (for 8-12 hrs.) samples were collected at different times of the experiment. Blood was drawn by cardiac puncture technique from anesthetized rats' intramuscular injection of Ketamine (90mg/Kg B.W.) and xylazine (40mg/Kg B.W.), blood was collected in plain tubes and used to separate serum which stored at (-20 Cº), then it used for measurement of biochemical parameters.

Biochemical assessment

The assessment of serum level of Triglycerides measurement done by use triglycerides kit *from Linear (Spain)*, leptin serum levels assessed by using leptin rat kit from *Shanghai Biological (China)*, while for assessment of serum troponin-l the *rat troponin kit* from *Beckman Coulter (USA)* have been used, Adiponectin measured by using *Rat Adiponectin Kit* from *Cusabio (USA).* All tests processed by ELIZA test and the apparatus from *TOSHO (JAPAN).*

Histopathological examination

Heart and adipose tissue are obtained after scarifying of the animals at the end of the experiment by high dose of anesthesia. Specimens with dimensions 1x1x1 cm were taken, the tissues were fixed in 10% buffer formaldehyde solution immediately after removal. After 72 hrs. of the fixation, the specimens were washed with tap water and then processing was routinely done with a set of upgrading alcoholic concentration from 70% to absolute 100% for 2 hrs in each concentration to remove water from the tissues, then clearance was done by xylol, then the specimens were infiltrated with semi-liquid paraffin wax at 58 °Ċ on two stages, then blocks of specimens were made with paraffin wax and sectioned by rotary microtome at 5µm for all tissues. all tissues were stained with Hematoxylin and Eosin (H&E) stain and the histopathological changes were observed under light microscope [11]. Adipocyte cell measurement was done by using a software (image J) for processing and analyzing scientific images [12]

Statistical analysis

Statistical analysis was applied by two ways ANOVA and the mean difference is significant at the 0.05 level in using statistical package for social sciences (SPSS), Version 10.

Results

The results of BW, BMI, Triglycerides, Leptin, Troponin-I, Adiponectin and Adipocyte size are shown in table (1). With regard to BW and BMI statistical analysis showed there are significant increase (P<0.05) among all treated groups in comparison with the Control negative group and a significant increase in mean value of BW and BMI in T3 group that treated with combination of clopidogrel and etoricoxib in comparison with negative control group. and significant decrease in comparison with T1 and T2 for BW and BMI. In addition, a statistical analysis shown a significant increase (P<0.05) in serum Triglycerides, Leptin, Troponin-I and Adipocyte size among treated groups (T1, T2 and T3) in comparison with C- group and a significance decreasing (P<0.05) when compared the data of treated groups (T1, T2 and T3) with C+ data. Furthermore, T3 treated group was recorded a largest decrement than (T1 and T2) in serum Triglycerides, Leptin, Troponin-I and Adipocyte size. While the serum Adiponectin levels in treated groups (T1 and T2) shown a significance decrease (P<0.05) in comparison with mean value of control negative group. The adiponectin level in control positive group reveal a significant (P<0.05) decrement in mean value in comparison with all another experiment groups. In addition, Adiponectin level of T1 and T2 revealed a significant increase (P<0.05) as compared with positive control group, as well as, the Adiponectin of T2 showed a significant increase (P<0.05) as compared with T1.

Table (1)
Effect of monosodium glutamate (4mg/g), Clopidogrel (10.97 mg/kg), Etoricoxib (8.48 mg/kg) and combination of Clopidogrel (4.03 mg/kg) and Etoricoxib (5.04 mg/kg) on Body weight (BW), Body Mass Index BMI, Triglycerides TG, Leptin, Troponin-I, Adiponectin, and Adipocyte size in male rats

Groups / Parameters	C-	C+	T1	T2	T3	LSD
BW (gm)	220.60±3.66 d	367.20±6.86 a	332.60±2.08 b	319.80±2.20 b	286.80±5.12 c	12.92
BMI	0.65±0.01 d	1.13±0.02 a	1.11±0.02 ab	1.04±0.02 b	0.84±0.02 c	0.06
TG (mg/dL)	77.28±2.42 d	154.68±5.40 a	126.89±1.85 b	112.60±2.55 c	81.64±1.33 d	9.03
Leptin (ng/ml)	4.68±0.73 d	23.86±1.30 a	17.66±1.45 b	12.86±1.21 c	6.42±0.0 d	3.28
Troponin-I (pg/ml)	10.70±0.66 c	27.61±1.16 a	21.14±1.04 b	18.71±087 b	12.63±0.73 c	2.69
Adiponectin ng/ml	5.08±0.48 a	0.63±0.11 d	1.77±0.19 c	2.81±0.34 b	4.23±0.41 a	1.00
Adipocyte size (μm^2)	23.48±0.78 e	60.67±0.65 a	51.40±0.80 b	38.34±0.60 c	29.26±0.82 d	2.08

*(P<0.05).
Mean having with the different letter in same parameter differed significantly.
C-:control negative, C+: MSG, T1: Clopidogrel, T2: Etoricoxib, T3: Clopidogrel & Etoricoxib.

Histopathological study
Adipose tissue

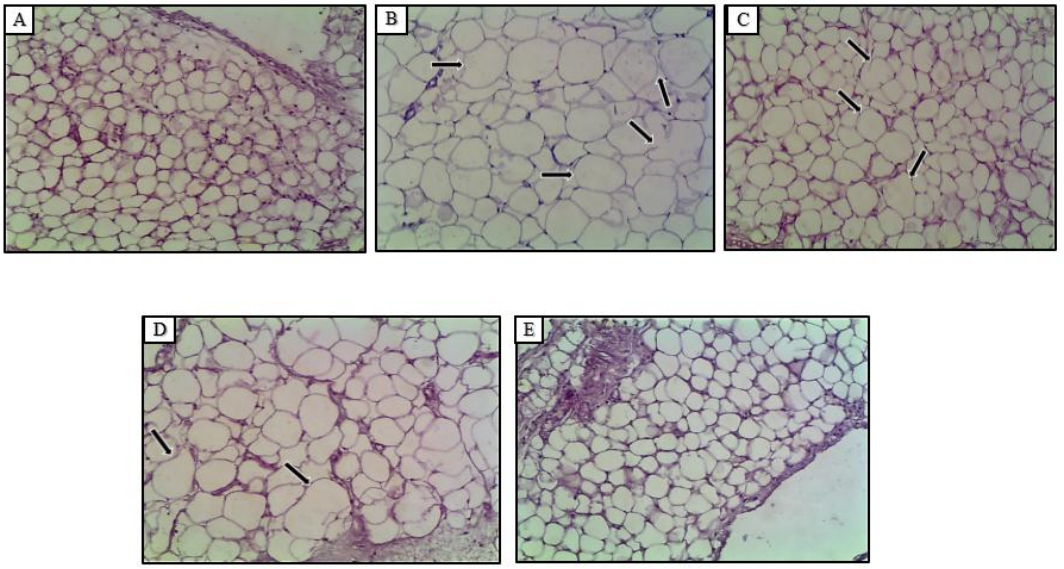

Figure (1): Photomicrograph of adipose tissue, A: control negative group rat shown normal architecture of adipose tissue, B: control positive group rat shown hypertrophy of lipocytes. Note the hypertrophy of lipocytes (Arrows) formed about 80% of tissue area compared with control negative group rats, C: T1 group rat shown hypertrophy of lipocytes. Note the hypertrophy of lipocytes (Arrows), however the hypertrophy of lipocytes formed about 30% of tissue area compared with control positive, where the hypertrophy of lipocytes formed about 80% of tissue area. D: T2 group rat shown hypertrophy of lipocytes. Note the hypertrophy of lipocytes (Arrows) formed about 50% of tissue area compared with control negative group rats and E: T3 group rat shown nearest to normal architecture of adipose tissue compared with control positive and T1 groups. H&E. X100.

Histopathological tissues of Heart

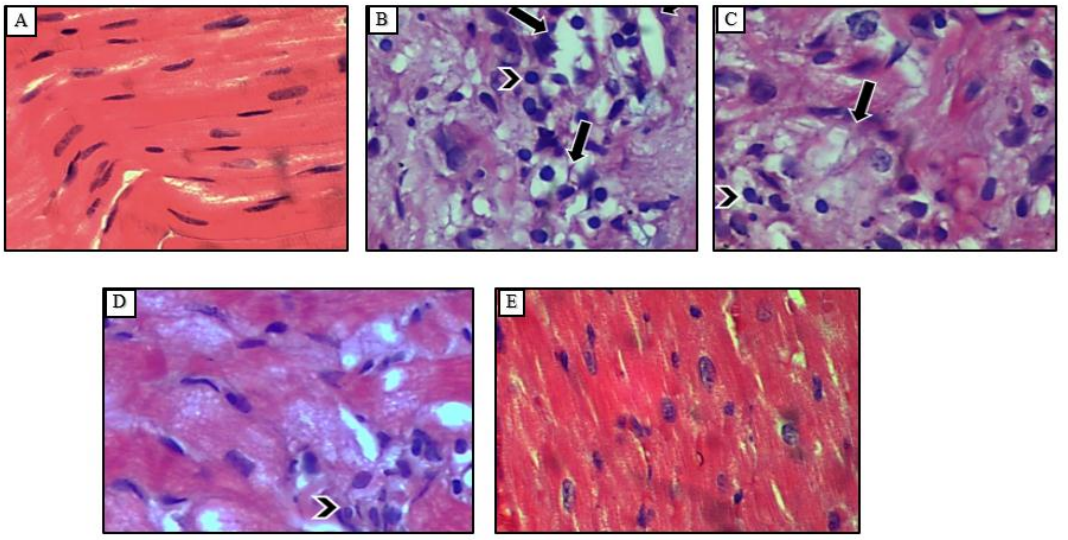

Figure (2): Photomicrograph of heart A: for control negative group rat shown normal heart architecture, B: control positive administered MSG group rat shown necrosis of cardiomyocytes forming a spaces (arrows) in heart parenchyma with presence of inflammatory cell infiltration (arrowheads) in affected area. Note the necrotic area was occupied by inflammatory cells, necrotic cell debris and exudate led to loss of heart normal architecture in affected area. C:T1 group administrated clopidogrel, shown necrosis of cardiomyocytes forming a spaces (arrows) in heart parenchyma with presence of inflammatory cell infiltration (arrowheads) in affected area. Note the necrotic area was occupied by inflammatory cells, necrotic cell debris and exudate led to loss of heart normal architecture in affected area, D: T2 group administrated etoricoxib, shown necrosis of cardiomyocytes forming a small spaces (arrows) in heart parenchyma with presence of limited inflammatory cell infiltration (arrowheads) in affected area, however normal heart architecture was observed in affected area and E: T3 group rat administered clopidogrel and etoricoxib in combination, shown nearest picture to normal heart architecture. H&E. X400.

Discussion

It has been hypothesized that MSG strongly correlate with obesity by influences energy balance through the disruption of the hypothalamic signaling cascade of leptin action [13], also MSG can be caused an increase food intake, induced metabolic disorders associated with oxidative stress, even in the absence of obesity lead to increasing of leptin levels [14]. enlargement of adipocytes and increase in lipid biosynthetic[15], as well as, decreased in adiponectin level due to insulin resistance in obesity and its revers correlation with state of inflammation and metabolic disorder [16]. Also MSG could be caused cardiomyopathy, artheroma, myocardial infarction and ischemia among other cardiovascular disease [17]. Furthermore, MSG considered as exotoxin which may be caused a damage to cells and may be lead to death in toxic doses [18]. As well as, MSG was

able to generation free radicals, proteases, phospholipases activation and endonucleases apoptotic programs activation and in rats, which may lead to cell damage and necrosis especially in heart tissue [19]. This clarify the results obtained in current study when MSG administered

The role of clopidogrel treatment in current study clarified by many authors demonstrations that clopidogrel had effect on lipid profile e.g. triglyceride by reducing platelet-dependent upregulation of inflammatory which induced by high fat diet in study performed in rabbits [20], also clopidogrel can improving metabolic syndrome disturbances, as well as, its known have anti-atherosclerotic effect by preventing platelet adhesion and atheroma which considered a most important complication of obesity and elevation of lipid profile e.g. triglycerides [21]. Leptin can be inhibited by clopidogrel action on platelet aggregation by blockading of platelet adenosine diphosphate (ADP) P2Y12 receptor and finally preventing leptin to exaggerate the state of obesity and its complications [22]. In addition clopidogrel can be enhanced recovering effect in obesity by its acting as antiplatelet and anti-inflammatory medication and its hydrolytic metabolite which increased in obese individual due to down-regulation of certain P450s in the liver [23]. However, clopidogrel remained the antiplatelet agent always shown to improve cardiovascular outcomes by its ability in suppressing the ADP receptors, which may contribute in decreasing of adipocyte size as well as improving obesity complications on cardiovascular system [24].

On the other hand, with regarding to etoricoxib, the investigation revealed that etoricoxib treatment significantly reduced the high plasma cholesterol, triglyceride, and LDL-cholesterol levels in high fat diet–fed rats [7], and this can be explained by a fact that COX-2 inhibitors may be beneficial in hyperlipidemia, hypercholesterolemia, metabolic disorder and atherosclerosis, since the pathogenesis of these diseases is closely linked with increasing of prostaglandins expression [25]. Also, etoricoxib can be clinically beneficial in the prevention of ischemia and reperfusion injury by its work as anti-oxidant that help in prevent a free radical that cause tissue damage as well as its anti-inflammatory properties [26]. In addition to its characteristic as anti-inflammatory medication that may be act to decrease metabolic syndrome that accompany with obesity state [27]. Furthermore, it was demonstrated in a study performed in rats that etoricoxib have a good beneficial role cardiovascular events versus another type of pain killer [28]. While, inflammation associated with increased adipose cells size by altered adipokine release which is tightly associated with an increased inflammatory response in obesity Lagathu, Bastard [29]; the decreasing of adipocyte size after treatment with etoricoxib can be clarified by a fact etoricoxib had the ability in suppression both inflammation and metabolic disorder resulted in decrease the size of adipocyte cell.

The maximum protective role against obesity complications induced by MSG e.g. heart tissue damage in combination treated group may be attributed by to an increase in the efficiency of inhibiting the inflammatory cell infiltration and necrosis by synergistic effect that produced from combination of these two drugs, as well as, combination medicinal therapy may be better than monotherapy by achieving synergistic therapeutic effect, dose and toxicity reduction, and to minimize or delay the induction of drug resistance.

Conclusion

The combination of etoricoxib and clopidogrel a medicinal therapy better than monotherapy by better therapeutic effect against obesity complications that induced by MSG, this can be seen on improvements of all parameters of this study, especially in histopathological pictures of heart and adipose tissue.

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