COMPARISON EFFECT OF DIFFERENT COMBINATIONS OF METFORMIN, ATORVASTATIN, CAPTOPRIL AND ASPIRIN ON OXIDATIVE STRESS MARKERS OF GASTRIC AND LIVER TISSUES OF DIABETIC RATS

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Some recent studies have been suggested high-dose aspirin consumption in diabetic patients because of its modifying effect on metabolism of glucose and lipid. In other side many researches showed aspirin induced gastrointestinal damage. Investigation of the effect of high-dose aspirin consumption in combinations with metformin, captopril and atorvastatin on oxidative stress markers in the stomach and liver tissues of diabetic rats. Rats divided into eleven groups: control (Cont), diabetic (D), and 9 treated groups with various combination of metformin (M), atorvastatin (AT), captopril (C), and aspirin (ASA). Animals were treated orally by M, C, AT and ASA daily for 6 weeks. Finally, oxidative markers were evaluated in stomach and liver tissues. The Malondialdehyde (MDA) level significantly boosted while total thiol content remarkably decreased in the diabetic group than the control. Administration of the different combinations of C, M, AT and ASA could significantly attenuate above parameters. Combination of the metformin, aspirin, atorvastatin and captopril has more marked effects on oxidative stress reduction in the stomach and liver tissues of diabetic rats and could ameliorated probably oxidative stress induced by aspirin.

Keywords: Diabetes, Stomach, Liver, Aspirin, Drugs combination

INTRODUCTION

There is an relation between diabetes and gastrointestinal and chronic liver disease¹. Gastrointestinal and liver disease are common in patients with diabetes mellitus². Evolving evidence proposes that oxidative stress has an key role in the diabetes complications pathogenesis including gastrointestinal and liver disease³.

Oxidative damage is arouse in hepatic and stomach cells by supra physiological glucose levels that lead to functional and morphological change in diabetic rats stomach and liver⁴.

Metformin is an anti-hyperglycemic drug that use in diabetic patients⁵. Oxidative stress is
decreased by metformin in addition to its effect on glucose decreasing in the treatment of Type 2 diabetes caused to this drug also introduced as attractive candidate for the prevention of stomach ulcer in diabetic patient.

Angiotensin converting enzyme (ACE) blockers such as captopril are consumed in diabetic patient for attenuate the diabetes-relevant cardiovascular disease. Antioxidant effects of ACE inhibitors such as captopril have been reported in many studies. New data demonstrate that RAS is well expressed and active in the GI tract however precise physiological function are to be defined. However, very rare researches have been focused on the effect of renin-angiotensin system (RAS) on the gastrointestinal (GI) system.

Diabetic are mostly suffering from dyslipidemia. One member of the statin’s family is atorvastatin that is consumed in diabetic subjects in order to lipid reduction. Also, many reports indicated the antioxidant and anti-inflammatory effects of statins. Statins can reduce the production of reactive oxygen species induced by NAD(P)H oxidase and antagonize the prooxidant effect of angiotensin II. Studies showed some statins significantly decrease stomach mucosal injury induced by indomethacin or ethanol in result of antioxidant properties.

Another drug that is widely prescribed for the primary prevention of cardiovascular disease in diabetes mellitus is aspirin (ASA). The protective effects of aspirin on cardiovascular disease in diabetic patients due to its antioxidant properties have been shown in many studies. Recent studies has been demonstrated modifying effect of high-dose of aspirin on lipid metabolism and glucose tolerance in type 2 diabetic subjects. Also, in our previous study, we observed the positive effects of co-administration of aspirin, metformin, captopril and atorvastatin on fasting blood glucose, serum lipid profile and oxidative stress markers of brain tissues of diabetic rats.

Considering the fact that our previous study suggested co-administration of these drugs had beneficial effect on brain tissues of diabetic rats, but in other side, many studies showed that nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin induced gastrointestinal damage and also experimental and clinical evidence propose that ROS have key role in NSAIDs induced stomach mucosal injury, hepatotoxicity and pathogenesis of gastrointestinal complications of diabetes. In this study we decided to examine the effect of different combinations of metformin, atorvastatin, captopril and aspirin on oxidative stress of stomach and liver tissues of streptozotocin (STZ) induced diabetic rat.

MATERIALS AND METHODS

Chemicals and drugs

Metformin, aspirin, captopril and atorvastatin prepared from Sigma Co.

Animals

In this study was used eighty-eight male Wistar rats (250–280 gm, 6 weeks old) obtained from Mashhad University of Medical Sciences, Mashhad, Iran. Rats were kept in standard condition in terms of food and water. All producers of the work were approved by Ethics Committee of Mashhad University of Medical Sciences.

Induction of diabetes

We used STZ (60 mg/kg) intraperitoneally for diabetes induction in rats. The fasting blood glucose (FBS) was measured three days after STZ administration. The FBS level ≥ 250 mg/dl was considered as diabetes induction improvement.

Grouping

Rats divided into eleven groups: control (Cont), diabetic (D), and treated groups with various combination of metformin (M), atorvastatin (AT), captopril (C), and aspirin (ASA). Animals were treated orally by M (300 mg/kg), C (50 mg/kg), AT (40 mg/kg) and ASA (120 mg/kg) daily for 6 weeks.

Sampling

The FBS was measured before STZ administration (day 0), 3 days after STZ administration (day 3), and 6 weeks after STZ administration (day 45). At the end animals were sacrificed under deep anesthesia, then the stomach and liver tissues were removed and then homogenized. Centrifugation of the homogenates was performed and supernatant
was stored at -80 °C until measurement of oxidative stress markers.

The oxidative stress markers including Catalase (CAT), Superoxide dismutase (SOD), tThiol content and Malondialdehyde (MDA) evaluated using related kits by biochemist.

Statistical analysis
Data was presented as mean ± SEM. After normality evaluating, the one-way ANOVA followed by the Tukey’s post hoc test using SPSS v.11 software. Statistical significance was considered as p< 0.05.

RESULTS AND DISCUSSION

Results
FBS on days 3 and 45 was significantly higher than the control in the diabetic and treated groups with drugs. FBS in all treated groups was significantly lower than the diabetic group on day 45 (p< 0.05 to p< 0.01) (Fig. 1).

MDA levels in the stomach and liver tissues collected from diabetic group were significantly higher than the control (p<0.001). Treated groups except D+M+C+ASA group in stomach tissue and also treated groups except D+M+C+ and ASA D+M+ASA groups in liver tissue, showed significant lower MDA levels than the diabetic group (p< 0.05) (Fig. 2 A and B).

![Fig. 1: Fasting blood glucose levels on days 0, 3, and 45 of the experimental periods. Data are shown as mean ± SEM. * p < 0.05, ** p < 0.01 and *** p < 0.001 show significant differences as compared to control group. And + p < 0.05 and ++ p < 0.01 show significant differences as compared to diabetic group (n=8 in each group).](image)

![Fig. 2: MDA concentration in the gaster (A) and liver (B) tissues. Data are shown as mean ± SEM. * p < 0.05, ** p < 0.01 and *** p < 0.001 show significant differences as compared to control group and + p < 0.05 show significant differences as compared to diabetic group (n=8 in each group).](image)
The tThiol content in the stomach and liver tissues collected from diabetic group were significantly lower than the control (p< 0.001 to p< 0.01). The tThiol content in stomach tissue of all drugs treated groups except D+ASA and D+M+ASA groups were significantly higher than the diabetic group (p<0.05). The tThiol content in liver tissue of treated groups was significantly higher than the diabetic group (p< 0.05) (Fig. 3-A and B).

The CAT and SOD activities of stomach and liver tissues of diabetic group did not different change than the control. In the stomach tissue of all drugs treated groups except D+ASA group significantly increase in levels of SOD activity were observed than the diabetic group (p< 0.05 to p< 0.01). The liver tissues of groups received combinations of three or four drugs had significantly high levels of SOD activity than the diabetic group (p< 0.05 and p< 0.01).

The significantly higher CAT activity also was determined in the stomach and liver tissues of treated groups than the diabetic group (p< 0.05) (Fig. 4 and 5 A and B).

Fig. 3: Total thiol concentrations in the gaster (A) and liver (B) tissues. Data are shown as mean ± SEM. * p < 0.05, ** p < 0.01 and *** p < 0.001 show significant differences as compared to control group. + p < 0.05 show significant differences as compared to non-treated diabetic group (n=8 in each group).

Fig. 4: SOD activity in the gaster (A) and liver (B) tissues. Data are shown as mean ± SEM. * p < 0.05 and ** p < 0.01 show significant differences as compared to control group; + p < 0.05, and ++ p < 0.01 show significant differences as compared to diabetic group; and $ p < 0.05 shows significant differences as compared to D+ASA treated group (n=8 in each group).
Discussion
As shown in this study, significantly increase in lipid peroxidation, SOD and catalase activities and reduce in total thiol content in the stomach and liver of non-treated diabetic group is similar to the results of studies that showed elevated TBARS levels and antioxidant enzymes activities during the progression of diabetes. Increased ROS lead to elevated levels of TBARS in liver and stomach and also this situation cause to increasing antioxidant enzymes activity in order to cope with ROS. These increasing could also be due to a reducing in the non-enzymic antioxidants such as total thiols.

Our results indicated that co-administration of the metformin, atorvastatin, captopril and aspirin can rectify the imbalance between ROS production and enzymes activity in the stomach and liver tissues of the diabetic rats. Many reports suggested the antioxidant role of atorvastatin, metformin and ACE inhibitors that led to decrease the peroxidation of lipid, increase in total thiol content, CAT and SOD activities that can be favorable for the liver and gastrointestinal system. Oxidative stress decreasing by metformin in addition to its effect on glucose decreasing in the treatment of Type 2 diabetes caused to this drug introduced as attractive candidate for the stomach ulcer prevention in diabetic patient. Also ACE blockers by remove of free radical and boost of prostaglandin E2 synthesis have gastro protective property. Some studies demonstrated atorvastatin exhibit gastro-protective effects and hepatoprotective effects via antioxidant properties. In the current work, the rate of oxidative stress markers in group received aspirin alone was similar to these markers in non-treated diabetic group. This is in line with other studies that have shown aspirin induced stomach damage by significantly increase in stress oxidative. However, in our study combination of aspirin with metformin, atorvastatin, and captopril drugs which usually consumed by diabetic patient, led to suppression of oxidative stress.

According to the results, the co-administration of these drugs synergistically enhances their antioxidant effect. Synergism effects of co-administration of these drugs present enhanced antioxidant effects. About the ROS scavenging effects of the metformin can mentioned to inhibition of complex I of the electron transport chain, activation of glucose-induced protein kinase C-β2, reduction of NAD(P)H oxidase activity, inducing thioredoxin expression through AMPK-FOXO3 activation and boosting the anti-oxidative system activities. Aspirin also can inhibits the ROS generation through inhibition of prostaglandins synthesis, and induction of heme oxygenase-1 (HO-1) expression that promotes the antioxidant system. The atorvastatin also affects on the Rac-1 and geranylgeranyl pyrophosphate.
(GGPP) and inhibits them, induces the HO, interference with NAD(P)H oxidase expression and activity and antagonized the pro-oxidant effect of angiotensin II. The captopril can reduced the ROS via suppression of angiotensin II and then superoxide radicals by NADPH oxidase and nitric oxide synthase.

In this study, treatment with various combinations of metformin, atorvastatin, aspirin, and captopril result in reduction in fasting blood glucose compared to diabetic group. Reducing blood glucose by these drugs can be one of the possible mechanisms for reducing oxidative stress.

Also results of our previous study that designed on the effect of combination of these drugs on lipid profiles showed that TG, cholesterol and LDL levels reduced while HDL level increased in drug-treated groups than the diabetic group. The study of the administration of combination of these drugs produced more highlighted beneficial effects compared to when each drug was administered alone. If we look at the effect of these drugs on the oxidative stress in this study and their effects on the lipid profile in our previous study, we find that there is a correlation between lipid profile improvement and oxidative stress reduction by these drugs. Maybe improving lipid profile was one of the possible mechanisms for reducing oxidative stress in patients treated with these drugs.

Two studies were indicated the combination of statins with the inhibition of angiotensin II system exhibit more potent effect on oxidative stress decreasing. Also another study reported putative additive effects of aspirin and statins in diabetes. Toussoulis showed co-administration of metformin and atorvastatin could be a useful combination in diabetic patients because of the increased antioxidant and antihyperlipidemic capacity of each drug by combination.

**Conclusion**

Our findings were demonstrated that co-administration of atorvastatin, metformin, aspirin and captopril in diabetic rat potentiate antioxidant effects of these drugs and decrease stress oxidative in the stomach and liver tissue of diabetic rat more than consumption these drugs alone. As a result, co-administration of aspirin, atorvastatin, metformin and captopril can rectify aspirin induced oxidative stress in stomach and liver. Improvement the lipid profile is one of the possible mechanisms for reduction of oxidative stress in co-administration of these drugs.

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مقایسه تأثیر تركیبات مختلفی از میتیوترومن وآتورفاستارین وکابوکربیل

ละเอیلست خوراکی بر علاوه علائم الکهاد التاکسیدی لانسارد المعدة والکبد لدى الجرذان

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افترشبت بعض الدراسات الحديثة استهلاك جرعة عالية من الأسبرین فی مرض السکري بسبب تأثیره المعدل على استقلب الجلیکوز والدهون. فی الجانب الآخر، ظهرت العديد من الاحقادات أن الأسبرین بسبب تأثیره في الجهاز الورمي، هدفت الدراسة إلى التحقق من تأثیر استهلاك جرعة عالية من الأسبرین وکابوکربیل وآتورفاستارین على علائم الکهاد التاکسیدی في أنسجة المعدة والکبد للفرائین المصابیة بالسکري. قسمت الجرذان إلى أربعة عشرة مجموعه: مجموعه الضابطة (D)، مجموعه السکري الغیر معالجة (M)، و 9 مجموعات معالجة مع مزجی (cont) مختلف من المیتیوترومن (ASA)، آتورفاستارین (AT)، کابوکربیل (C)، والاسبرین (M). تم علاج الحیوانات تحت تأثیر الدم من قبل يوميا لمدة 5 أسابيع. تُقسم SYSA و AT و C و M علائم الأکسیدا فی أنسجة المعدة والکبد. تُقسم الاحیان تحت تأثیر الفم باليوميا لمدة 5 أسابيع. تُقسم علامات الأکسیدا فی أنسجة المعدة والکبد. ارتفع مستوى MDA بشكل ملحوظ بينما انخفض إجمالي محتوى الثیول بشكل ملحوظ موضعی مشابه موضعی موضعی تأثیرًا أكثر ودعا على الحد من الکهاد التاکسیدی في أنسجة المعدة والکبد للفرائین المصابیة بالسکري ويمكن أن يخفف من الکهاد التاکسیدی الناجم عن الأسبرین.