The effect of ginkgo biloba extract in experimental strangulation ileus in a rat model

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ÖZET: **Deneysel strangulasyon ileusunda ginkgo biloba ekstraktının etkisi**

Ratlarda deneysel strangulasyon ileusu oluşturularak, ginkgo biloba ekstraktının (EGb) etkisi araştırıldı. Ratlar kontrol (n= 7) , strangulasyon ileusu (SI), (n= 11) ve strangulasyon ileusu +EGb (SI-EGb) (n= 11) olarak üç gruba ayrıldı. Kontrol grubuna cerrahi işlem uygulanmadı. İkinci ve üçüncü gruplarda 2.5 saat süreyle strangulasyon ileusu oluşturuldu. İkinci gruba serum fizyolojik ve üçüncü gruba 100 mg/kg 1 ml. serum fizyolojik içerisinde seyreltilmiş EGb, strangulasyon sonunda intraperitoneal olarak verildi. Strangulasyonun sonlandırılmasından 24 saat sonra relaparotomi uygulandı. Kan örnekleri alındı ve histopatolojik inceleme yapmak üzere ratlar öldürüldü. Doku çalışmalarında SL grubuna göre SI-EGb'nin doku redükte glutatyon düzeylerinin ve glutatyon peroksidaz aktivitelerinin arttığı gözlendi. Serum CK, AST, ALT, ALP aktiviteleri ve Pi düzeyleri her üç gruptada ölçüldü. Histopatolojik incelemede EGb verilmeyen grupta konjesyon, hemoraji, villus atrofisi, nekroz, goblet epitel kaybı ve infiltrasyon prosesinin daha şiddetli olduğu görüldü. İstatistiksel olarak da bu farklılık anlamlı bulundu. Sonuç olarak, strangulasyon ileusunda EGb'nin koruyucu etkileri olduğu gözlendi.

Anahtar kelimeler: **Strangulasyon ileusu, ginkgo biloba ekstraktı, redükte glutatyon, glutatyon peroksidaz**

SUMMARY:

Effect of the ginkgo biloba extract (Egb) in experimental strangulation ileus in rat model was investigated. The rats were divided into three groups as control (n= 7), strangulation ileus (SI, n= 11), and strangulation ileus-EGb (SI-EGb, n= 11). No surgical procedure was applied to the control group. In the second and third groups strangulation ileus was created for 2.5 hours. In the second group serum physologic and in the third group 100 mg/kg EGb diluted within 1 ml of serum physiologic was administered intraperitoneally, by the end of strangulation. Relaparatomies were done 24 hours after the termination of strangulation. Blood samples were taken and the rats were sacrificed for tissue and histopathological examinations. In the tissue studies, EGb was shown to increase tissue GSH levels and GSH-Px activities as compared with the SI group. Serum CK, AST, ALT, and ALP activities and Pi levels were determined in the three groups as well. In the histopathological examination, in the groups given no-EGb, congestion, hemorrhage, villus atrophy, necrosis, goblet epithelial loss, an infiltration process, were found to be much more severe. The difference was statistically significant as well. As a result, in the strangulation ileus, EGb was shown to have a preventive effect.

Key words: **Strangulation ileus, ginkgo biloba extract, reduced glutathione, glutathione peroxidase**

THE obstruction of intestinal lumen, and subsequent thrombosis of the related vessels to that part of the intestine result in strangulation ileus. Strangulation is the most common cause of mortality and morbidity in intestinal obstruction [1,2]. Ischemic injury to the intestinal mucosa occurs as the tissue is deprived of oxygen and other nutrients necessary to maintain cellular metabolism and integrity [3,4].

Parks and Granger have shown that relatively little injury to the intestinal mucosa occurs during the ischemic period, but the majority does occur as the begining of reperfusion [5]. In the cellular damage arised with the reperfusion of the ischemic intestinal segment, the reactive oxygen metabolites as such O_2-, H_2O_2, and OH were shown to have an important role [6,7]. Another important source of reactive oxygen metabolites in postischemic tissues is polymorphonuclear leucocytes [8].

Platelet activating factor (PAF) is a kind of phospholipid accompaning inflammatory reactions and it is shown to start releasing superoxide anion and H_2O_2 from the stimulated macrophages and polymorphonuclear leucocytes [8].

In this experimental study, we examined PAF antagonist extract of ginkgo biloba (EGb) effect on the tissue injury subsequent to intestinal and reperfusion insult. For this aim, we determined tissue reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) activities, following the examination of tissue histopathologies.

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Table 1. *GSH levels and GSH-Px activities in the control, SI and SI-EGb groups (Means ± SEM).*

	GSH (µmol/ mg prot)	GSH-Px (nmol /min/mg pr)
Control group	24.2±1.8	140.7±4.5
SI group	11.9±0.6	78.2±2.6
SI-EGb group	16.3±1.2	92.3±3.5
Control/SI	p<0.001	p<0.001
Control/SI-EGb	p<0.01	p<0.001
SI/SI-EGb	p<0.01	p<0.01

MATERIALS AND METHODS

Swiss-Albino rats, weighing between 190 vand 250 g were selected

for study. Animals were randomized to either receive saline solution (strangulation group, n= 11) or EGb at a dose of 100 mg/kg (EGb group n= 11). Also a healthy control group was build up with 7 animal. After and overnight fast, laparatomy was performed via a midline incision under ether anesthesia.

1. Control group: After the induction of anesthesia, the abdomen was opened by a midline incision. Blood samples were obtained by cardiac puncture and collected into heparinized tubes, and the rats were sacrificed. Then a 8 cm small intestinal segment was removed throughly and washed with cold saline solution. Tissue was prepared for histopathologic examination and for the determination of enzyme activity.

2. Strangulation ileus group: Following the mid-abdominal incision, an ileum segment of 8 cm, 4-5 cm proximal to the cecum was ligated with it's meso by 1 no. silk sutures, so that a clinical strangulation ileus was tried to be created experimentally. Subsequently the abdomen closed by silk sutures and 1 ml isotonic saline solution injected intraperitoneally. By 2.5 hours of ischemia period relaparatomy was done and strangulated segment was tied off and reperfusion was established subseqently. 1ml of isotonic saline solution administration repeated by the same route. At the 24'th hours of the reperfusion time blood samples were taken and the rats were sacrificed. Tissue specimens were taken for determination of tissue analysis and for histopathological examination.

3. Strangulation ileus-EGb group: In addition to the procedures in the group two, in this group following the strangulation, 100 mg/kg EGb was administered intraperitoneally, and following a period of 24 hours reperfusion time, blood and tissue samples were taken.

Blood samples were centrifuged for 10 minutes (4°C, 2000 xg) (Hermle ZK 510). Plasma samples were assayed in the same day. CK, AST, ALT, ALP and Pİ levels were measured by BM-Hitachi 911 automated analyzer using it's original commercial kits.

The small intestine tissue was stored at-20°C until analyzed. GSH levels and GSH-Px activity in the small intestine were assessed within a month. The tissues were thawed and homogenized in phosphate buffer (1M, pH= 7.0) for GSH-Px and in potassium chloride buffer (0.15M) for GSH with an Ultra-Turrax (T 25, Janke Kunkel, IKA Labortechnik). The homogenates were then sonicated with a Bandelin Sonopuls HD 70. After centrifugation for 20 minutes (4°C, 10.000xg), enzyme activity and protein analysis was performed on the supernatants. The GSH levels was determined according to the method of Beutler [9]. The GSH-Px activity was determined according to the method of Paglia and Valentine [10]. Protein concentrations were analyzed using a Lowry protein kit purchased from Sigma.

Tissues were fixed in 10% formaldehyde and processed routinely to paraffin wax, after the surgical procedure was done. Thin section were stained with H-E and observed objectively by an experienced histopathologist in blind by a light microscope. The specimens were ranked by the degree of congestion, hemorrhage, villus atrophy, necrosis, goblet epithelial infiltration.

Statistical analysis was done by student's t test and by chi-square test. Differences with p values lower than 0.05 were considered statistically significant.

RESULTS

Tissue levels of GSH and GSH-Px activities in the control, strangulation ileus (SI) and SI-EGb groups and statistical evaluation between the groups are shown on the Table 1. GSH levels in the control, SI and SI-EGb groups were 24.2±1.8, 11.9±0.6 and 16.3±1.2 mmol/mg prot. respectively. In the statistical study, except the control group in the remaining two groups GSH levels were found to have decreased significantly ($p<0.01$ respectively). As the SI and SI-EGb groups were compared the GSH levels decreased significantly, that is in the SI group it was highly reduced ($p<0.01$).

GSH-Px activity in the control, SI and SI-EGb groups measured as, 140.7±4.5, 78.2±2.6 and 92.3±3.5 nmol/min/mg prot. respectively. As the control group was compared with the other two groups, the decrease in these groups found to be statistically significant ($p<0.001$ for both). SI group and SI-EGb group were compared increase in the enzyme activity in the SI-EGb group was shown to bee statistically significant ($p<0.01$).

Serum CK, AST, ALT, ALP and P levels in the control, SI and SI-EGb groups are shown on the Table 2.

Figure 1. Decreased lieberkühn criptas, atrophy of villi and severly damaged organelles in lamina propria (HE x 64).

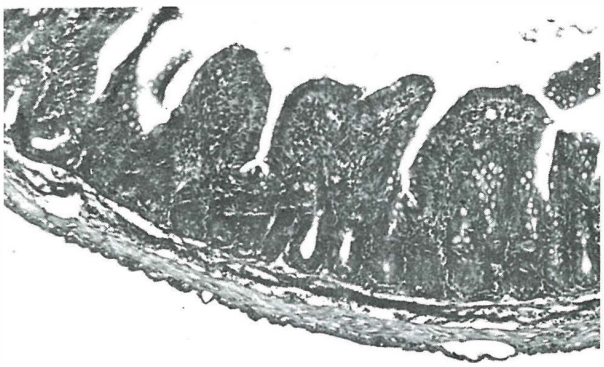

Figure 2. The intestinal villi and other organels are recovered with Egb tretament (HE x 64).

The results of the histopathological study as a degree of injury in the SI and SI-EGb groups are shown in the Figures 1,2.

In the histopathological examination of the SI and SI-EGb groups, the specimens were classified as, congestion, hemorrhage, villus atropy, necrosis, goblet epithelial loss and infiltration. The degree of these events evaluated as, none, mild, moderate and severe. In the statistical study, as the SI-EGb group was compared with SI group, tissue damage in the SI-EGb group was found to be decreased significantly ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.001$, $p<0.01$, $p<0.001$ recpectively). The results of histopathological examination are on the Table 3.

DISCUSSION

Vasoconstriction at the ischemic area caused by neurogenic, hormonal or locally active vasoconstrictive agents, is one of the most important events resulting in injury to small intestine [11,12]. A second important factor is the clustering of the red blood cells, leucocytes and platelets that all together obscure the capillary lumen [13].

In recent studies have shown that, PAF administered intraarterially not only increased intestinal microvascular permeability but also leucocyte adhesion in the mesenteric venules [14,15]. PAF activates platelets and leucocytes that in turn causes release of reative oxygen metabolites which subsequently result in endothelial damage [16].

In this experimental study, a strangulation ileus model, we investigate the effect of intraperitoneally administered EGb on small intestine pior to reestablishment of tissue perfusion following strangulation. For this aim, we determined tissue GSH levels and GSH-Px activities following of 2.5 hours strangulation and 24 hours perfusion period. We also measured serum CK, AST, ALT, ALP and Pi levels. Tissue histopathologies were studied as well.

Specific role of the GSH against post ischemic tissue injury is well known. GSH is a cofactor for GSH-Px and also an important cellular antioxidant and hydroxyl radical scavenger. GSH directly interact with free radicals, and metabolizes lipid hydroperoxides and hydrogen preoxides by GSH-Px [17]. In this study, as the control group was compared with SI and SI-EGb groups, in the latter two GSH was found to be decreased significantly ($p<0.001$, $p<0.01$ recpetively). For these two groups GSH-Px activity was reduced significantly as well ($p<0.001$ for both). As the SI group was compared with SI-EGb group, EGb was shown stimulate that is also statistically significant ($p<0.01$ for both, Table 1). In on experimental study by Younes et al. GSH level was found to

Table 2. *Serum enzyme activities and Pi levels in the control, SI and SI-EGb groups (Means ± SEM).*

	CK (U/L)	AST(U/L)	ALT(U/L)	ALP(U/L)	Pi (mg/dl)
Control	141±9	142±11	39±3	126±7	3.9±0.1
SI	422±69	259±17	53±5	203±24	5.1± 0.3
SI-EGb	227±32	255±13	52±3	165±15	4.3 ±0.3
Control/SI	p<0.01	p<0.001	p<0.05	p<0.05	p<0.05
Control/SI-EGb	p<0.05	p<0.001	p<0.01	p>0.05	p>0.05
SI/SI-EG b	p<0.05	p>0.05	p>0.05	p>0.05	p>0.05

Table 3. *The results of histopathological examination of tissues.*

	Congestion		Hemorhage		Villus Atropy		Necrosis		Loss of Goble		Infiltration	
	SI	EGb[a]	SI	EGb[b]	SI	EGb[c]	SI	EGb[c]	SI	EGb[b]	SI	EGb[c]
None	–	3	1	9	–	7	–	9	–	7	2	10
Slight	4	8	4	2	2	4	4	2	4	4	9	1
Moderate	1	–	6	–	4	–	3	–	3	–	–	–
Severe	6	–	–	–	5	–	4	–	4	–	–	–
Total	11	11	11	11	11	11	11	11	11	11	11	11

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

be decreased during the reperfusion, and SOD was shown to prevent this [18].

We measured serum CK, AST, ALT, ALP and Pi levels, in order to understand the relation betwen biochemical parameters and the tissue damage occuring in SI and SI-EGb groups. Serum CK ($p<0.01$), AST ($p<0.001$), levels were rather increased in the SI group as compared with control group, as well as the ALT, ALP and Pi values ($p<0.05$ respectively). The difference between the control and SI-EGb groups was statistically significant for AST ($p<0.01$) and ALT ($p<0.01$) that increased in the SI-EGb group, however CK, ALP and Pi values were not significant for these two groups. When the SI-EGb and SI groups were compared, CK found to be increased significantly ($p<0.05$), but the changes in the other parameters were not statistically valuable ($p>0.05$, Table 2). Previous studies shown that, those enzymes and Pi could be a statistical value in advanced intestinal ischemia and could be valuable in the long term follow up, but still not a specific indicator [19-22].

In our study, the benefit of the postocclusive EGb administration was histopathologically. As the SI-EGb and SI groups were compared, the drug was shown to decrease congestion ($p<0.05$), hemorrhage, goblet epithelial loss ($p<0.01$), villi atrophy, necrosis and infiltration ($p<0.001$) significantly. In the other studies in the literature, EGb was proven to decrease postocclusive histopathological damage, in the gastrointestinal system significantly [7, 23].

In recent years, EGb was shown to have benefical effects in cerebral ischemia, cardiovascular disorders, retinal diseases, and peripheral vascular disorders [24-26]. In this experimental study, we observed that, in the experimental strangulation ileus during the postocclusive stage, EGb prevented strangulated intestinal segment from the ischemia-reperfusion successfully both as the cellular levels and histopathologically. As conclusion, depending on our results and literature data, we can say that, EGb can be used for the patients admitted with strangulation ileus we believe that it will yield a good outcome.

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