

## **Anandamide mitigates ischemia/reperfusion (I/R)-induced gastric ulcer: modulation of inflammation and oxidative stress**

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### **ABSTRACT**

Anandamide is a cannabinoid receptors agonist that showed a protective effect against intestinal I/R model. However, its modulatory effect on I/R-induced gastric ulcer has not been previously elucidated. Therefore, the aim of this study was to delineate this modulatory effect of anandamide on gastric ulcer and the possible involvement of the liver. Rats were randomized into sham, I/R and anandamide treated group (1.5 $\mu$ mol/kg). Gastric I/R caused an increase in the gastric ulcer index in addition to elevation of the inflammatory mediators TNF- $\alpha$ , nuclear factor kappa-B (NF- $\kappa$ B), and MPO along with a significant decrease in adenosine monophosphate protein kinase (AMPK) activity; this effect was shown both in the stomach and liver. Moreover, the gastric insult resulted in an imbalance in the redox system, where it elevated the lipid peroxidation content along with a noticeable decrease in the defense system (GSH, CAT and SOD). Additionally, I/R caused a marked increase in the serum activity of the aminotransferases ALT and AST, documenting the involvement of liver injury with gastric I/R model. Pre-administration of anandamide proved its anti-inflammatory effect by decreasing the measured inflammatory parameters and increased that of AMPK. Regarding its endowed antioxidant property, anandamide enhanced tissue activity/content of CAT, SOD, as well as GSH, besides normalizing the MDA level. **Conclusion:** the protective mechanisms of anandamide against I/R-induced gastric ulcer and liver injury may be mediated by modulation of oxidative stress through the improvement of the endogenous defense system. In addition, the anti-inflammatory effect of anandamide may be facilitated *via* the AMPK and NF- $\kappa$ B pathways.

**Key words:** Gastric ischemia/reperfusion, anandamide, AMPK, TNF- $\alpha$ , NF- $\kappa$ B, MPO, lipid peroxidation, catalase, superoxide dismutase, liver, ALT, AST.

### **INTRODUCTION**

The gastric ischemia/reperfusion (I/R) insult is one of the major causes of gastric ulcer and this model resembles the myocardial ischemia-reperfusion (I/R) clinically (Cour et al., 2014). The I/R-induced gastric ulcer is accompanied by overproduction of reactive oxygen species (ROS) (Balk, 2000) resulting in elevation of lipid peroxidation and imbalance in redox defense system (El-Abhar et al., 2003). Moreover, this insult in the gut causes the infiltration of neutrophils (Horie *et al.*,

1997), as well as the enhancement of pro-inflammatory mediators, as TNF $\alpha$ , which is a powerful pro-inflammatory cytokine that plays a central role in the pathogenesis of I/R-induced gastric ulceration (Seyyed *et al.*, 2012). Moreover, ample of evidence revealed a close link between the reduced adenosine 5'-monophosphate protein kinase (AMPK) activity and inflammation, which is a crucial regulator of energy metabolic homeostasis at the cellular and organismal survival during stress (Jing et al., 2013). The

I/R insult suppresses the AMPK level along with the elevation of TNF- $\alpha$  and NF- $\kappa$ B by up-regulating the expression of protein phosphatase 2C, an inhibitor of AMPK signaling (Steinberg et al. 2006; Bai et al., 2010). The liver is the first distant organ affected by the intestinal I/R due to its coupled vasculature with the intestine organ (Turnage *et al.*, 1991) an effect that is mirrored by the activation of aminotransferases *viz.*, AST and ALT (Kurabayash et al 2005; Harada et al., 2007; Trocha et al., 2014). Anandamide is cannabinoid receptor agonists derived from the female plant of *Cannabis sativa L* family Cannabaceae (Green Candy Press 2005; Howlett et al., 2002). It has a potent anti-ulcerogenic and anti-secretory activity *via* the activation of the cannabinoid receptor CB<sub>1</sub> (Sofia et al., 1978; Coruzzi et al., 1999; Izzo et al., 2001). The present study aimed to assess the possible modulatory effect of anandamide on I/R-induced gastric ulcer. Moreover, the current work assessed the possible effect of the gastric I/R on a distant organ as the liver.

## **MATERIAL and METHODS**

### ***1-Animal***

Male Wistar rats, weighing 180-220 g (Research Institute of Ophthalmology, Giza, Egypt) were kept on a 12h light/dark cycles, constant environmental conditions and were maintained on a proper diet chow and water *ad libitum*. Prior to experiment (24 hrs) all animals were kept individually in wide mesh bottom cages and deprived of food only. All the surgical processes were performed under urethane anesthesia, and all efforts were made to minimize suffering.

### ***2- Drug***

Anandamide was purchased from Sigma-Aldrich Chemical Company (MO, USA). All other chemicals and reagents used were of

analytical grade. Anandamide (1.5  $\mu$ mol/kg, i.p) was dissolved in 99% absolute alcohol (1 ml) and completed with saline to 100 ml.

### ***3- Induction of gastric I/R***

The I/R model was performed according to the method previously described by Kotani et al. (2007). Briefly, under urethane anesthesia (1.25 g/kg, i.p), the celiac artery was occluded using a small disposable vascular clip to induce ischemia for 30 min.; afterwards reperfusion was allowed for 90 min. by declamping the artery.

### ***4- Experimental design***

Rats were divided into 3 groups (n=7-8), where in the first one, animals were anaesthetized and the celiac artery was manipulated only to serve as the sham-operated control group. Animals in the following groups were subjected to I/R; the untreated (second) group was denoted as the positive control (I/R), in which animals received the vehicle, 45 min. before I/R. Rats in the 3<sup>rd</sup> group received anandamide (1.5 $\mu$ mol/kg, i.p) 45 min. before the induction of gastric I/R.

### ***5- Biochemical measurements***

At the end of the reperfusion time, the abdomen was surgically opened and blood was drawn from the inferior vena cava to prepare serum, then animal were then killed and the stomach was excised, opened along the greater curvature, rinsed with ice cold saline and the extent of gross mucosal damage was assessed and calculated as the ulcer index (UI). This parameter was measured and expressed as the sum of ulcer lengths per stomach in mm (Nishida et al., 1998). Consequently, glandular mucosa was homogenized in ice-cold saline to obtain 20% homogenate using a stainless steel homogenizer (MPW-120, Poland). Moreover, the liver was collected from each rat, weighed and homogenized in ice-cold saline to obtain 20% homogenate. Different tissues (stomach, intestine, liver, kidney &

pancreas) homogenates were kept in aliquotes and stored at -80 °C till assessment of biochemical parameters.

### 5.1- Estimation of serum parameters

The serum level of the following parameters was evaluated using the corresponding kits and the catalogue number as shown in parentheses. AST and ALT (Teco diagnostics, CA, USA), serum TNF- $\alpha$  (Koma Biotech, seoul, Korea), NF- $\kappa$ B (EIAab, Wuhan, China) and serum AMPK (Abcam, Cambridge, UK).

### 5.2 Estimation of tissue parameters

#### 5.2.1. Estimation of the activity of meyaloperoxidase (MPO)

MPO (U/gm), a marker for tissue neutrophil infiltration, was assessed according to Bradley et al. (1982). The method is based upon measuring the hydrogen peroxide-dependent oxidation of o-dianisidine, catalyzed by MPO, which results in the formation of a compound exhibiting an increased absorbance at 460 nm.

#### 5.2.2. Estimation of malondialdehyde (MDA)

Lipid peroxidation (nmol/g tissue) was determined according to the method of Mihara and Uchiyama (1978), where the thiobarbituric acid reactive substances (TBARS), measured as malondialdehyde (MDA), was used as an index of lipid peroxides. The MDA-TBA adduct develops pink color, which was extracted by n-butanol and measured at two wave lengths, viz., 520 and 535nm.

#### 5.2.3. Estimation of reduced glutathione (GSH)

The method described by Ahmed et al. (1991) was adopted to assess the gastric non-protein sulfhydryl groups (mainly GSH) by reacting with Ellman's reagent after precipitation of protein SH-groups. The reaction with Ellman's reagent forms a

stable yellow color of 5 mercapto-2-nitrobenzoic acid, which was measured colorimetrically at 412 nm (mg/gm).

#### 5.2.4. Estimation of superoxide dismutase (SOD)

SOD activity was determined in the cytosolic fraction of the stomach homogenate according to method of Marklund & Marklund (1974). It depends on that the spontaneous autoxidation of pyrogallol, at alkaline pH, produces superoxide anion radical ( $O_2^{\cdot-}$ ), which in turn enhances more pyrogallol oxidation. The presence of SOD in the reaction medium inhibits pyrogallol autoxidation by scavenging the superoxide anion radical.

#### 5.2.5. Estimation of catalase (CAT)

This enzyme was assessed using ELISA colorimetric kit catalase (EIAab, Wuhan, China) as the manufacturer instructions.

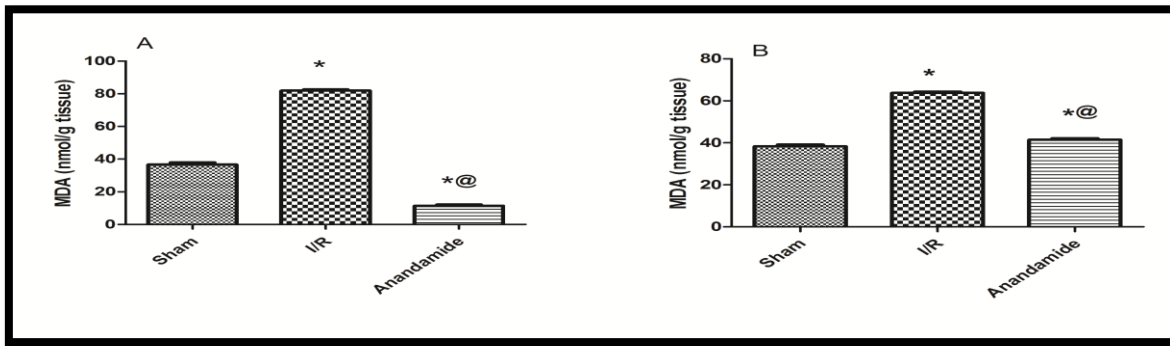
### 6. Statistical analysis

Data were expressed as mean  $\pm$  S.E.M of 7-8 animals. Statistical comparisons between means were carried out using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test. Statistical significance of difference was considered at  $P < 0.05$ .

## RESULTS

### 1- Effects on oxidative stress

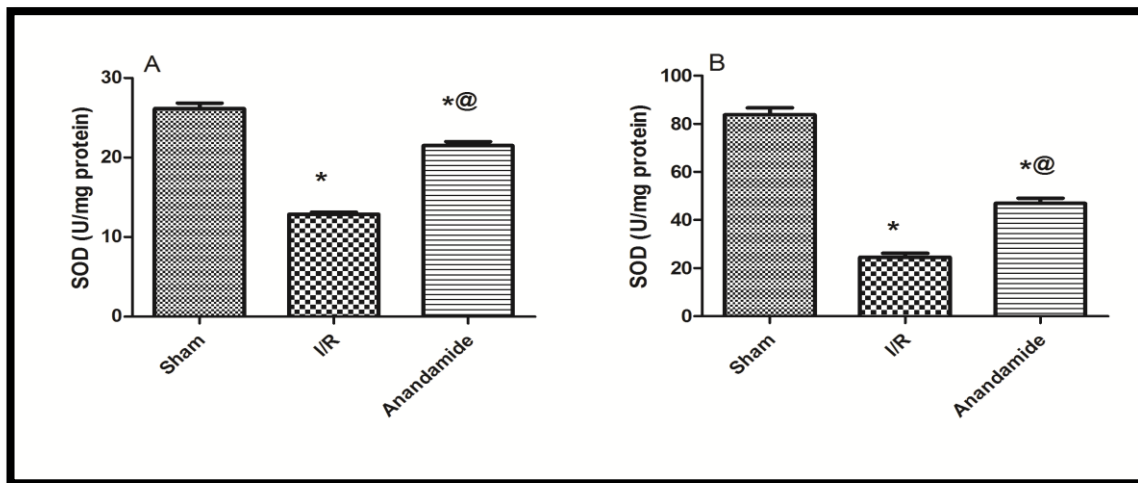
As depicted in **figure 1**, ischemia/reperfusion insult induced in (A) the stomach elevated lipid peroxides level to reach 2.2 folds compared to the sham control group. The same effect was observed in (B) the remote organ; viz., liver; this elevation was mounted to 166%, as compared to the corresponding sham control group. Pretreatment with anandamide, 45 min (Agurell, 1986) before I/R, has significantly  $p < 0.05$  hampered the elevated MDA level in gastric tissue (86%) and liver (35%) as compared to the I/R group.



**Figure (1):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on MDA content in (A) stomach and (B) liver of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (<sup>@</sup>) control groups, P<0.05.

Induction of I/R insult to stomach organ, inhibited one of the enzymatic defense system, SOD. In the (A) gastric tissue the SOD content significantly p <0.05 subsided by 51% as compared to the sham value. Similarly, SOD content was abated

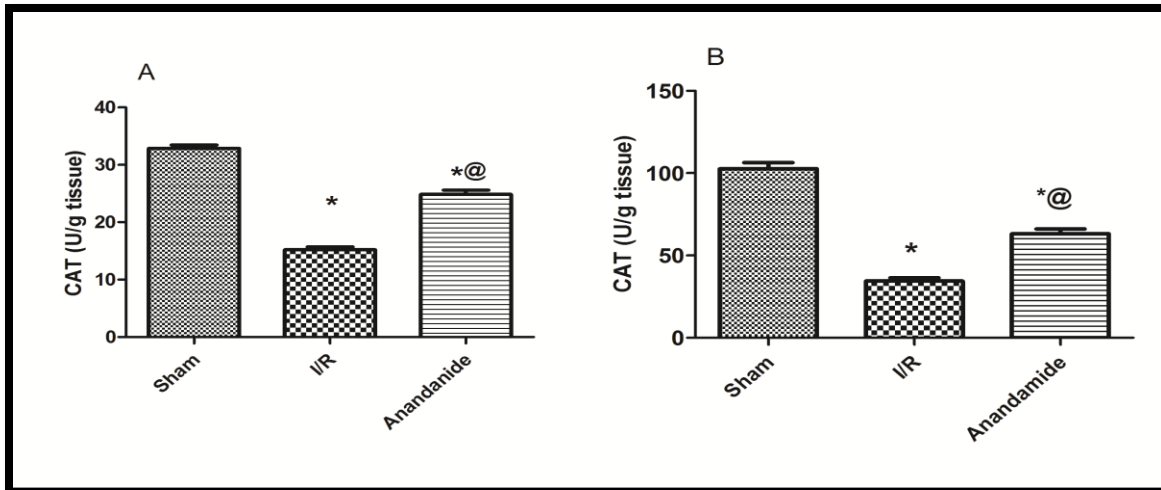
in the (B) liver to reach 24.6 U/mg proteins vs 83.8U/ mg in the corresponding sham group. All these altered values were significantly p <0.05 opposed in the anandamide pretreated group as illustrated in **figure 2**.



**Figure (2):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on SOD content in (A) stomach and (B) liver of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (<sup>@</sup>) control groups, P<0.05.

The amount of the CAT enzyme was also evaluated as another anti-oxidant enzyme in both organs; after I/R mediated in the stomach, the insult leveled off the gastric CAT content, as well as the other remote

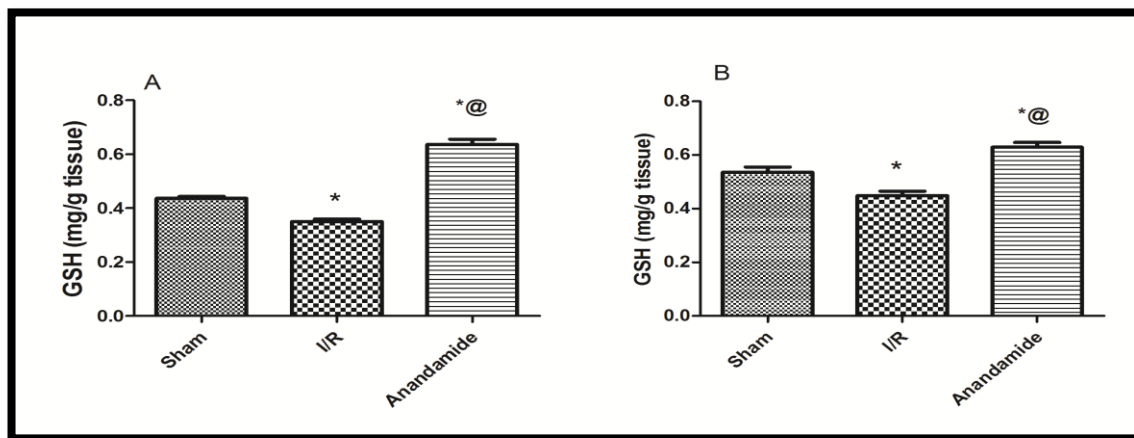
organ, liver. Prophylactic treatment with anandamide before I/R induction restored the level of CAT by 64% in the stomach and 83.4% in the liver as seen in **figure 3**.



**Figure (3):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on CAT content in (A) stomach and (B) liver of rats subjected to ischemia/reperfusion (I/R; 30/90 min.) Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (@) control groups,  $P < 0.05$ .

One of the non-enzymatic defense systems is GSH was evaluated in gastric mucosa and liver after I/R insult. The GSH content in the stomach was significantly  $p < 0.05$  inhibited by 20% in the I/R group; alike the effect on the stomach, I/R sharply

lowered the GSH content in the liver (84%), as compared to the normal sham group. The I/R-induced alteration in GSH level was significantly  $p < 0.05$  hindered by the prophylactic injection of anandamide as depicted in **figure 4**.



**Figure (4):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on GSH content in (A) stomach and (B) liver of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (@) control groups,  $P < 0.05$ .

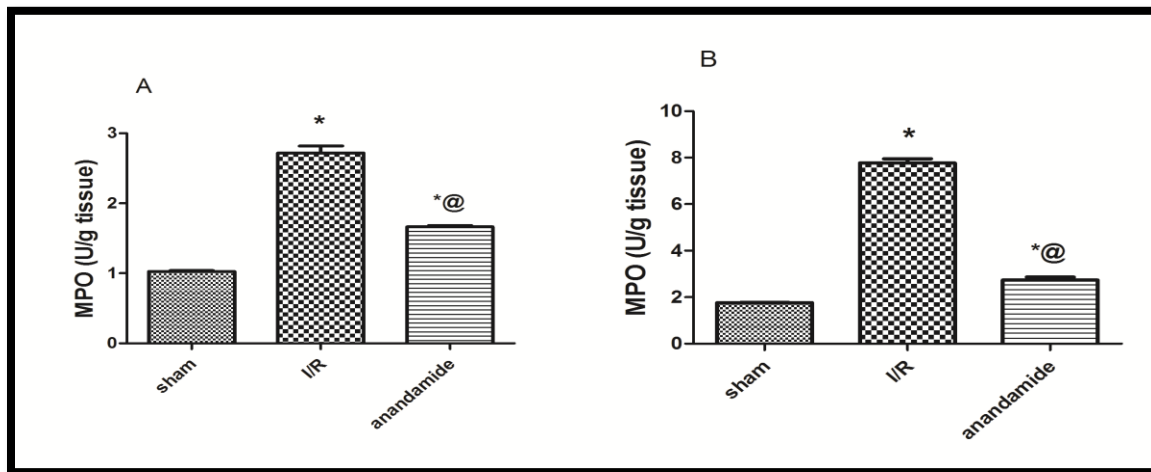
## 2- Effect on neutrophil infiltration

The I/R effect on the tested organs boosted the infiltration of neutrophils intracellularly, as manifested by the significant rise in the MPO level. The

intraperitoneal administration of anandamide guarded against the I/R effect; in the stomach and liver, the MPO activity was significantly  $p < 0.05$  limited

to 65% and 38.25%, respectively, as

compared to the I/R effect (Fig. 5).

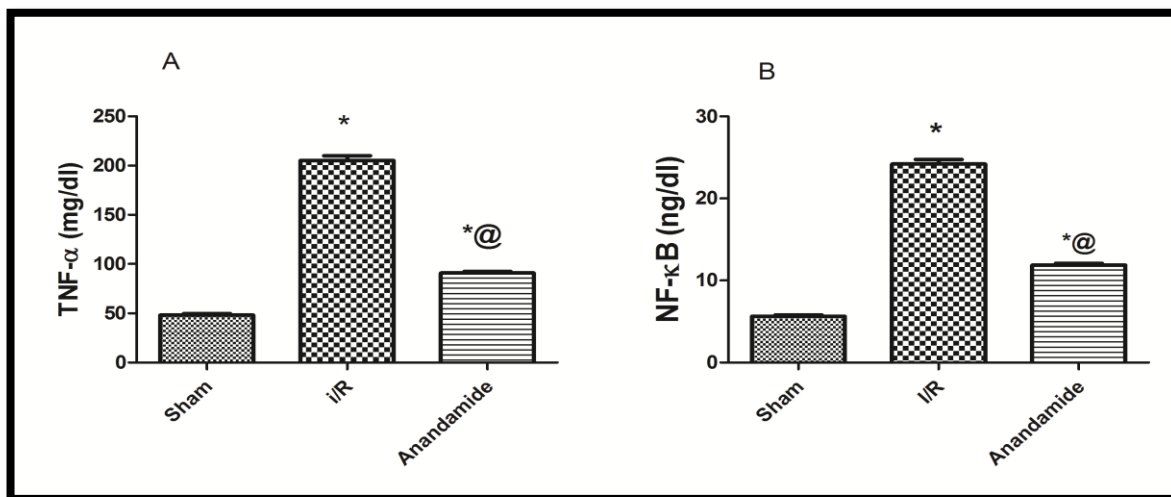


**Figure (5):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on MPO content in (A) stomach and (B) liver of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (<sup>@</sup>) control groups,  $P < 0.05$ .

### 3- Effect on pro-inflammatory mediators

Following the induction of I/R insult the serum TNF- $\alpha$  level was boosted to be around 4 times higher than the sham group, as a consequent to the inflammatory cascade. Pretreatment with anandamide significantly  $p < 0.05$  evoked a noticeable protection against the I/R insult (Fig. 6). By inspecting the serum content of NF- $\kappa$ B, the insult induced

by I/R on stomach and liver significantly  $p < 0.05$  augmented the level of the pro-inflammatory mediator by 4 folds in comparison with sham group, while by the pre-treatment with anandamide, the harmful effect of I/R has been significantly  $p < 0.05$  halved.

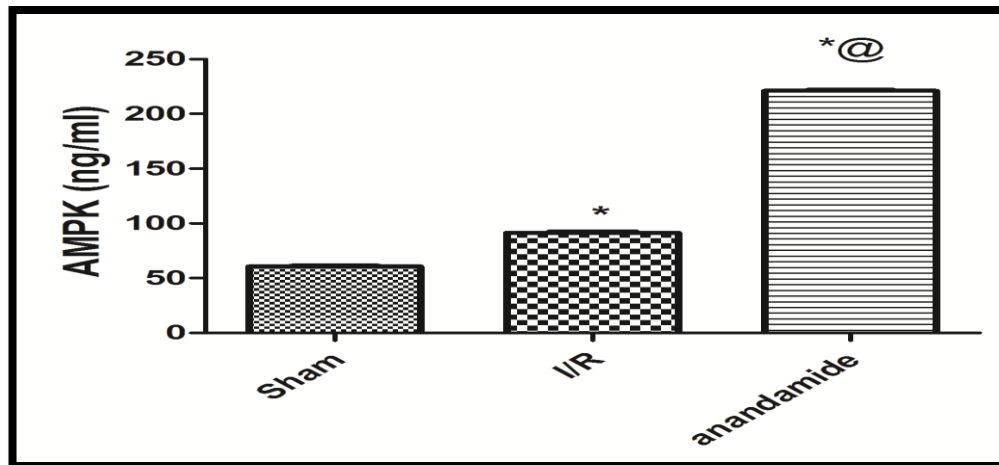


**Figure (6):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on (A) serum TNF  $\alpha$  and (B) NF- $\kappa$ B levels in rat subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (<sup>@</sup>) control groups,  $P < 0.05$ .

#### 4- Effect on serum adenosine 5'-monophosphate protein kinase (AMPK)

AMPK acts as an inhibitor of the inflammatory mediators signaling. However, its level was significantly  $p < 0.05$  increased

by I/R to reach to 1.5 fold compared to sham group. The pre-administration of anandamide significantly  $p < 0.05$  elevated it further to reach 3.6 times that of the sham control rats and 2.4 folds of the I/R group (Fig. 7).

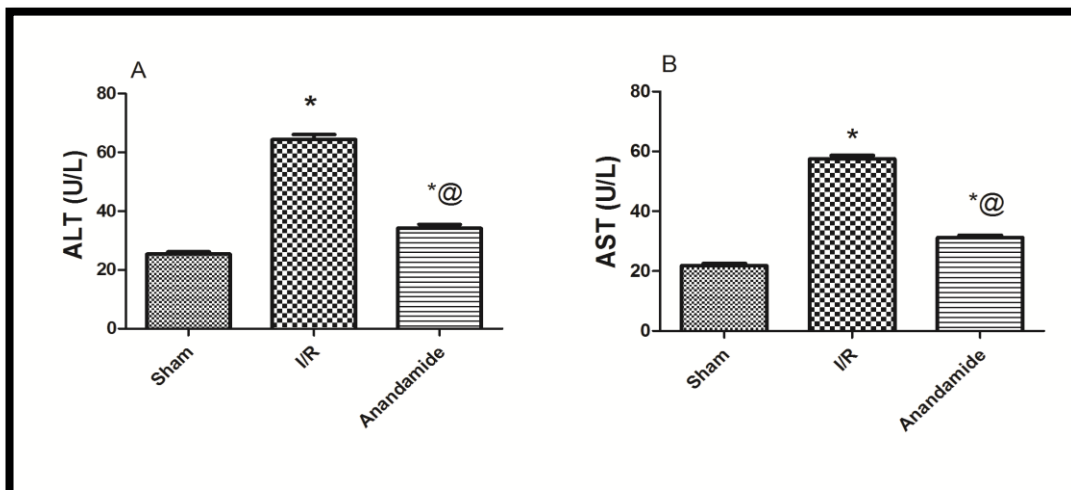


**Figure (7):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on serum AMPK activity of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (@) control groups,  $P < 0.05$ .

#### 5- Effect on liver function

As depicted in **figure 8**, (A) the activity of ALT and (B) AST enzymes showed a significant surge caused by I/R; this activation was 2 and 2.6 folds that of the

sham values. However, the anandamide pre-treated group significantly  $p < 0.05$  leveled off ALT and AST by 47% and 45.4%, respectively, compared to the I/R group.



**Figure (8):** Effect of 30 min. pre-administration of anandamide (0.4 ml/kg, i.p) on serum activities of (A) ALT and (B) AST of rats subjected to ischemia/reperfusion (I/R; 30/90 min.). Values are expressed as mean  $\pm$  SEM (n=8 animals). Statistical analysis was carried out using one way ANOVA followed by Tukey's test for multiple comparisons. Significantly different from sham (\*) and I/R (@) control groups,  $P < 0.05$ .

## DISCUSSION

I/R injury is an interruption of organ's blood flow with its subsequent lack of oxygen and nutrient supply followed by reflow of blood and oxygen supply ending in ATP depletion, loss of cell membrane integrity and cellular damage (Serracino-Ingloft et al., 2001; Romanque et al., 2005). In the current work and as a result to the I/R-overproduction of ROS and FRs, lipid peroxidation level was significantly increased in the tested organs, *viz.*, the stomach and liver. Redox imbalance was further verified by the decrease in the assessed endogenous enzymatic antioxidants, such as SOD and CAT, as well as the non-enzymatic antioxidant GSH, leaving the present organs more vulnerable to the increased shower of ROS. The I/R-induced gastric injury was reflected by the increased ulcer index, an effect that can be explained by the inhibition of NO and the protective prostaglandins (Yusifzade et al., 2009).

In the present experimental study, the rats pretreated with anandamide exhibit a significant attenuation in the I/R-mediated oxidative stress in both stomach and liver. It lowered lipid peroxidation and enhanced the content of the endogenous enzymatic (SOD, CAT) and non-enzymatic (GSH) antioxidant defense systems, to reach a normal level or even to exceed it, especially in the GSH content. Moreover, the anti-oxidant effect of anandamide was supported by other *in-vitro* (Jia et al., 2014) and *in-vivo* studies following testicular torsion (Beheshtian et al., 2008) and transient global cerebral ischemia (Zhang et al., 2009). The NADPH-dependent oxidase (Nox) system is one source of ROS overproduction (Klcbanoff, 1988; Reilly et al., 1991). The Nox2, a subunit of Nox, contributes to oxidative damage of the tissues (Ano et al., 2010) and its inhibition can attenuate oxidative stress injury (Ha et al., 2010; Ye et al., 2012).

Another potential source of ROS production is neutrophils through the Nox system (Weiss and Ward, 1982). In the present study, the I/R insult encouraged the infiltration of neutrophils as evidenced by the gastric and hepatic increase in their marker, MPO. Since the stomach is in a constant state of controlled inflammation, thus, amplification of the inflammatory response triggers pathological responses to I/R, beside the infiltration of the inflammatory cells, assessed as MPO. Former researchers have shown that induction of I/R stomach (Horie *et al.*, 1997) increased the level of MPO in the affected organ, as well as the remote ones, findings that mimic the present results.

Upon injecting the rats with anandamide, it lowered the MPO level in both organs, pointing to its anti-inflammatory, as well as its anti-oxidant properties. The anti-neutrophil infiltration effect of anandamide may be attributed to its ability to activate the peroxisome proliferator-activated receptor- $\alpha$ -dependent receptors (PPAR- $\alpha$ ), as reported by Alhouayek *et al.* (2014). These authors stated that increasing PPAR- $\alpha$  reduces neutrophils infiltration, an effect that may be partly responsible for the decreased oxidative stress. The anandamide-mediated inhibition of oxidative stress and neutrophil infiltration can explain the minimized gastric ulcer seen in the anandamide group, results that coincide with previous ones, pointing to its upregulating effect on CB2 protective receptors, in addition to reduce the mucosal oxidative stress (Warzecha et al., 2011).

In the present work, both TNF $\alpha$  and NF- $\kappa$ B were elevated in the two organs. TNF $\alpha$  is a signature cytokine of inflammation, where it triggers the activation of the NF- $\kappa$ B pathway, and in a vicious cycle pattern, the activated NF- $\kappa$ B signaling pathway results in the induction of TNF  $\alpha$  as a downstream pathway (Sancho et al., 2003). Anandamide, in the present study, verified its anti-inflammatory character by inhibiting the



amount of TNF  $\alpha$  and NF- $\kappa$ B. These findings coincide with that of previous studies, stating that anandamide decreases the level of TNF $\alpha$  (Rettori et al., 2012) and NF- $\kappa$ B (Sancho et al., 2003). The latter effect was attributed to the inhibition of TNF $\alpha$ -mediated NF- $\kappa$ B activation *via* direct inhibition of the I $\kappa$ B kinase (IKK)- $\beta$  with the subsequent inhibition of NF- $\kappa$ B binding to DNA, lowering, thus, the NF- $\kappa$ B-dependent transcription. Furthermore, the study of Esposito and coworkers (2014) offered another explanation of the anandamide anti-inflammatory effect through the involvement of the toll like receptor (TLR) pathway.

AMPK is a crucial regulator of energy metabolic homeostasis at the cellular and organismal survival during stress (Jing et al., 2013). Several studies have revealed a close link between the reduced AMPK activity and inflammation (Gauthier et al., 2011). Steinberg et al. (2006) observed that TNF- $\alpha$  suppressed the activity of AMPK by up-regulating the expression of protein phosphatase 2C, an inhibitor of AMPK signaling (Bai et al., 2010). However, this was not the case in the present work, where I/R slightly increased the AMPK level, an effect that can be considered as a compensatory mechanism of the body against the I/R injury.

Administration of anandamide reversed the I/R action, as it elevated the AMPK by 4 folds and at the same time inhibited the inflammatory markers. There is emerging evidence that through its signaling network, AMPK can suppress the activation of NF- $\kappa$ B system indirectly, where NF- $\kappa$ B by itself is not a direct target of phosphorylation by AMPK; however, several downstream targets of AMPK, e.g., SIRT1, PGC-1 $\alpha$ , p53, and Forkhead box O (FoxO) factors are responsible for inhibiting the NF- $\kappa$ B signaling (Salminen et al., 2011). Moreover, AMPK is linked also to oxidative stresses as documented by the findings of the present

work and by Salminen et al. (2011). In a previous study, Li et al. (2009) revealed that AMPK signaling induced the expression of mitochondrial uncoupling protein-2 (UCP-2), hence, reducing the production of superoxide radicals in hyperglycemic endothelial cells. Besides the AMPK-mediated induction UCP-2 protein, the production of ROS is inhibited by AMPK through suppression of the Nox system (Wang et al., 2010). In an interplay between AMPK, NF- $\kappa$ B and oxidative stress, Wang et al. (2010) stated that the mechanism whereby AMPK inhibits the NF- $\kappa$ B signaling involves the downregulation of different Nox subunits leading to the alleviation of oxidative stress, besides the anti-inflammatory action.

Several studies point to the injurious effect of intestinal I/R on liver, which is considered the first distant organ affected by this insult (Towfigh *et al.*, 2000) reported that the liver is, due to its coupled vasculature with the intestine, however, this is the first study to correlate the influence of gastric I/R on liver function. An ample of evidence reported that the liver is a major site for dysfunction after I/R insult and this injury is coupled by a significant elevation of its aminotransferases *viz.*, AST and ALT (Kurabayash et al., 2005; Harada et al., 2007; Trocha et al., 2014). These reports support the current findings, where the gastric I/R resulted in hepatic dysfunction manifested by elevation in both AST and ALT. Similar results were reported by many investigators using gut I/R. In the present investigation, the pretreated rats with anandamide hampered the I/R effect on the measured aminotransferases, indicating a protective effect of anandamide mediated by its antioxidant and anti-inflammatory characters. In a recent study, B $\acute{a}$ tkai et al. (2012) supported the present findings, where anandamide, in their study also protected liver injury mediated by a hepatic I/R model

by virtue of its antioxidant/anti-inflammatory characters.

In conclusion, the present study showed that gastric I/R-induced damage can entail remote organs as liver and that anandamide protected against this harmful effect by virtue of its antioxidant and anti-inflammatory characters.

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## الانانداميد يقوم بتخفيف القرحة وتغيير الالتهاب وعوامل الاكسده الاجهاديه المحدثه بالقصور الدموي

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الانانداميد هو ناهض مستقبلات القنب الذي أظهر وجود تأثير وقائي ضد قصور الامداد الدموي في الامعاء . مع ذلك ، الأثر التغييرى على قرحة المعدة الناجم عن قصور الامداد الدموي لم يتم توضيحها سابقا. لذلك ، كان الهدف من هذه الدراسة هو لتحديد هذا الأثر التغييرى من الانانداميد على قرحة المعدة و احتمال تورط الكبد. تم اختيار الفئران بصورة عشوائية في الشام ، مجموعته قصور الامداد الدموي و مجموعة المعالجة با الانانداميد ( 11.5 µmo / كغ ) . قصور الامداد الدموي في المعدة تسبب زيادة في مؤشر قرحة المعدة ، بالإضافة إلى ارتفاع وسائط الالتهابات مثل: عامل نخر الورم ألفا، العامل النووي كابا بي و انزيم ميالوبيروكسيداز جنبا إلى جنب مع انخفاض ملحوظ في نشاط أدينوزين مونو فوسفات بروتين كيناز ؛ وقد ظهر هذا التأثير سواء في المعدة و الكبد. علاوة على ذلك فإن تدمير المعدة أدى الى حدوث خلل في نظام الأوكسدة والاختزال ، حيث ارتقى محتوى الدهون المؤكسدة مع انخفاض ملحوظ في نظام الدفاع(السوبر اوكسيد ديسميوتيزر, الكاتلاز والجلوتاثيون).بالإضافة إلى ذلك ، قصور الامداد الدموي تسبب زيادة ملحوظة في نشاط أنزيمات الكبد مثل: أسبرتات أمينو ترانسفيراز (AST) و الألبين أمينو ترانسفيراز(ALT) وهذا دليل توثيقي على تأثر الكبد بنوذج قصور الامداد الدموي المحدث في المعده . أثبت الانانداميد قبل اعطائه تأثيره المضاد للالتهابات عن طريق خفض معلمات الالتهابات و زياده في مستوى أدينوزين مونو فوسفات بروتين كيناز بالاضافه الى ذلك وجود زياده في مستوى الانزيمات المضاده للاكسده مثل: السوبر اوكسيد ديسميوتيزر, الكاتلاز والجلوتاثيون جنب الى جنب مع ارجاع ال (إم دي إيه)الى المستوى الطبيعي.

تتلخص هذه الدراسة في ان الاليات الوقائيه لماده الانانداميد لقرحة المعده الناجمه عن قصور الامداد الدموي و إصابة الكبد قد تكون بوساطه تعديل الاكسدة من خلال تحسين نظام الدفاع الذاتية. بالإضافة إلى ذلك ، فإن التأثير المضاد للالتهابات لماده الانانداميد قد يتيسر عن طريق مسارات الادينوزين مونو فوسفات بروتين كيناز و العامل النووي كابا بي.