

Uric Acid Is a Key Player in Salt-Induced Endothelial Dysfunction: The Therapeutic Role of Stigma Maydis (Corn Silk) Extract

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ABSTRACT

Hyperuricemia has been implicated in the pathogenesis and complications of cardiovascular diseases with associated elevated oxidant events. There is evidence that excessive salt intake results in cardiometabolic disturbances but the mechanism is elusive. Also, *Stigma maydis* (corn silk) is noted for its antioxidant properties among other beneficial roles. This study, therefore, aimed to establish the effect of high salt diet (SD) on uric acid (UA) production and the role of *Stigma maydis* in salt-induced phenotypes. Four groups of randomly selected rats (n=5) were fed with normal rat feed (CTR), corn silk extract (CS; 500mg/kg), SD (8%) and corn silk extract plus high salt feed (SD+CS). After six weeks of the experimental procedure, each animal was anesthetized by exposure to chloroform vapor and blood samples collected by cardiac puncture. Data were expressed in mean $\pm$ SEM and p-values < 0.05 were accepted as significant. High salt diet resulted in reduced plasma superoxide dismutase (SOD), nitric oxide (NO) and glutathione peroxidase (GPx) but not endothelial nitric oxide synthase (eNOS). Also, plasma UA and vascular cell adhesion molecule-1 (VCAM-1) increased in the SD group compared with control. However, *Stigma maydis* extract in SD-exposed group increased NO and GPx and not SOD. Also, *Stigma maydis* extract attenuated UA and VCAM-1. In conclusion, high salt intake may initiate deleterious cardiovascular events through UA- dependent mechanism and *Stigma maydis* extract has therapeutic potential in high salt-induced oxidative damage and/or UA-dependent endothelial pathologies.

KEYWORDS: Hyperuricemia, Corn silk, High Salt, Uric acid, Wistar rats, Endothelial dysfunction

INTRODUCTION

Oxidative stress is involved in the advancement of endothelial dysfunction (Silva et al. 2012) and vascular oxidative stress is usually triggered by many pathways, including the xanthine oxidase (XO) pathway (Kelley et al. 2010). Xanthine oxidase catalyzes the production of uric acid (UA) during purine catabolism and generates reactive oxygen species in the process (Saugstad 1996). The UA status of a biological system can be used to reflect the level of oxidative stress and also a strong predictor of macrovascular complications (Bos 2006). The mechanism(s) by which UA may engender organ damage is still partly understood, but there is growing evidence that an endothelial dysfunction is a fundamental event and in association with UA affects cardiovascular function and structure (Johnson et al. 2003). An array of experiments in rats demonstrated that elevated plasma uric acid elicited by uricase inhibition triggers hypertension and disrupts nitric oxide (NO) production in the macula densa; a point on the distal end of the thick ascending limb containing densely packed granules, while treatment with NO precursor (L-arginine) reduces hypertension and renal injury (Mazzali et al. 2001; Sanchez-Lozada et al. 2005).

Exposure of the cells to excess oxidants damage the cell proteins, provoke inflammation and promote apoptosis (Droge et al. 2002). Although oxidants are required for immune response signaling, cellular antioxidant molecules play a vital role in maintaining physiological redox status. SOD plays a key antioxidant role in nearly all living cells exposed to oxygen as singlet oxygen is one of the main reactive oxygen species in the cell. Also, GPx is a selenium-containing antioxidant enzyme that effectively reduces H_2O_2 and lipid peroxides to water and lipid alcohols respectively, and in turn oxidizes glutathione to glutathione disulfide (Hecker et al. 2013). Reduced glutathione has also been shown to play a major role in the regulation of intracellular redox reactions (Hecker et al. 2013).

Corn belongs to the grass family *Graminae* (*Zea mays*). It is a highly successful cereal grass among others in the equatorial region. Corn silk (*Stigma maydis*) is made from stigmas, the yellowish thread like strands from the female inflorescence of maize. Corn silk (CS) is an important herb used traditionally by the Chinese, and Native Americans to treat many diseases. It is also used as traditional medicine in many parts of the world such as Turkey, United States and France (Khairunnisa et al. 2012). It is a waste material from corn cultivation and is in abundant supply (Maksimović et al. 2005). It has been consumed for a long time as a therapeutic remedy for various illnesses and is important as an alternative natural-based treatment. Other beneficial treatments of corn silk include anti-fatigue activity, anti-depressant activity and diuretic activity (Hu et al. 2010). In addition, it possesses an excellent antioxidant capacity (Ebrahimzadeh et al. 2008); and demonstrated protective effects in radiation and nephrotoxicity (Bai et al. 2010; Sepehri et al. 2011).

High salt diet, on the other hand, is a diet that has high sodium content in it. Estimates indicate that 8% salt diet in rats is equivalent to 40g/day human, an intake that is significantly higher than what is obtainable in the normal human diet (Luft 1990). There is growing evidence that excessive dietary salt promotes the development of not only insulin resistance but also hypertension, in humans and animal models (Donovan et al., 1993; Ogihara et al. 2001; Ogihara et al. 2002). Furthermore, a high-salt diet in Sprague Dawley rats led to insulin resistance, decreased glucose utility in skeletal muscle, and elevated blood pressure (Ogihara et al. 2002). Therefore, this study aimed at testing the hypothesis that uric acid-led oxidative events are involved in salt-induced endothelial derangements and stigma maydis extract has therapeutic potential to resolve this salt-engendered condition.

MATERIAL AND METHODS

Animals.

Twenty (20) female Wistar rats with a mean weight of 135 ± 1 g were acclimatized for one week and randomly allotted into 4 groups of 5 rats each. They were maintained at a standard temperature (28 °C) in a well-controlled lit room with food and water provided ad libitum. The number and suffering of animals were reduced to the barest minimum. The study was carried out in line with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was ratified by the ethical review committee, University of Ilorin, Ilorin, Nigeria.

Extract preparation

Freshly collected stigma maydis (Figure 1) ~50 g was thoroughly washed with distilled water, cut into shreds and air dried at room temperature for 7 days and then dissolved in 200 ml of 100 % methanol (MeOH) in a conical flask. This was then placed in a shaker for approximately 120 hours at $28 \pm 2^\circ\text{C}$. The resulting extracts were then transferred to clean vessels, evaporated to dryness, and re-dissolved in dimethyl sulfoxide (DMSO) according to the methods of Erica et al., 2016 to yield a stock concentration of approximately 200 mg/ml. The stock concentration was filtered with a membrane filter and stored in an amber bottle at room temperature. Extracts were administered at a dose of 500 mg/kg body weight of rats in distilled water p.o. (Ha et al. 2018).

Treatment

After a week of acclimatization, groups were fed with normal rat chow (CTR), extract of corn silk (CS), high salt diet (SD) and high salt diet with corn silk extract (SD+CS). After 6 weeks of the experimental procedure, the animals were anesthetized with chloroform by inhalation and blood samples were collected by cardiac puncture and dispensed into lithium heparinized sample bottles. The blood was then centrifuged at 5000 rpm at 10 °C for 15 minutes to separate the plasma from the cells.

Determination of Sodium ion content in Feed

Atomic emission spectroscopy method was used to determine the Na⁺ content of the feed sample. High salt feed consisting of 8 % NaCl (Sofola et al. 2002) was prepared and fed to the SD group instead of 0.3% NaCl found in the normal rat chow.

Phytochemical Analysis

Phytochemical screening (PS) of the local CS with voucher number UILH/001/1219 was done for this study by standard photochemical screening according to the methods of George, 2016 and the proximate compositions of the extract obtained. PS of the CS reflected that alkaloids contain the highest % by weight/kg (115.69 mg/log) followed by terpenoids (90.36 mg/log).

Biochemical Assay

Plasma vascular cell adhesion molecule-1 (VCAM-1), nitric oxide (NO), uric acid (UA) were determined by using ELISA kit (E-EL-R2434) from Elabscience (Wuhan, China). Glutathione peroxidase (GPx), superoxide dismutase (SOD) and endothelial nitric oxide synthase (eNOS) were measured by the standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, United Kingdom),

Statistical Analysis

Results were presented as means $\pm$ SEM. The test for significance was done using one-way ANOVA and LSD post hoc test. $P < 0.05$ was accepted as significant. All statistical comparisons and test were done using version 20 of the Statistical Package for Social Sciences (SPSS).

RESULTS

Stigma Maydis extract heightens circulating glutathione peroxidase in high salt-fed rats.

Plasma glutathione peroxidase (GPx) was significantly reduced compared with control in the SD group. However, Stigma Maydis extract treatment with or without salt diet led to elevated plasma glutathione peroxidase compared with control and SD groups (*Figure 2*).

Stigma Maydis extract without a high salt diet increases plasma superoxide dismutase.

Plasma superoxide dismutase did not change compared with control in SD and SD+CS groups but significantly increased in CS when compared with control. The impact of high salt intake on redox activities revealed is independent of superoxide dismutase (*Figure 3*).

Stigma Maydis extract increased nitric oxide synthesis in high salt-fed rats

In the SD group, NO but not endothelial eNOS reduced significantly compared with CTR. Nevertheless, NO and eNOS increased in the CS group compared with control and SD groups. However, both NO and eNOS in the plasma were augmented and normalized in the SD+CS groups (*Figure 4a & 4b*).

Stigma Maydis extract attenuated vascular cell adhesion molecule-1 (VCAM-1) in high salt-fed rats.

Plasma vascular cell adhesion molecule-1 increased significantly in the SD group, whereas it was attenuated significantly in both CS and SD+CS groups (*Figure 5*).

Stigma Maydis extract suppressed plasma uric acid in high salt-fed rats.

Plasma uric acid (UA) was elevated in the SD group and was reduced in the CS group compared with control. However, the plasma UA in SD+CS group is reduced and is comparable with the CTR group (*Figure 6*).

DISCUSSION

The links between high salt intake and cardiometabolic disturbances are still being investigated. The present study probed the possible relationship between high salt intake and markers of endothelial dysfunction and antioxidant capacity. In this study, high salt intake disrupts markers of the endothelial function indicated by a reduction in plasma NO but not eNOS and elevation of plasma VCAM-1. This disruption is associated with elevated plasma uric acid and deteriorated systemic antioxidant defense. Nitric oxide is a vasodilator that augments blood flow. Adequate perfusion of organs plays a pivotal role in their function and sustenance. The expression of adhesion molecules (VCAM-1 and ICAM-1) has been shown to be negatively regulated by NO through the suppression of superoxides and down-regulation of inflammatory factors (Space et al. 2000). Data here showed that high salt intake raised the blood level of VCAM-1, a molecule known to increase in non-quiescent inflamed endothelium. Increased inflammatory and proliferative activities in the endothelium is a strong risk factor for atherosclerosis and heart failure. Hence, high salt may induce endothelial dysfunction through dysregulated inflammatory response leading to adverse events such as macrovascular diseases, which are associated with high mortality.

Oxidative stress has been implicated in cardiometabolic disturbances and usually causes damage to cellular structures resulting in loss of functionality. Despite the fact that these oxidants have physiological signaling roles, a cellular antioxidant defense is paramount in regulating the amount of oxidant present in the cell. Depressed antioxidant capacity of the cell will make the cell more susceptible to oxidative damage. In this study, high salt intake caused depression in circulating GPx and SOD. These two enzymes reduce superoxides and reactive oxygen species (ROS) protecting the cell from redox imbalance and cell damage (Mates et al. 1999). The reduction in these enzymes might be as a result of overwhelming excessive ROS and superoxides generation.

Among the processes that liberate ROS, UA production via xanthine oxidase (XO) activity is very important. Studies have shown that inhibition of xanthine oxidase with allopurinol reduced uric acid levels and improved renal derangements (Kosugi et al. 2009). Also, hyperuricemia has been implicated in inflammatory and cardiometabolic diseases (Johnson et al. 2005 Leyva et al. 1998). Despite the fact that uric acid agreeably functions as an anti-inflammatory molecule, previous studies have shown the relationship between UA and surrogate markers of cardiometabolic diseases like endothelial dysfunction (Khosla et al. 2005). This study, however, is the first to show that high salt intake increased UA production with concomitant depression of antioxidant defense systems (G6PD-dependent GPx and SOD) and nitric oxide synthesis with evidence of increased endothelial adhesion to inflammatory cells. It, therefore, shows that salt-induced derangement might be UA/oxidative stress-dependent. Uric acid has been shown to augment arginase activity thereby increasing the clearance of L-arginine which is the substrate for eNOS. This status reduces NO synthesis and may contribute to endothelial dysfunction (Zharikov et al. 2008). Taken together, salt-induced elevated uric acid indirectly (through oxidative stress) or directly (through increased arginase activity) can modulate NO synthesis, which eventually determines the inflammatory or proliferative status of the endothelium through regulation of adhesion molecules. This study shows that NO reduced in high salt diet-fed rats without a corresponding reduction in eNOS. This status shows that uric acid might have reduced NO by reducing eNOS substrate L-arginine. Conversely, corn silk extract treatment in this study showed significant antioxidant potentiating capacity and elicited attenuation of salt-induced deleterious alterations. This study, however, showed that the therapeutic effect of the extract might be mediated by its suppression of circulating UA levels (Figure 6). In summary, the model presented in this study demonstrates that salt-induced cardiometabolic disruption might be indicated by both endothelial dysfunction and

depressed antioxidant capacity. Nevertheless, since an array of literature supports the antioxidant potentiating effect of stigma maydis extract (Ebrahimzadeh 2008); among other reportedly therapeutic effects (Hu *et al.*,2010); we administered the extract as a therapeutic intervention in salt-induced derangement of endothelial dysfunction mediators. Considering the dearth of information in the effect of stigma maydis on UA production, the study also investigated the effect of the extract on plasma UA. It was found that corn silk extract ameliorated salt-induced elevated endothelial dysfunction markers and buffered redox status in high salt-fed rats. These effects were accompanied by suppression of plasma UA:

1. UA was significantly reduced in rats treated with the extract alone compared with the control group (Figure 6). This shows that the extract has a uric acid-lowering capacity at least in healthy rats but the mechanism remains elusive. However, we propose enhanced uricase activity, inhibition of xanthine oxidase and increased UA excretion/clearance since reports indicate that the extract has diuretic property (Hu *et al.* 2010).
2. The extract reduced plasma uric acid to levels comparable to the control group from the elevated salt-induced levels in high salt-fed rats (Figure 5). This advances the therapeutic potential of the extract in conditions characterized by hyperuricemia. The therapeutic potential is hence affirmed by the reversal of endothelial dysfunction markers and improved antioxidant capacity in rats fed with high salt diet and treated with the extract.

CONCLUSION

In conclusion, this study demonstrates that uric acid production is involved in the pathogenesis of salt-induced elevated endothelial dysfunction markers and that corn silk extract has therapeutic potential in uric acid heightening conditions. Therefore, it is recommended that the mechanism of these striking findings and the link between the extract's phytochemicals and anti-UA effect should be studied towards characterizing novel pharmacologic and therapeutic candidates in the treatment of cardiometabolic diseases.

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CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have affected its outcome.

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FIGURE CAPTIONS

Fig.1. A diagram showing the identification of stigma maydis (corn silk) on a corn plant.

Fig. 2 Effect of corn silk (CS) with high salt diet on glutathione peroxidase (GPx). The animals were fed with normal chow (CTR), high salt (8%) feed, corn silk extract (500mg/kg) and both high salt (8%) and corn silk extract (500mg/kg). Corn silk extract increased GPx singly or in combination with high salt diet. Data were *analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p <0.05 vs control; # p <0.05 vs SD).

Fig. 3 Effect of corn silk (CS) with high salt diet on plasma superoxide dismutase (SOD). The animals were fed with normal chow (CTR), high salt (8%) feed, corn silk extract (500mg/kg) and both high salt (8%) and corn silk extract(500mg/kg). Corn silk extract increased SOD singly but not in combination with high salt diet. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p <0.05 vs control; # p <0.05 vs SD).

Fig. 4 Effect of corn silk (CS) with high salt diet on plasma uric acid (UA). The animals were fed with normal chow (CTR), high salt (8%) feed, corn silk extract (500mg/kg) and both high salt (8%) and corn silk extract (500mg/kg). Corn silk extract reduced UA singly or in combination with high salt diet. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p <0.05 vs control; # p <0.05 vs SD).

Fig. 5 Effect of corn silk (CS) with high salt diet on plasma nitric oxide (NO) (4B) and endothelial nitric oxide synthase (eNOS) (4B). The animals were fed with normal chow (CTR), high salt (8%)

feed, corn silk extract (500mg/kg) and both high salt (8%) and corn silk extract (500mg/kg). Corn silk extract increased NO singly and maintained NO at normal levels in combination with high salt. Corn silk extract increased eNOS singly or in combination with high salt diet. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p <0.05 vs control; # p <0.05 vs SD).

Fig. 6 Effect of corn silk (CS) with high salt diet on plasma vascular cell adhesion molecule-1 (VCAM-1). The animals were fed with normal chow (CTR), high salt (8%) feed, corn silk extract (500mg/kg) and both high salt (8%) and corn silk extract (500mg/kg). Corn silk extract reduced VCAM-1 singly but not in combination with high salt diet. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p <0.05 vs control; # p <0.05 vs SD).

Figure 1



Figure 2

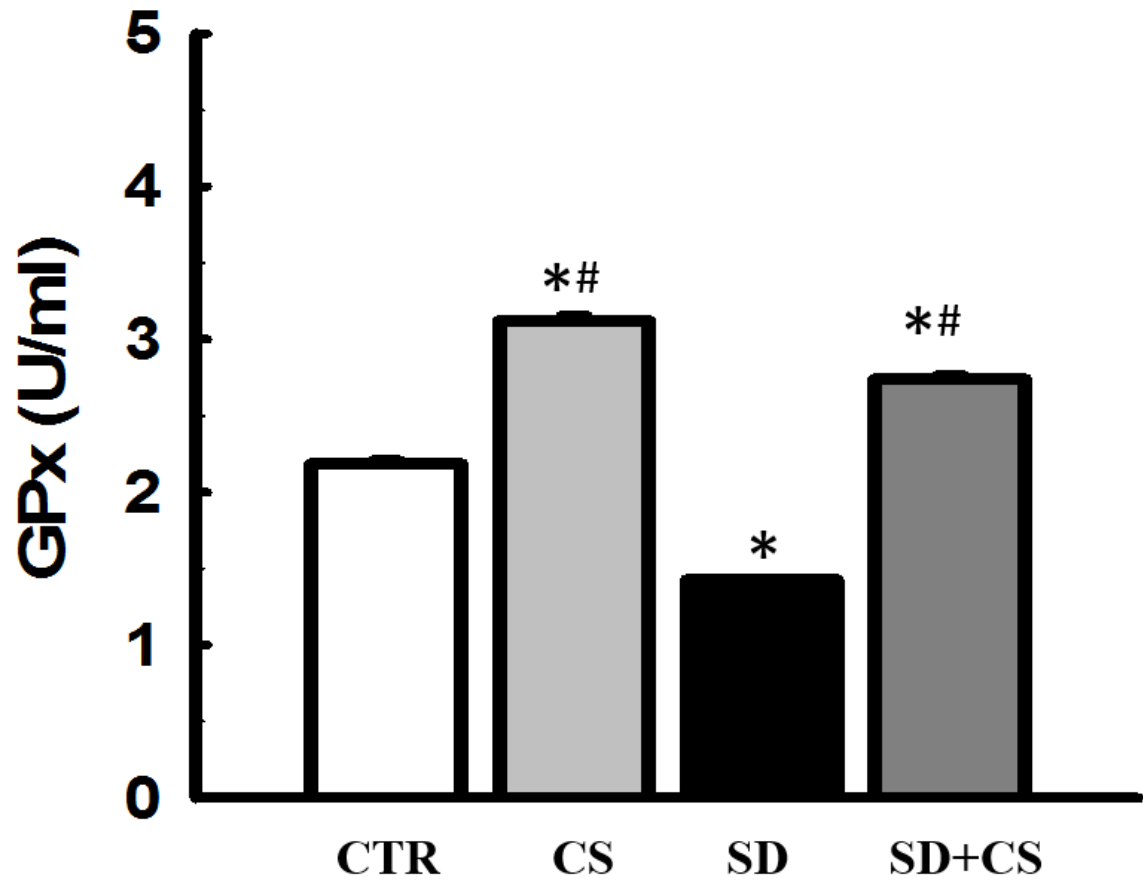


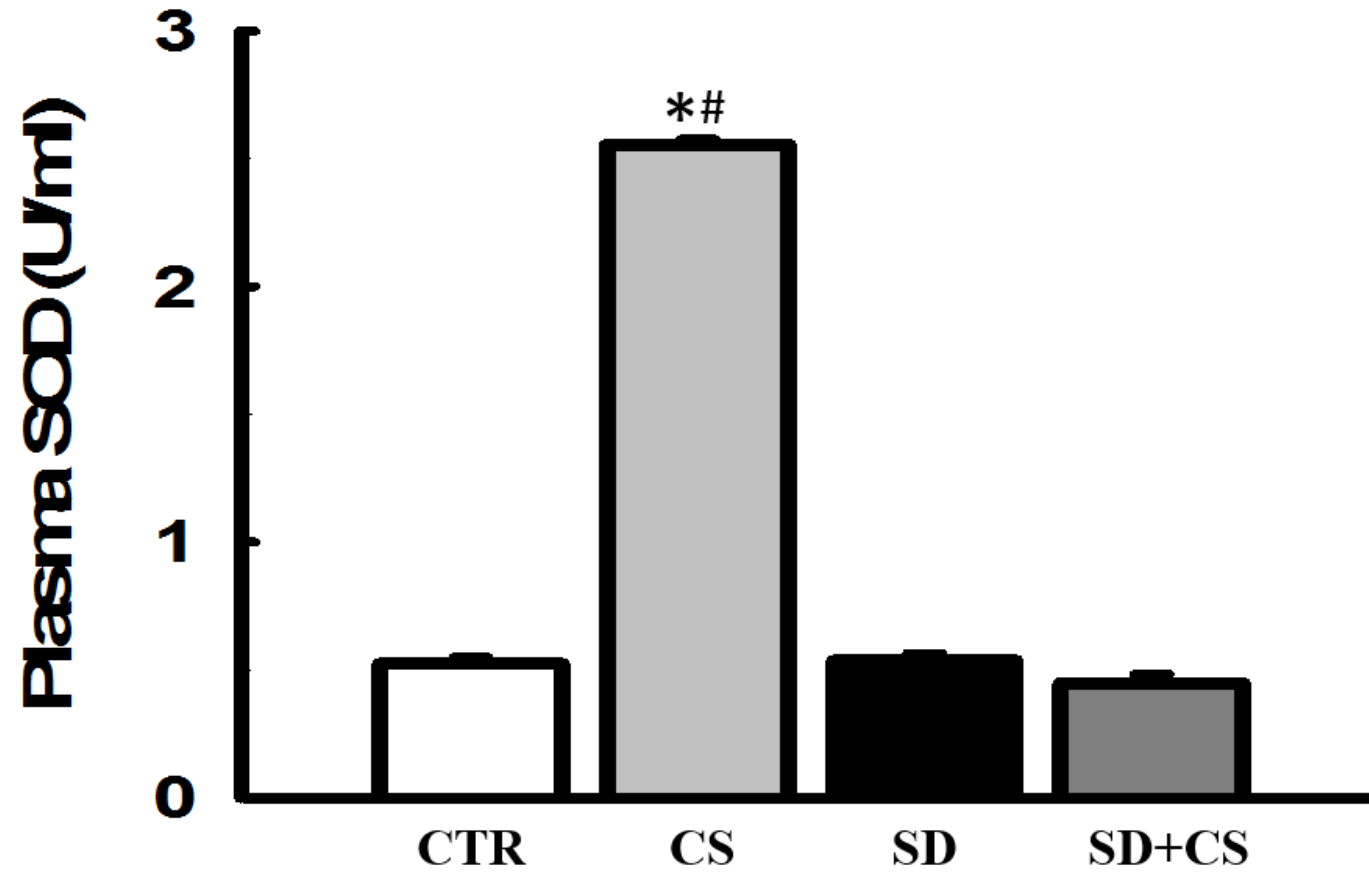
Figure 3

Figure 4

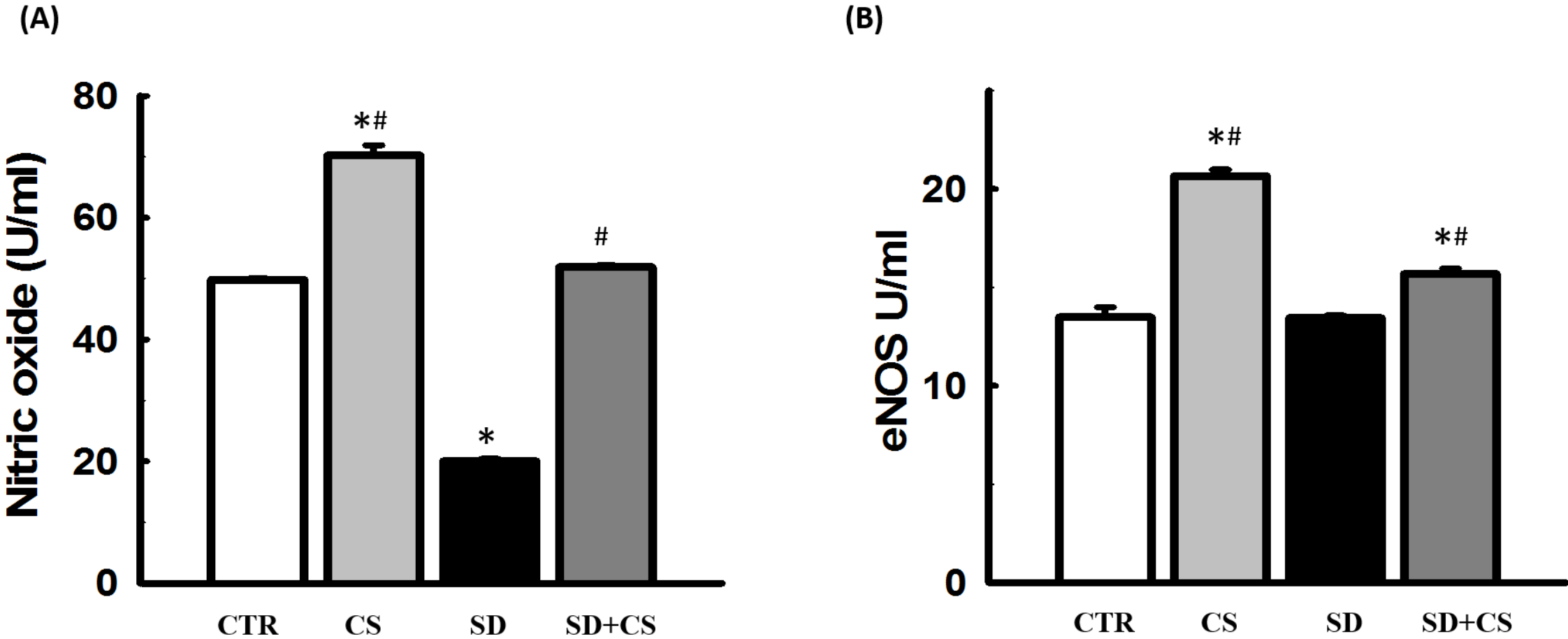
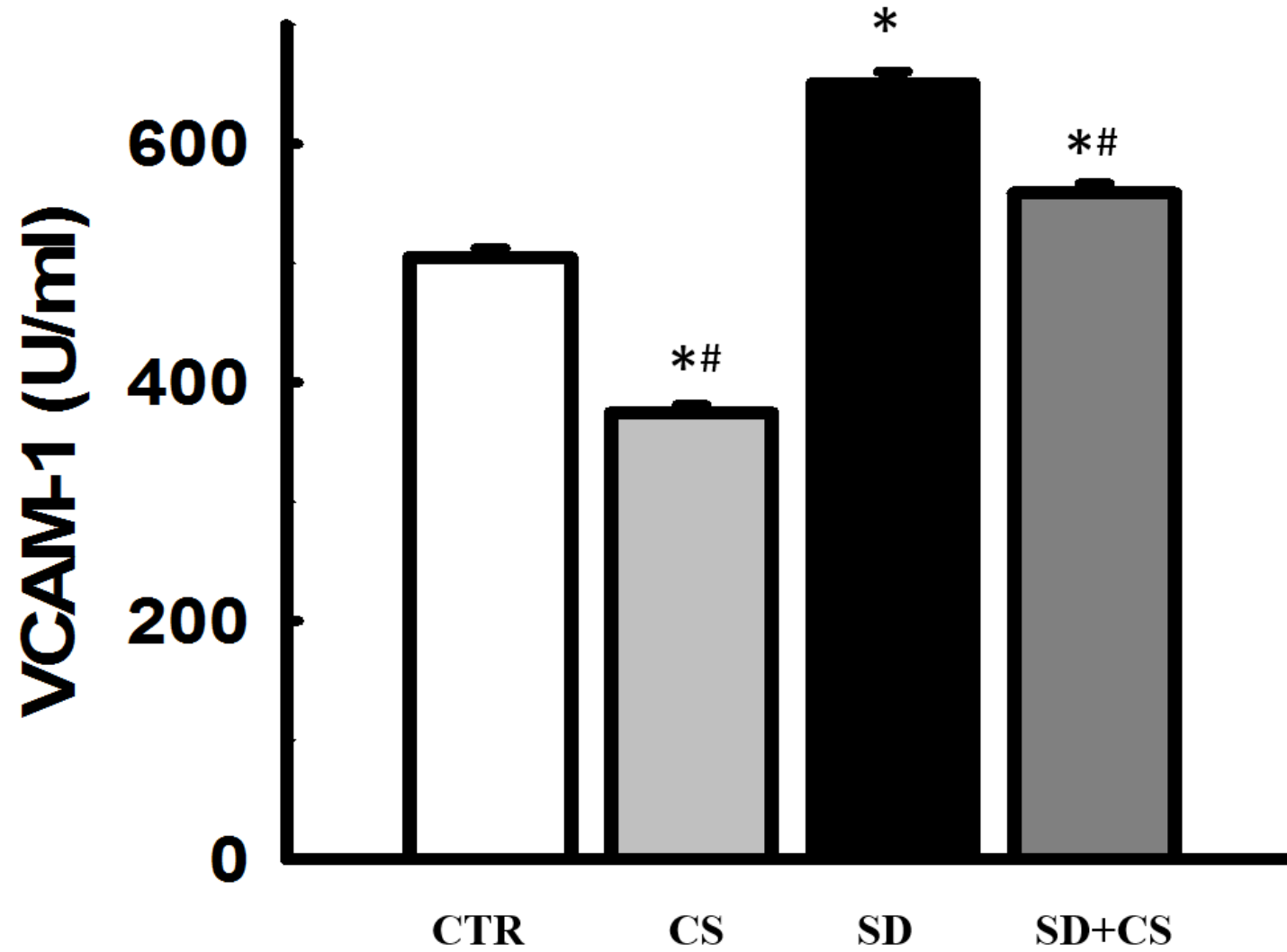
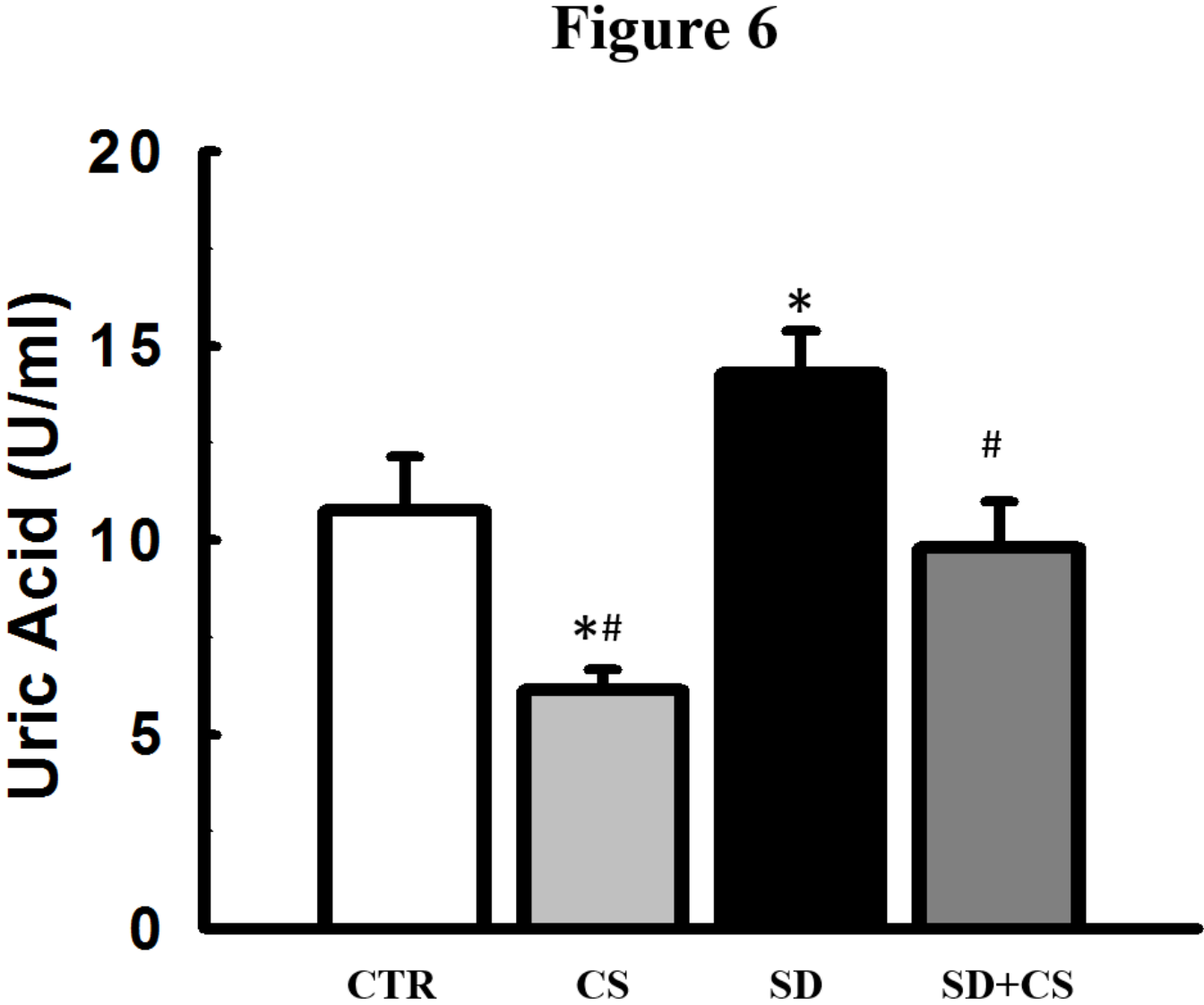
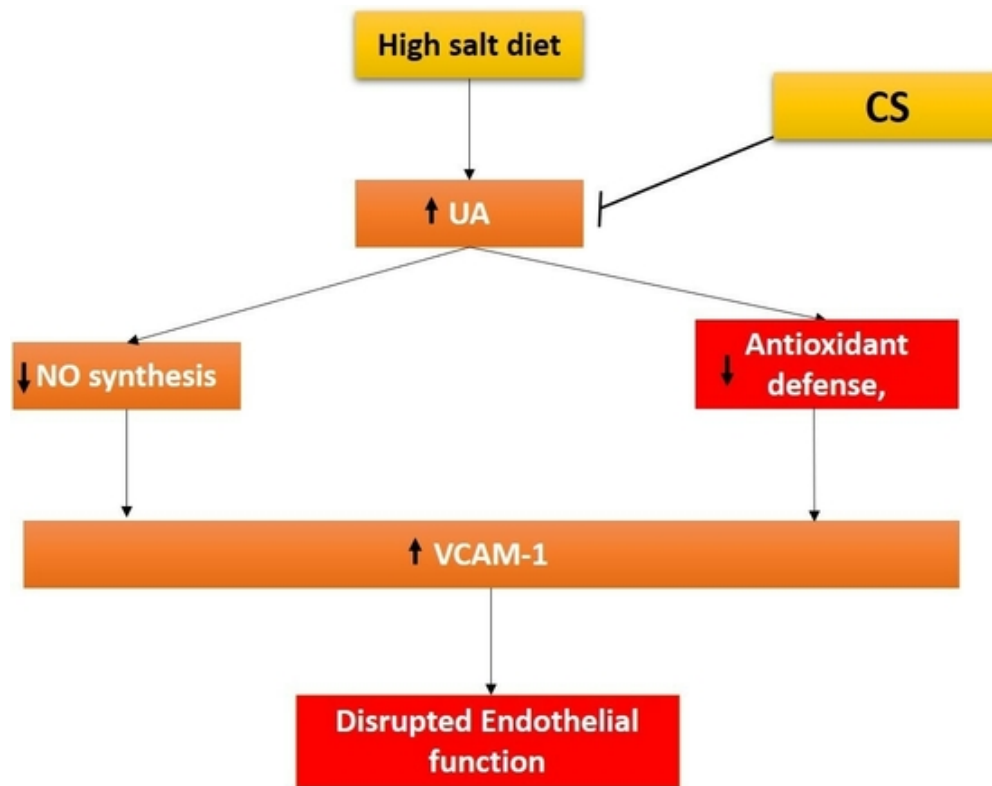


Figure 5





46x37mm (300 x 300 DPI)