

Monosodium Glutamate Induced Oxidative Stress in Accessory Reproductive Organs of Male Sprague-Dawley Rats (Monosodium Glutamate Mengaruh Tekanan Oksidatif pada Organ Aksesori Reproduksi Tikus Jantan Sprague-Dawley)

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ABSTRACT

Monosodium glutamate (MSG) is widely used as a food additive but its excessive intake leads to oxidative stress of several organs. However, the oxidative effect of MSG on male accessory reproductive organs remains unclear. Therefore, the aim of this study was to evaluate the effect of MSG on the status of oxidative stress and morphological alterations in the male accessory reproductive organs such as epididymis, prostate glands and seminal vesicle of Sprague-Dawley rats. A total of 24 male Sprague-Dawley rats were randomly divided into three groups with 8 rats per group. Control group received distilled water (1 ml/kg) while MSG60 and MSG120 received 60 mg/kg and 120 mg/kg of MSG, respectively. All the substances were administered via force feed oral for 28 consecutive days. At the end of the study, the rats were sacrificed to obtain the accessory organs for biochemical analysis and histological observations. The SOD activity in the epididymis showed a significant increase in MSG60 and MSG120 compared to control ($p < 0.05$). The GSH levels in the epididymis of MSG 120 showed a significant reduction ($p < 0.05$) compared to the control group. The levels of MDA and PC in the epididymis and prostate gland of MSG60 and MSG120 showed a significant increased ($p < 0.05$) compared to the control group. Histological alterations were found in the epididymis and prostate gland of MSG treated rats. In conclusion, MSG at both doses induced oxidative stress in the epididymis and prostate gland of experimental rats.

Keywords: Epididymis; free radicals; monosodium glutamate; prostate gland; seminal vesicle

ABSTRAK

Monosodium glutamate (MSG) digunakan secara meluas sebagai perasa tambahan namun pengambilannya secara berlebihan telah menyebabkan tekanan oksidatif pada pelbagai organ. Walaubagaimanapun, kesan oksidatif MSG ke atas organ aksesori reproduktif lelaki masih kurang dijalankan. Oleh itu, kajian ini dilakukan untuk menilai kesan MSG ke atas tekanan oksidatif dan perubahan morfologi organ aksesori reproduktif tikus jantan Sprague-Dawley seperti epididimis, kelenjar prostat dan seminal vesikel. Sejumlah 24 ekor tikus jantan Sprague-Dawley dibahagikan secara rawak kepada 3 kumpulan dengan 8 ekor setiap kumpulan. Kumpulan kawalan menerima air suling (1 ml/kg) sementara kumpulan MSG60 dan MSG 120 menerima 60 mg/kg dan 120 mg/kg MSG, setiap satunya. Kesemua bahan ini diberikan secara oral paksa berturutan selama 28 hari. Pada akhir kajian, tikus dikorbankan untuk mendapatkan organ aksesori reproduktif untuk analisis biokimia dan pemerhatian histologi. Aktiviti SOD pada epididimis tikus kajian menunjukkan aras yang lebih tinggi secara signifikan pada kumpulan MSG60 dan MSG120 berbanding kumpulan kawalan ($p < 0.05$). Hanya organ epididimis kumpulan MSG120 sahaja yang menunjukkan aras GSH yang lebih rendah secara signifikan berbanding kumpulan kawalan ($p < 0.05$). Aras MDA dan PC bagi epididimis dan kelenjar prostat pula menunjukkan aras yang lebih tinggi secara signifikan ($p < 0.05$) pada kumpulan MSG60 dan MSG120 berbanding kumpulan kawalan. Perubahan histologi diperhatikan pada epididimis dan kelenjar prostat yang diaruh MSG. Kesimpulannya MSG pada kedua-dua dos telah menyebabkan berlakunya tekanan oksidatif pada epididimis dan kelenjar prostat tikus kajian.

Kata kunci: Epididimis; radikal bebas; monosodium glutamate; kelenjar prostat; vesikel semen

INTRODUCTION

Monosodium glutamate (MSG) is a well-known food enhancers that has been used widely to improve palatability of food (Insawang et al. 2012). It also acts as food preservatives in food industry especially in the preparation of fast food products (Rangan & Barceloux 2009). MSG is naturally occurring sodium salt that contains 78% of glutamic acid and 22% of sodium and water (Walker & Lupien 2000). Generally, MSG has been categorized as a

safe food enhancer in which its consumption has increased globally with average daily intake of approximately 10 g/day (Nakanishi et al. 2008). Therefore, its usage has been unintentionally abuse in food industry because of its abundance and improper labelling in many food ingredients (Egbonu et al. 2009).

Repeated exposure of MSG had cause adverse effects on human and animal studies (Farombi & Onyema 2006; Shimada et al. 2013; Husarova & Ostatnikova 2013; Tawfik Al-Badr 2012). In China, the risk of weight gain

has been increased in the population who continuously consuming the MSG (He et al. 2011). Administration of MSG in high doses has increased the risk of diabetes in mice by increasing the levels of glucose and reducing the levels of insulin (Sant'diniz et al. 2005; Collison et al. 2009). Furthermore, MSG in high dose increased the formation of Reactive Oxygen Species (ROS), thus cause oxidative stress to several important organs such as the brain (Farombi & Onyema 2006), liver (Onyema et al. 2006) and kidney (Sharma et al. 2014).

MSG has been reported to induce damage to male reproductive system by disrupting the hypothalamic-pituitary-axis (HPA) pathway and through oxidative stress. High dose of MSG also induced oxidative damaged to the testes of rats (Vinodini et al. 2008; Nayanatara et al. 2008). Besides, MSG also caused disruption to the HPA pathway through its excitotoxicity effects in the brain (Igwebuike et al. 2011; Cattani et al. 2014). The disruption of HPA pathway may lead to the decreasing of testosterone hormone levels which eventually led to the reduction of sperm quality (Igwebuike et al. 2011).

The male accessory reproductive organs especially the epididymis is surrounded by adipose tissue that makes this organ as a target of ROS (Pratt, Tallman & Porter 2011). Furthermore, the epididymis, prostate gland and seminal vesicle are androgen-dependent organs (Li et al. 2006). Therefore, any disruption on the androgen hormones such as testosterone might influence the healthy status of these organs. In addition, the effects of MSG on male accessory reproductive organs are still lacking. Therefore, this study was done to assess the effects of MSG on the accessory reproductive organs such as epididymis, prostate gland and seminal vesicle of male Sprague Dawley rats.

MATERIALS AND METHODS

CHEMICALS

MSG was purchased from local market located at Kuala Lumpur. All other chemicals were purchased from Sigma-Aldrich, United States of America except for nitroblue tetrazolium and thiobarbituric acid was purchased from ICN Biomedicals, Unites States of America.

ANIMAL TREATMENT SCHEDULE

Twenty-four male Sprague-Dawley rats weighing between 170-200 g were purchased from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The rats were taken a week earlier for acclimatization, placed in polypropylene cage with standard environmental condition (12 h light/dark cycles, 25-28°C) and maintained on standard pellet diet and water *ad libitum* throughout the study period. All the animals handling protocols were approved by the Animal Ethics Committee (UKMAEC) with the resolution number: FSK/2016/IZATUS/23-NOV./807-NOV.-2016-FEB.-2019.

The rats were randomly divided into three groups with eight rats per group. Control group received distilled water (1 mg/kg) while MSG 60 and MSG 120 received MSG in the dose of 60 mg/kg body weight and 120 mg/kg body weight, respectively. The dose was chosen based on the Acceptable Daily Intake (ADI) where the limitation of MSG intake is 120 mg/kg of body weight/day (WHO 1971). All the substances were orally force feed for 28 consecutive days. At the end of study period, the rats were anaesthetized using 50 mg/kg KTX and sacrificed. The male accessory reproductive organs such as epididymis, prostate gland and seminal vesicle were obtained. The obtained organs were weighed, chopped and homogenized in PBS Sucrose with a ratio of 10 g/ml (w/v) at a speed of 8000 rpm for 20 min at 4°C. The homogenate were kept at -20°C for biochemical analysis. A part of the obtained organs were cut and soaked in a solution of 10% formalin for histological observation.

DETERMINATION OF ANTIOXIDANT STATUS AND OXIDATIVE DAMAGE BIOMARKER

For determination of antioxidant status, the homogenate of accessory reproductive organs were assessed for superoxide dismutase (SOD) activity and glutathion (GSH) level by method of Beyer and Fridovich (1987) and Ellman (1959), respectively. Meanwhile for determination of oxidative damage biomarker, the lipid peroxidation was measured by malondialdehyde (MDA) (Stocks and Dormandy 1971) and protein oxidation was measured by protein carbonyl (Levine et al. 1990).

HISTOLOGICAL OBSERVATIONS

A routine histological procedure was carried out for all male accessory reproductive organs: epididymis, prostate gland and seminal vesicle (Ochei & Kolhatkar 2006). The formalin fixed epididymis, prostate gland and seminal vesicle were processed by an automated tissue processor Leica EG1160 (EquipNet, Massachusetts, United States of America). Paraffin section of all organs were cut into 3-4 µm thicknesses and stained with haematoxylin and eosin (H & E). The histological changes of all organs were observed under X40 magnifications using light microscope. All the observations were verified by a histopathological expert.

STATISTICAL ANALYSIS

All the data were tested for normality test using Shapiro-Wilk. The data with normally distributed were further evaluated using one-way analysis of variance (ANOVA) followed by Tukey HSD for multiple comparisons (Statistical Packages for the Social Sciences (SPSS) version 22.0). All the results were considered statistically significance difference at $p < 0.05$ and expressed by mean \pm SEM.

RESULTS AND DISCUSSION

ANTIOXIDANT STATUS ANALYSIS

Figure 1 and 2 showed the SOD activity and GSH level of experimental rats, respectively. The SOD activity in the epididymis of MSG60 was significantly higher compared to control group at $p < 0.05$. Furthermore, the SOD activity

in the epididymis of MSG120 was significantly higher as compared to control and MSG60 groups ($p < 0.05$). In addition, the SOD activity in the seminal vesicle of MSG120 group is significantly higher compared to control group ($p < 0.05$). For GSH level, only the epididymis of MSG120 group is significantly lowered when compared to control group at $p < 0.05$.

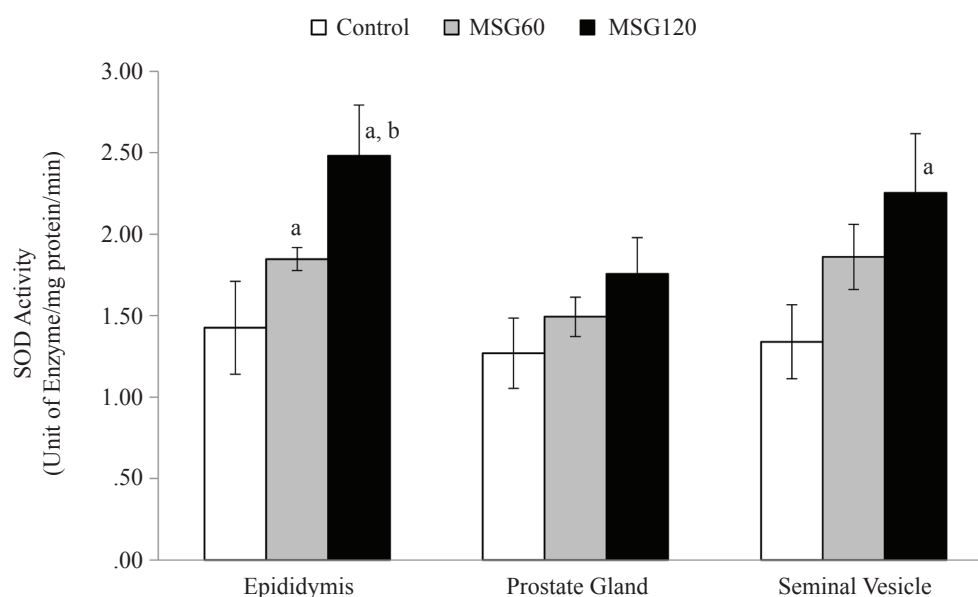


FIGURE 1. SOD activity of all experimental groups. a is significantly different compared with control group ($p < 0.05$); b is significantly different compared with control group ($p < 0.05$).

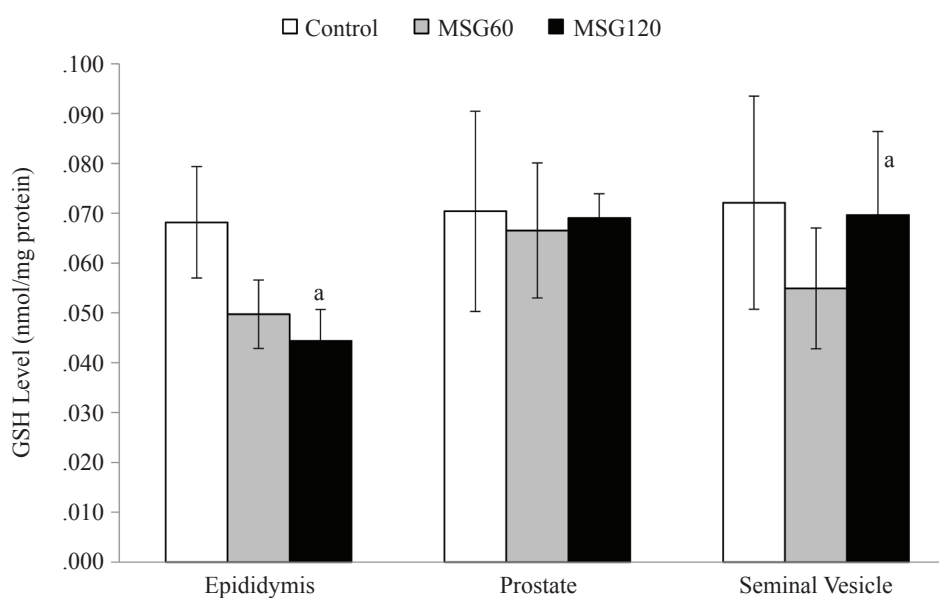


FIGURE 2. GSH level of all experimental groups. a is significantly different compared with control group ($p < 0.05$).

Antioxidants are needed in the body as a shield against ROS produced by physiological and pathological processes. SOD is an enzymatic antioxidant which acts in catalysing the breakdown of superoxide anion into oxygen and hydrogen peroxide (H_2O_2) (Zelko, Mariani & Folz 2002), while GSH is a non-enzymatic antioxidant that involved

in dissociation of this reactive molecule, H_2O_2 to water and oxygen (Pompella et al. 2003). In line with previous study done by Abdel-Reheim et al. (2014), MSG increased the activity of SOD and decreased the GSH level in the epididymis of rats.

The reduction of GSH levels in this study may be due to the increased activity of SOD. This is because SOD catalyzes the conversion of superoxide anion to H_2O_2 and lead to the increase of H_2O_2 production (Zelko, Mariani & Folz 2002). When this situation was beyond the ability of GSH, which plays a role in converting H_2O_2 to a non-toxic product, it will cause a reduction in GSH levels. In addition, GSH reduction may also be due to the inhibition of cystine/ glutamate antiporter system (Bridges et al. 2012). This antiporter plays an important role in mediates the exchange of extracellular cystine and intracellular glutamate across the cellular plasma for the synthesis of GSH (Dringen 1999). The increase of glutamate levels results from MSG metabolism prevent the cystine to enter the cells, thus

lead to the decreasing in GSH synthesis (Dringen 1999). This might explain the reduction of GSH level in present study.

OXIDATIVE DAMAGE BIOMARKER ANALYSIS

For oxidative stress biomarker, the level of MDA and PC of experimental rats were shown in Figure 3 and 4, respectively. The level of MDA and PC in epididymis and prostate gland were significantly increased at $p < 0.05$ in MSG60 and MSG120 compared to control group. The MDA and PC are an important indicator of oxidative damage to macromolecules such as lipid and protein, respectively (Alghasham et al. 2013). The present result showed that

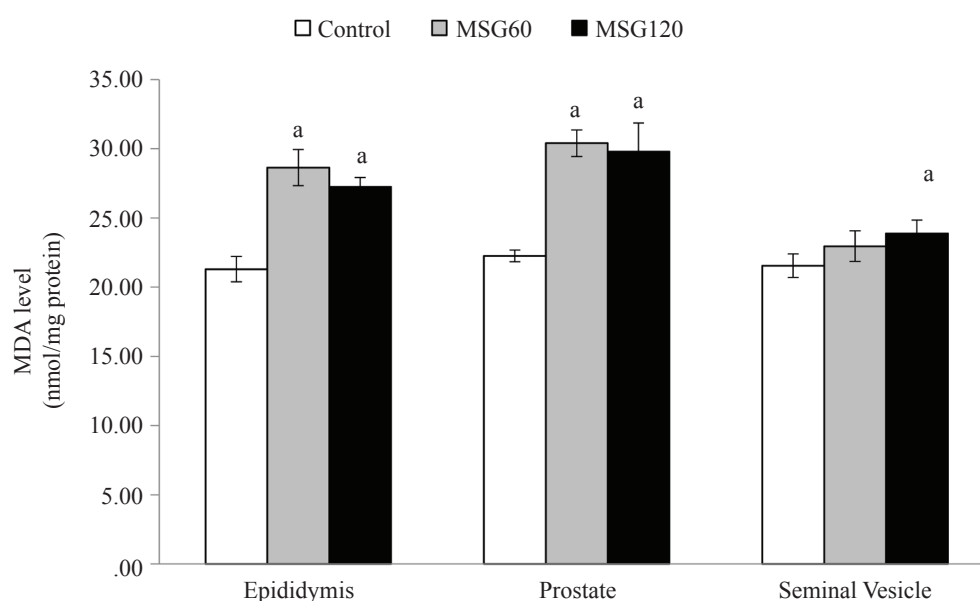


FIGURE 3. MDA level of all experimental groups. a is significantly different compared with control group ($p < 0.05$).

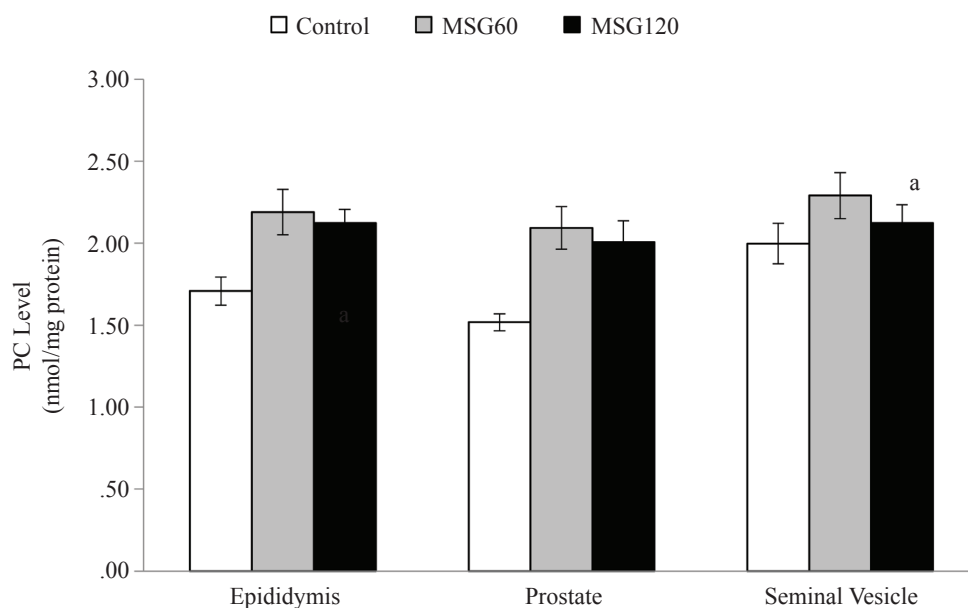


FIGURE 4. PC level of all experimental groups. a is significantly different compared with control group ($p < 0.05$).

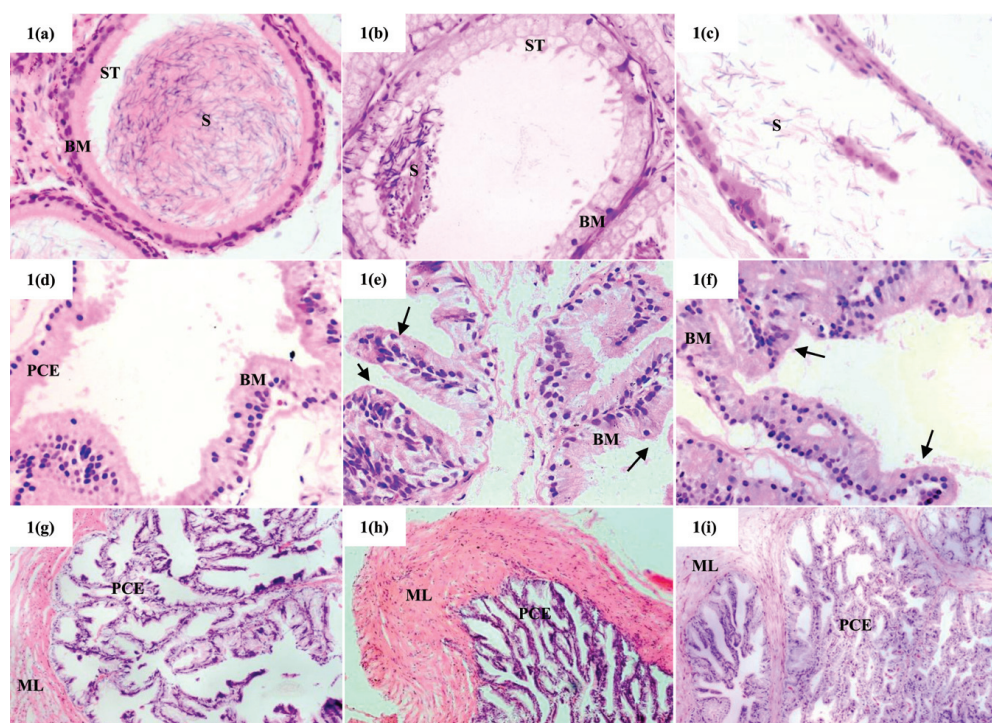
MSG increased the level of MDA and PC in epididymis and prostate gland. Administration of aspartame, an artificial sweetener used in the food was found to cause increased in MDA level of sperm due to the increased formation of ROS (Ashok et al. 2017). Furthermore, the excessive intake of MSG leads to the increased of glutamate level in circulation. High level of glutamate resulted in increased of intracellular calcium (Jaiswal et al. 2009). It will cause the excessive influx of calcium ions into the mitochondria and consequently increases the ROS production (Szydłowska & Tymianski 2010). This might explain the increased of MDA level in the present study.

Since protein is the main component of the biological system and plays an important role in various cellular functions such as signal transduction, mitosis and cellular transport system, the risk of protein to expose with ROS is high. Previous study found that increased ROS formation lead to an increase in the level of PC (Şahin & Gümürlü 2007). The increased of protein oxidation observed in this

study may be associated with a direct impact on the increase in ROS generation as a result of the excessive intake of MSG. The same finding has previously been reported in studies involving other organs such as liver (Onyema et al. 2006), brain (Farombi & Onyema 2006), male reproductive organs (Husarova & Ostatnikova et al. 2013) and kidney (Sharma et al. 2014).

HISTOLOGICAL EXAMINATION

Picture 1 showed the histology of epididymis, prostate gland and seminal vesicle of experimental rats at x40 magnification. The control group showed a normal morphology of epididymis comprised of basement membrane (BM) with stereocilia (ST) lining the epididymis and the lumen was filled with sperm (S) (Picture 1a). A reduction of sperm concentration in the lumen of epididymis was found in MSG60 (Picture 1b) and MSG120 (Picture 1c) groups.



PICTURE 1. The histology of accessory reproductive organs of experimental groups (H&E staining; X40 magnification). Picture 1a – 1c shows the epididymis of experimental rats. A normal morphology of epididymis was shown in control rats which consists of basement membrane (BM), stereocilia (ST) and the lumen of epididymis was compact with sperm (S) (Picture 1a). A reduction of sperm concentration in lumen of epididymis were found in MSG 60 (Picture 1b) and MSG 120 (Picture 1c) groups. Picture 1d – 1f shows the prostate gland of experimental rats. A normal morphology of prostate gland was shown in control group (Picture 1d) which the gland is lining with pseudostratified columnar epithelium (PCE). The basal membrane (BM) is more folded in MSG 60 (Picture 1e) and MSG 120 (Picture 1f) groups. Picture 1g – 1i shows the seminal vesicle of experimental rats. No morphological alterations were found in all experimental groups which the seminal vesicle is composed of pseudostratified columnar epithelium (PCE) and muscular layer (ML).

A normal morphology of prostate gland was shown in control group (Picture 1d) which the gland is lining with pseudostratified columnar epithelium (PCE). The basal membrane (BM) is more folded in MSG60 (Picture

1e) and MSG120 (Picture 1f) groups. The abnormalities found in both epididymis and prostate gland supports the biochemical findings. The anatomical structure of epididymis which surrounded by adipose tissue makes

this organ more vulnerable to oxidative stress (Pratt, Tallman & Porter 2011). However for seminal vesicle, no morphological alterations were found in all experimental groups composed of pseudostratified columnar epithelium (PCE) and muscular layer (ML). The accessory organs glands are androgen-dependent, thus insignificant decrease of testosterone hormone is not sufficient to cause any damage to seminal vesicle (Kumari & Singh 2013). This might explain the histological and biochemical findings found in seminal vesicle of MSG-treated rats.

CONCLUSION

In conclusion, the MSG at the dose of 60 mg/kg and 120 mg/kg cause oxidative damage to the male reproductive accessory organs such as epididymis and prostate glands in Sprague Dawley rats. Further study on the effects of MSG on the androgenic receptor of male reproductive accessory organs might be done in order to evaluate the function of these organs.

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