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AMELIORATING EFFECTS OF COCONUT WATER ON SPERM QUALITY AND SELECTED ORGANS HISTOLOGY IN MONOSODIUM GLUTAMATE PRE TREATED MALE MICE (*Mus musculus*)

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ABSTRACT

This study was conducted to determine the effect of both monosodium glutamate (MSG) and coconut water on sperm quality of *Mus musculus*. Twenty adult mice weighing between 25-30 g were randomly divided into four groups. Group A (Control), received saline solution; Group B received 2 g/kg b.wt of MSG; Group C received administration of both coconut water (10 mL/kg b.wt) and MSG (2 g/kg b.wt); and Group D was given coconut water (10 mL/kg b.wt) only. Treatments were given daily for a duration of 15 days. Results revealed the MSG treated group showed a significant reduction (p<0.05) in sperm concentration, motile sperm, and viable sperm with values 23×10^6/ml, 19.59%, and 16%, respectively. Group C which received both of MSG and coconut water showed significant improvement (p<0.05) in sperm concentration, motile sperm, and viable sperm which were 47.4×10^6/ml, 66.69%, and 30.6%, respectively. Several histological alterations were observed in the testes of MSG treated mice. However, the co-administration of MSG and coconut water was proven to be beneficial for the improvement of sperm concentration, motility, and viability. Also, coconut water group showed improved architectures of testes compared to the MSG treated mice. In conclusion, MSG had severely affected sperm quality but co-administration of both coconut water and MSG had indicated ameliorating effects of coconut water towards its negative impact.

Key words: Monosodium glutamate, coconut water, infertility, sperm quality, testes

INTRODUCTION

Monosodium glutamate (MSG) is one of the famous flavouring agents or food additives that is commonly used in processed food to preserve flavour and enhance taste (Rosa *et al.,* 2015). Regardless of its acceptance in some countries and popularity on its capability to improve taste of foods, the use of MSG in daily life is a major concern as its toxicity has been expressed towards human and rodents (Quines *et al.,* 2014). Apart from that, MSG at a high dose can lead to negative impact on male reproductive system, hence, increases the risk of reduced sexual function, which can lead to male infertility (Igwebuike *et al.,* 2011; Sukhorum & Lamsaard, 2013).

One of the most versatile natural products used in the food industry is coconut fruits *Cocos nucifera* L.. One of the consumeable parts of the coconut fruit is the coconut water. Various studies indicated that it consists of many vitamins, minerals, proteins and is nutritious and beneficial to the body health (Yong *et al.,* 2009). It also represents as one of the rapid expanding essential beverages because of its natural hydrating abilities, to enhance flavour, medical health purpose and natural benefits (DebMandal & Mandal, 2011; Preetha *et al.,* 2012). Not only that, coconut water also contains antioxidant properties (Nurulain, 2006). The current study objective is to determine the effect of both coconut water and monosodium glutamate (MSG) on sperm quality and the effect on selected organs in particular the testes and kidney.

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MATERIALS AND METHODS

Animals

Male mice, *Mus musculus*, aged between 6-8 weeks and weight between 25-30 g were randomly selected and divided into four groups with five mice each. The mice were handled according to the Guidelines of Care and Use of Laboratory Animals, UiTM Care (Ref No. 112/2015). They were acclimatised to the laboratory condition for at least 7 days before administration. The mice were housed under conditions of controlled temperature (27ºC) with 12 hours light and dark cycle throughout the experiment. The mice received standard pellet diet and distilled water was given *ad libitum.*

Collection of *Cocos nucifera* L.

Young and fresh coconut fruits, *Cocos nucifera* L. were purchased from a shop located in Sungai Buloh, Selangor. The coconut water was collected and accumulated in a sterilised conical flask, which was then measured by using a measuring cylinder and filtered into a sterilized 15 mL falcon tube. Then it was immediately frozen at -20ºC to prevent any microbial or enzymatic activity. Prior to treatment, the coconut was thawed at 37ºC in a water bath for 5 minutes.

Preparation of Test Substances

The dosage of MSG used is 2 g/kg body weight. The MSG was weighed and diluted in 0.25 mL saline solution. The coconut water dosage amount of 10 mL/kg body weight was used in the study. In coconut water with MSG treated group, the MSG was diluted with 0.25 mL of coconut water. Next, the test substances was given through forced feeding for 15 days.

Euthanasia and Preparation of Sperm Suspension

The mice were euthanised by cervical dislocation and the abdominal cavity of the mice were opened by cutting through a midline abdominal incision to take out the testes and the cauda epididymis. The epididymis was minced and squeezed by using forceps and scissors to release the sperm out from the tissues. The sperm were suspended in the sperm media (30 µL) (DMEM) and kept at 37ºC in a water bath for further analysis (Nakane *et al.,* 2005; Marysia *et al.,* 2015).

Sperm Concentration Analysis

Sperm concentration and sperm motility were analysed. Briefly, 10 µL of the sperm suspension was kept inside a 56ºC water bath to kill all the sperm for the easier counting process. Sperm count was performed by using a Makler counting chamber and the concentration of sperm was expressed in million per mL (Nakane *et al.,* 2005).

Sperm Motility Analysis

Sperm motility was properly conducted by keeping the sperm suspension inside a 37ºC water bath to make sure that the sperm stayed alive when observed. A 10 µL of the sperm suspension was placed on the Makler counting chamber. The sperm motility was expressed as the percentage of progressive and non-progressive cells (Nakane *et al.,* 2005).

Sperm Viability Analysis

Viability of sperm was evaluated by using Eosin-Nigrosin (EN) staining method, which consisted of 4% eosin and 5% nigrosin. A 10 µL of sperm suspension was mixed with equal volume of EN stain and smeared onto a glass slide and air dried. The smears were examined under a 100 X oil immersion objective lens of a light microscope (Golshan & Rezazadeh, 2013). Approximately 200 sperm were scored for the determination of live or dead sperm (Klimowicz-Bodys *et al.,* 2012) and the values were expressed in percentage.

Histological Studies

Collected organs which are testes and kidneys were fixed using 10% formalin for 24-48 hours at room temperature. Next, the tissues were dehydrated through increasing ethanol concentrations for few hours followed by clearing process by dipping the sample into xylene for 20 mins. Then the tissues were individually embedded in paraffin wax. The block to be sectioned was inserted into the microtome block holder so that the wax faced the blade in the upright plane. Sections of 4 µm thick were made and stained with haematoxylin and eosin (H&E).

Statistical Analysis

The results obtained were expressed as mean ± SEM. The comparison between the means were analysed using One Way Analysis of Variance (ANOVA) of the Statistical Program for Social Sciences (SPSS) software with significant value at $p<0.05$.

RESULTS AND DISCUSSION

Sperm Concentration Analysis

The epididymal sperm concentration in mice treated with MSG was significantly lower ($p<0.05$) (23×10^6/ml) than that of the control (68.6×10^6/ml). This results were in agreement with the data of Nayatara *et al* (2008), Igwebuike *et al* (2011), Ekaluo *et al* (2013) and Iamsaard *et al* (2014) who reported that MSG administration reduced the epididymal sperm concentration significantly. A decrease in ascorbic acid level was also reported in

which it induces ROS towards damaging the germ cells that leads to significant decline in sperm concentration (Nayatara *et al.,* 2008).

However, in the present work, mice treated with both MSG and CW showed significant improvement (p<0.05) in sperm count (47.4×10^6/ml) compared to MSG treated group, and this may be due to ascorbic acid contained in the coconut water that was proven to increase sperm concentration (Sandhya & Rajamohan, 2014). Ascorbic acid is responsible for scavenging free radicals, thus, protecting bio membrane from oxidative stress (Nayatara *et al.,* 2008). Figure 1 shows the results on the effect of MSG and CW on sperm concentration in mice.

Sperm Motility Analysis

Figure 2 shows the percentage of motile and non-motile sperm. The results demonstrated that mice treated with coconut water had the highest motile sperm with 82.99 ± 0.82%. For mice treated with MSG have significantly lowest (p<0.05) motile sperm with 19.59 ± 4.56% compared to other groups. This suggests that consumption of high-dose MSG (2 g/kg b.wt) impaires motility of the sperm. Similar

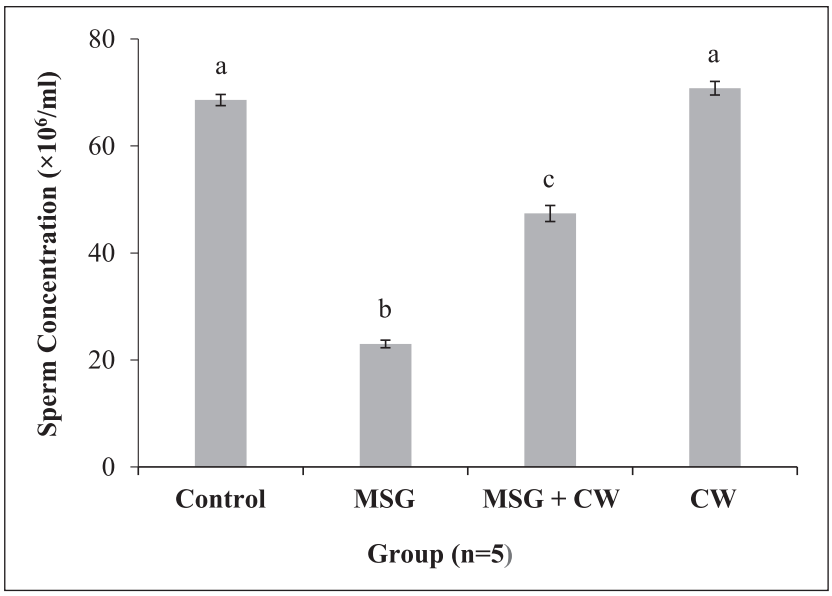

Fig. 1. The effect of MSG and coconut water on sperm concentration in all treatment groups (n=5), different letters (a, b, c) represent significant difference at (p<0.05).

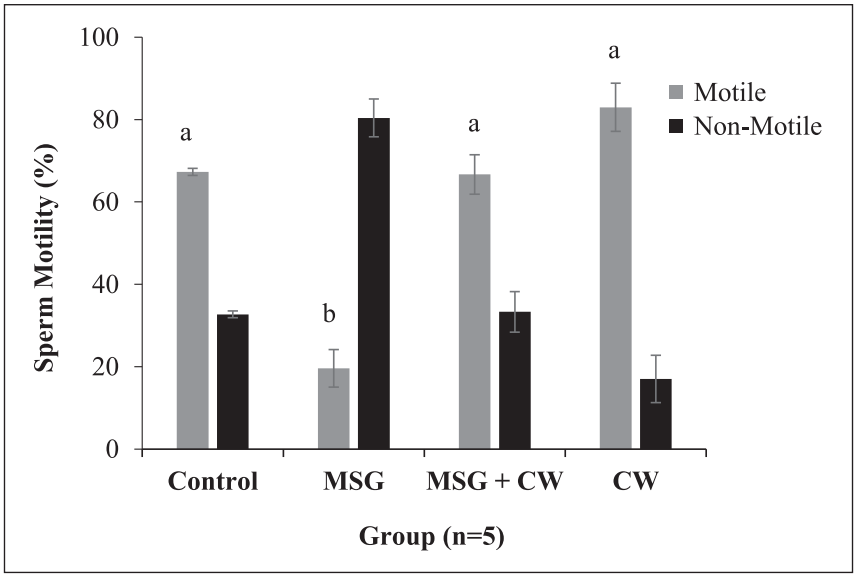

Fig. 2. The effect of MSG and coconut water on sperm motility in all treatment groups (n=5), different letters (a, b) represent significant difference at (p<0.05).

finding was previously reported by Kadir *et al* (2011).

However, mice treated with both MSG and CW showed significant improvement ($p<0.05$) on the sperm motility with 66.69 ± 4.77% which was approximately 20% higher than that of MSG group. This proves that coconut water supplementation may contain nutritive substances such as L-arginine which protects the sperm from the damaging effects of MSG (Sandhya & Rajamohan, 2014). L-arginine also exhibits positive effects on spermatozoa through the involvement of nitric oxide synthesis (Srivastava *et al.,* 2006).

Sperm Viability Analysis

The percentage of live sperm of mice treated with MSG showed a significant decrease ($p<0.05$) compared to other groups as shown in Figure 3 with value 16 ± 2.42%. This observation was corroborated by previous studies (Kadir *et al.,* 2010; Hilwani *et al*., 2014) who demonstrated that high dose of MSG consumption could cause damage to the testes, and the reduction in percentages of viable sperm might be due to the oxidative damaged through decreased levels in free radical scavenges (Nayatara *et al*., 2008).

The percentage of viable sperm in mice treated with a combination of MSG and CW showed a significant increase ($p<0.05$) compared to the MSG and CW group with value 30.6 ± 3.14%. This could be due to beneficial biologically active components found within coconut water such as L-arginine, ascorbic acid and some minerals which include calcium, magnesium and potassium that may be vital for the sperm (Sandhya & Rajamohan, 2014). Meanwhile, groups of mice treated with saline (control) and coconut water showed no significant changes on the percentages of viable sperm which were 40.6 ± 1.86% and 43.2 ± 2.72%, respectively.

Histological Analysis of Testes

Cross sections of testes stained with Haematoxylin and Eosin (H&E) are shown in Figure 4. Normal histological architecture of the seminiferous tubules with different stages of spermatocytes and supporting cells were demonstrated by the control mice (Figure 4A). Meanwhile, testes of mice treated with MSG (Figure 4B) showed disruption of the germinal epithelium which was irregularly placed on the basement membrane and detached away from the lumen.

The present result has been previously observed by Hilwani *et al* (2014) where disorganization of the normal architecture of seminiferous tubule as well as the change in tubular lumen diameter in mice treated with MSG. These changes may be due to indirect effect via hypothalamic lesions where previous study proved that MSG has the ability to damage nerve cells of the hypothalamus, hence, disrupting the hormone secretion through the hypothalamic pituitary gonadal regulatory axis (Alalwani, 2014). Also, earlier studies had proved the presence of functional glutamate transporters and receptors in testes of mice, which in turn, indirectly affects neuronal activity on the receptors and facilitating the flooding of calcium into the cell, causing cytotoxic effects (Ghosh *et al.,* 2011). One of the mechanisms may be a direct effect of

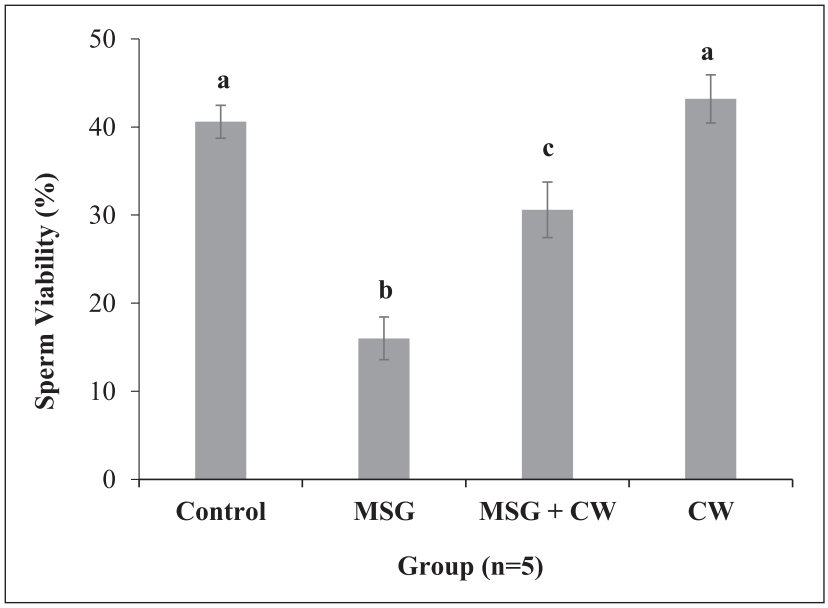

Fig. 3. The effect of MSG and CW on sperm viability in all treatment groups (n=5). Different letters (a, b, c) represent significant difference at ($p<0.05$).

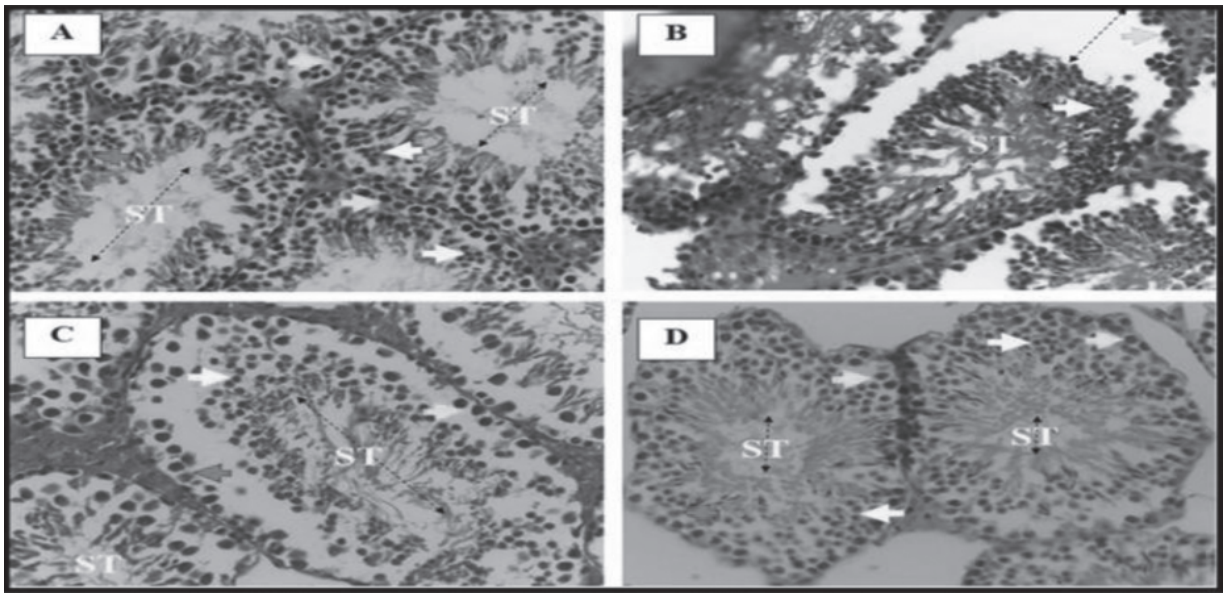

Fig. 4. Histology of testes (H&E, 40× Magnification). (A): Control- showing normal architectures of the seminiferous tubule (ST); spermatids (yellow arrows), spermatogonia (green arrows) and sertoli cells (red arrow); (B): MSG group- showing sloughing of germinal epithelia of the seminiferous tubule (black dotted arrow) and large tubular lumen (black dotted arrow); (C): MSG + CW group- showing improvement of germinal epithelia of the seminiferous tubule (black dotted arrow) and tubular lumen (black dotted arrow); (D): CW group- showing normal architectures of seminiferous tubule (ST), spermatids (yellow arrows), spermatogonia (green arrows), and sertoli cells (red arrow).

Table 1. The mean diameters (µm) of the tubular lumen in mice (mean ± SEM)

Organ	Treatment groups			
	Control	MSG (2g/kg b.wt)	MSG (2g/kg b.wt) + CW	CW
Tubular lumen (µm)	10.62±0.80[a]	22.10±2.19[b]	10.52±0.55[a]	10.86±0.99[a]

Superscripts [a, b] represent significant difference (p<0.05) among groups.
MSG - Monosodium glutamate; CW - Coconut water.

MSG on the epithelial cells of the seminiferous tubules (Alalwani, 2014).

The histological structures of mice treated with both MSG and CW (Figure 4C) showed an improvement of the germinal epithelia of the seminiferous tubule. Besides that, Table 1 shows significant improvement (p<0.05) on the diameter of the tubular lumen by decreasing in its diameter. This may be due to the antioxidant effects of coconut water. Selenium, vitamin C and vitamin E have been proven to reduce the toxicity of MSG (Hamza & Al-Harbi, 2014). Selenium has been proven to enhance other antioxidant activity by increasing the content of the antioxidant (Su *et al.,* 2008) and surprisingly, these antioxidants have been reported to be found in coconut water (Sandhya & Rajamohan, 2014).

Table 1 shows a significant increase (p<0.05) on the diameter of the lumen of the seminiferous tubule of the MSG treated mice. These results were in paralleled with findings of other studies on the effect of MSG (Das & Ghosh, 2010; Saber & Gamal, 2010; Mohamed, 2012) who reported that sloughing of the germinal epithelium from the basal lamina. On the other hand, histological architectures of the seminiferous tubules in control and CW groups in Figure 4A and Figure 4D showed normal seminiferous tubular structures, with no significant difference on the diameter of the tubular lumen.

Histological Analysis of Kidney

Histology of the kidney of mice treated with MSG is shown in Figure 5B. There were dilation of proximal convoluted tubule (PCT), swelling in Bowman's capsule and significant reduction (p<0.05) in the diameter of glomerulus (4.39 ± 0.21 µm) compared to the other treated groups. Necrosis on the urinary tubules was also recorded in other

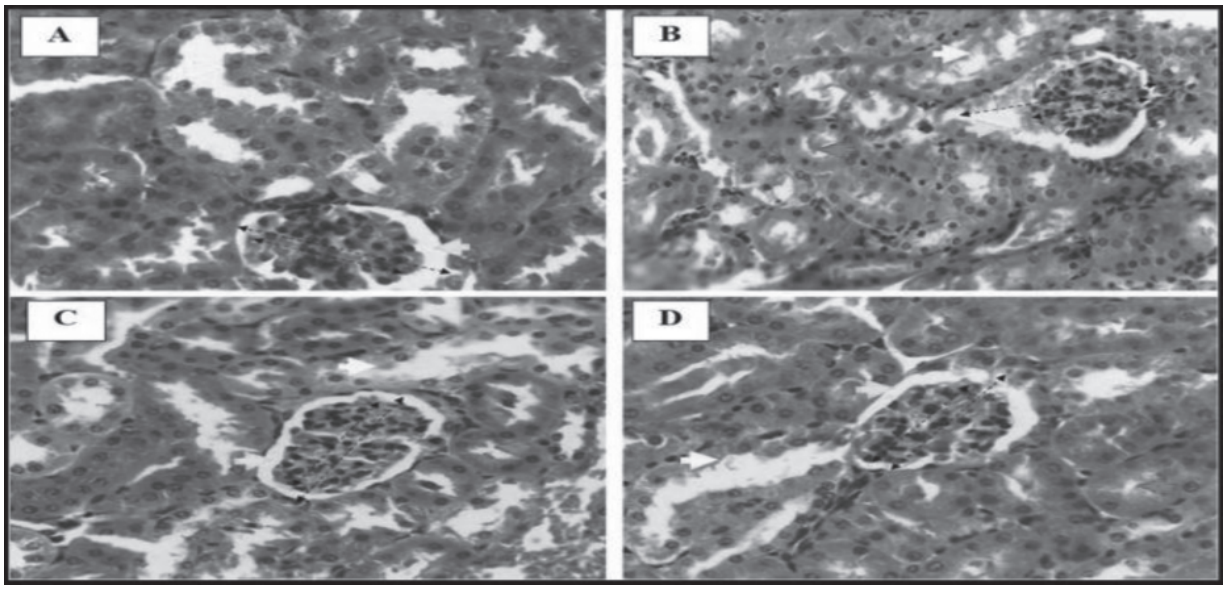

Fig. 5. Histology of kidney (H&E, 40× Magnification); (A): Control- showing normal histological architecture of glomerulus (white arrow), Bowman's capsule (green arrow), proximal (red arrow) and distal convoluted tubule (yellow arrow); (B): MSG group- showing dilation of Bowman's capsule (green arrow), shrinkage glomerulus (black dotted arrow and white arrow), dilation of proximal (thin arrow) with sloughing of the renal tubules cells (yellow arrow); (C): MSG + CW group- showing an improvement at most of the kidney cortex. Bowman's capsule (green arrow), proximal (red arrow) and distal convoluted tubule (yellow arrow), however, degeneration of the renal tubules cells is still present with increased in lumen space; (D): CW group- showing normal histological architectures of the kidney cortex as in the control group.

Table 2. The mean diameters (μm) of glomeruli in mice (mean ± SEM)

Organ	Treatment groups			
	Control	MSG (2g/kg b. wt)	MSG (2g/kg b. wt) + CW	CW
Glomeruli (μm)	8.00±0.15[a]	4.39±0.21[b]	8.12±0.27[a]	8.08±0.11[a]

Superscripts [a, b] represent significant difference (p<0.05) among groups.
MSG - Monosodium glutamate; CW - Coconut water.

groups of mice. These findings are mostly in conformity with previous studies (Nagata *et al.,* 2006; Cappelletti *et al.,* 2012; Onaolapo *et al.,* 2013; Singh *et al.,* 2014). The histological architecture of kidney tissues showed dilation of the proximal convoluted tubule (PCT) and distal convoluted tubule (DCT), swelling in Bowman's capsule, shrinkage of glomerulus and necrosis on the urinary tubules. Swelling of cells would be due to the drop of aerobic respiration and decreased O_2 levels in the presence of MSG that was responsible for the production of O_2 and O_2-free radicals (Dixit *et al.,* 2014).

Perhaps, the nephron of the kidney tried to excrete the l-glutamate from MSG which arrived in high concentration through the renal artery. The initial blood-filtering components which is the renal corpuscle received the l-glutamate through the afferent arteriole, then, going through the process of absorption and filtration, it crosses the membrane and damage the cells (Ortiz *et al.,* 2006). Besides that, the increase in lipid peroxidation products was also reported by Ortiz *et al* (2006) were malondialdehyde (MDA) and 4-hydroxy-2-nonenals in the response of the kidney damage and at the same times, there were also an increased in alanine aminotransferase (ALAT) and aspartate amino-transferase (ASAT) enzymes which consistent with hydropic degeneration and necrosis of the renal tubules.

The histological study showed that supple-mentation of coconut water in mice had reduced the cellular changes of the kidney and less tubular alteration (Figure 5C). Most of the Bowman's capsules and glomeruli appeared as normal. The mean diameter of the lumen of most PCT (8.12 ± 0.27 μm) in the mice as shown in Table 2 were significantly larger (p<0.05) compared to the MSG

group (4.39 ± 0.21 µm). This could be due to dietary antioxidants found in coconut water which provide protective potential against oxidative stress induced by the MSG. This suggested that reactive oxygen species (ROS) caused by MSG metabolism can be overcome by the administration of coconut water. Farombi & Onyema (2006) reported active oxygen species played an important role in MSG toxicity. Both the control and coconut water treated mice showed the normal histological architectures of the kidneys Figures 5A, 5D respectively.

CONCLUSIONS

The administration of 2 g/kg b.wt monosodium glutamate (MSG) had caused toxic effects on sperm and the architectures of the kidney and testes. Reduced sperm concentration, sperm motility, and sperm viability are the hallmarks of diminished fertility in males. This study suggests that continuous consumption of monosodium glutamate (MSG) in the dosage range herein may impair male fertility. Varying degrees of kidney and testicular injuries supported the claims that monosodium glutamate (MSG) may bring negative impact toward the body health. However, the present study demonstrated that, coconut water administered in combination with monosodium glutamate (MSG) had minimized the hazards caused by monosodium glutamate (MSG). This could be possibly due to the fact that coconut water has the biologically effective components in ameliorating the MSG effect on cell and tissues of selected organs. It is suggested that coconut water is recommended as the remedy for MSG induced toxicity.

It is recommended to further study on the effect of sub-acute toxicity of monosodium glutamate (MSG) in combination with other possible anti-oxidants in order to extrapolate the results of the present study to human and the use of coconut water as the therapeutic agent. Histological studies on other organs such as liver, spleen, and pancreas should be included in a comprehensive study**.**

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