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EFFECT OF PARTIAL REPLACEMENT OF MONOSODIUM GLUTAMATE BY 5´-INOSINE MONOPHOSPHATE ON THE FERTILITY OF MALE RATS

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ABSTRACT

This work aimed to study the effect of partial replacement of monosodium glutamate (MSG) by 5´ inosine monophosphate (5´-IMP) on the fertility of male rats after oral administration for 90 days. Before the biological treatment, a half amount of MSG as flavor enhancer in chicken burger was replaced by 5´IMP. The sensory assessment of cocked chicken burger confirmed that this used mixture (1:1 w/w) had a synergistic effect and led to improve the flavor intensity compared to that with MSG. Treatments were applied by stomach tube (mg/kg BW); (i) MSG, [60 for adult; 30 for weaned rats]; (ii) Mixture (1:1; w/w) of MSG and 5´-IMP [30:30 for adults; 15:15 for weaned rats] and (iii) 5´-IMP [30 for adult; 15 for weaned rats]. Body weight gain (BWG%) and weight of some reproductive organs including testes, prostate, cauda epididymes and seminal vesicles were measured. Serum testosterone and interstitial cell-stimulating hormone (ICSH) and seminal fructose content were assayed. Spermatozoa activity and the histology of reproductive organs were also studied in adult and weaned male rat groups. Severe negative effects on most studied parameters were demonstrated in MSG-groups, where the lowest fructose content in prostate, testosterone levels and spermatozoal activities were recorded. Reduction in the weight of cauda epididymes and testes was recorded in MSG-treated weaned rats. Histologically, all studied reproductive organs were dramatically affected by MSG-treatment. Considerable enhancements in the studied parameters and normal histological profiles were obtained due to the partial replacement of MSG by 5´-IMP. In conclusion, 5´-IMP has a potential protective effect against MSG-hazards in reproductive organs.

INTRODUTION

The flavoring enhancer monosodium glutamate (MSG) is one of the controversial food additives. The MSG has a characteristic taste called *umami* "savory deliciousness", which is considered distinct from the four other basic tastes (sweet, sour, salty, and bitter). The optimal palatability concentration for MSG in foods is between 0.2 – 0.8% with the largest palatable dose for humans being about 60 mg/kg body weight (BW) (Walker and Lupien, 2000; Elhariry et al., 2004). Despite anecdotal reports of MSG triggering headaches or exacerbating asthma, Joint FAO/WHO Expert Committee of Food Additive (JECFA), the European Community's Scientific Committee for Food, the American Medical Association, and the National Academy of Sciences have all affirmed the safety of MSG at normal consumption levels **(Tarasoff and Kelly, 1993; Walker and Lupien, 2000; Yoneda et al 2011).** The average intake in Europe and Asia is about 1- 10 g/day. These levels in addition to the natural intake have been considered as safe **(Walker and Lupien, 2000; Beyreuther et al 2007)**.

Although, several researches have supported the MSG safety, recently public concern about the health hazards of MSG remains high **(Simon, 2000; Yoneda et al 2011)**. These hazards included injury and/ or damages in brain **(Mautes et al**

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2001; Elhariry and El-Dakak, 2009a), kidney **(Ortiz et al 2006; Elhariry and El-Dakak, 2009b),** liver **(Ortiz et al 2006; Nakanishi et al 2008; Elhariry and El-Dakak, 2009b)**, pancreas **(Elhariry and El-Dakak, 2009a)**, reproductive organs **(Pizzi et al 1977; Miskowiak et al 1993; Das and Ghosh, 2011; Igwebuike et al 2011)**, lungs **(Yoneda et al 2011)** and imbalance of hormone secretion **(Mautes et al 2001; Elhariry and El-Dakak, 2009a)**.

MSG had been reported as a causer of obesity and decreasing weight of pituitary glands and testes of male rats **(Miskowiak et al 1993).** In the same study, blood serum levels of interstitial cell-stimulating hormone (ICSH), also known as Lutenizing hormone (LH) and Follicle-stimulating hormone (FSH) as well as the height of epithelial cells of accessory sexual glands remained unchanged, whereas testosterone level has been lowered **(Miskowiak et al 1993)**. More recently, the effects of oral administration of varied doses of MSG on the morphology of the testes and cauda epididymal sperm reserves of rats were studied in young and adult male Sprague-Dawley rats **(Igwebuike et al 2011)**. In that study, age variation did not influence the effect of MSG on these parameters. There was a significant reduction (*P<0.05*) in the cauda epididymal sperm reserves and the serum testosterone levels of the rats received MSG relative to the control ones. On the other hand, the histomorphology of the testes of the rats that were given MSG did not differ from those of the rats in the control.

Following the success of microbial large-scale production of L-glutamate, synergism of 5´-ribonucleotides with MSG greatly stimulated research on nucleotide overproduction greatly **(Abbouni et al 2004; Elhariry, 2004; Elhariry et al 2004; Elhariry et al 2005)**. The intensity of *umami* taste is markedly enhanced by mixing glutamate with 5´-ribonucleotides such as 5´-inosine monophosphate (5´-IMP) and 5´-guanisine monophosphate (5´-GMP). A 1:1 mixture of MSG and these 5´-ribonucleotides acts synergistically and gives flavor intensity 30 times stronger than that of monosodium glutamate alone **(Maga, 1994)**. In a previous work, taste and overall acceptability of beef burger contained the mixture of MSG and 5´-IMP (1:1 w/w) have been significantly (*p<0.05*) enhanced compared with samples contained MSG only **(Elhariry and El-Dakak, 2009a; 2009b)**. Moreover, the partial replacement (1:1 w/w) of MSG with 5´-IMP has been reported to reduce the MSG-induced hazards in adult and weaned rats. These hazards included neural damages in the brain, kidney, liver, pancreatic injury and imbalance of hormone secretion.

Based on this assumption and hence the toxicity of MSG administration on the reproductive organs has been extensively investigated without proposing a suitable solution, the aim of the present study was to evaluate the role of partial replacement of MSG by 5´-IMP for reducing the MSG-induced toxicity in the reproductive organs of male rats.

Materials and methods

Materials

Chicken meat and ingredients were obtained from the local market in Cairo. Forty eight male Sprague-Dawely strain rats were obtained from Research Institute of Ophthalmology, Giza, Egypt. 24 were adult rats (145 g ± 2 and 10 weeks age) and 24 were weaned rats (45 g ± 2 and 3 weeks age). Kits for all biochemical testes were purchased from Bio-diagnostic, Egypt.

Chemicals

Monosodium glutamate (MSG) and 5′ inosine monophosphate (5´-IMP) were purchased from Sigma-Aldrich Chemical Company, Louis, U.S.A. The kits for fructose determination were obtained from Stanbio Laboratories, Boerne, Texas, USA; Testosterone and lutenizing hormone (LH) kits were from AccuBindTM, Monobind Inc., USA. The other chemicals used were of analytical grade and purchased from Sigma-Aldrich, USA.

Methods

Sensory assessment of flavor enhancement effect of MSG/5´-IMP mixture

The MSG was added as flavor enhancer to chicken burger at level of 0.5 % according to the Egyptian Standards **(E.S. 2005)**. A 1:1 w/w mixture of MSG and 5´-IMP was applied as a partial replacement of MSG with 5´-IMP (Maga, 1994). Cooked chicken burger samples were assessed for their sensory attributes by ten panelists according to **Klein and Bardy (1984)** The panelists were asked to score the different samples for their appearance, color, aroma, taste, juiciness, tenderness and overall acceptability as follows: very good 8-9, good 6-7, fair 4-5, poor 2-3, and very poor 1-2.

Treatments

The procedures were carried out in compliance with the guidelines for the ethical use of animals in scientific research **(Festing and Wilkinson, 2007)**. Briefly, the animals were housed in separate stainless steel cages raised in a well-ventilated room with 12-h light/dark cycle and were fed ad-libtum (free access to feed and water) throughout the experimental period (90 days). After adaptation period (10 days), rats were divided into two main classes; adult class and weaned class. The adult class was one confined to rats aged over 10 weeks (weighed 151±2 g). It was randomly divided into four groups; G1, G2, G3 and G4. The weaned class was confined to weaning rats (weighed 47±2 g) and was randomly divided into four groups; G5, G6, G7 and G8. Each group contained six rats. All animals were fed on basal diet (10% casein, 10% corn oil, 5% cellulose, 1% vitamin mixture, 4% salt mixture, 70% corn starch for 90 days **(Lana Peter and Pearson, 1971).** Rats of G1 and G5 were used as controls. The other six groups were administrated with different concentrations of MSG and/or 5´-IMP by stomach tube. Stock solutions of MSG and/or 5´-IMP were prepared in double distilled water and the given amounts were calculated according to the BW of rats. Rats of G2, G3 and G4 were orally administrated using stomach tube with 60 mg MSG/ kg BW, 30 mg MSG +30 mg 5´-IMP (1:1 w/w), and 30 mg 5´-IMP / kg BW, respectively. Rats of G6, G7 and G8 were administrated with 30 mg MSG/ Kg BW, 15 mg MSG + 15 mg 5´-IMP (1:1 w/w) and 15 mg 5´-IMP / kg BW, respectively. The doses of MSG and 5´-IMP were doubled when the rats of G6, G7 and G8 reached to the weight of adult age (7 weeks from the beginning of the experiment).

Body weight gain

Rats were observed daily for the appearance of any symptoms of discomfort that might be related to studied treatments. BW of the rats was recorded daily before administration of studied additives. At the end of experimental period, the percentage of weight gain was expressed [(final weight – beginning weight) / beginning weight] × 100**.**

Weight of reproductive organs

At the end of the experimental period, rats were weighted and killed by diethyl ether. Testes, epididymes, seminal vesicles and prostate were cut off, washed in ice-cooled saline solution 0.15M KCl to remove blood and weighed. Organ weights were recorded after autopsy and represented as weight per 100 g body weight **(Bajaj and Gupta, 2012)**.

Spermatozoal activity

The Resazurin Reduction Test (RRT) was applied for determining spermatozoal activity **(Reddy and Bordekar, 1999)**. RRT depends on the ability of metabolically active spermatozoa to reduce the resazurin dye (blue), with maximum absorption at 615 nm (A_{615}), to resorufin (pink) with a maximum absorption of 580 nm (A_{580}). The ratio of the optical densities of reduced to oxidized form (i.e. 580 to 615 nm) can be used to evaluate the various grades of semen sample. Semen samples were centrifuged at 2500 g for 20 min. The seminal plasma supernatant was applied for test according to manufacturer's constructions using kits.

Biochemical assay

At the end of experimental period, blood samples were collected from the eye plexuses of animals by a fine capillary glass tubes and placed immediately on ice. Blood serum samples were collected into dry clean centrifuge tubes; the serum was separated after centrifugation for 10 min at 3000 rpm (1500 xg) and kept at –20 ºC until analysis. Testosterone and interstitial cell-stimulating hormone (ICSH) concentrations were measured in triplicate by ELISA according to the producer's instructions.

Histopathological studies

Different sections of studied reproductive organs (testes, cauda epididymes, seminal vesicles and prostate) were prepared for histological examination **(Bancroft and Stevans, 1996)**.

Statistical analysis

All values are means ± SD obtained from eight animal groups (six of each). Data were analyzed with SAS software (SAS Institute, Cary, N.C.) using SAS analysis of variance (PROC ANOVA). Significant differences between means were determined by Duncan's multiple range test ($P < 0.05$).

Results and discussion

Sensory assessment of flavor enhancement effect of MSG/5´-IMP mixture

The main objective of the present study was to evaluate the role of the partial replacement of MSG with 5´-IMP for reducing the neuronal hazards in-

duced by the oral intake of MSG. Therefore, half amount of MSG in chicken burger was replaced by 5´-IMP. Insignificant changes were recorded for the appearance, color, juiciness, and tenderness due to the partial replacement of MSG with 5´-IMP. However, overall acceptability, aroma, and taste of chicken burger prepared using the mixture of MSG and 5´-IMP were significantly enhanced ($p<0.05$) compared with that contained MSG alone (untabulated data). On the other hand, this finding confirmed that the 1:1 mixture of MSG and 5´ IMP has a synergistic effect and led to improve the flavor intensity compared to that with MSG alone **(Maga, 1994; Delwiche, 2004)**.

Since (i) the dose of MSG added to foods is between 0.2 – 0.8% depending on the product itself and (ii) the ADI is not specified, the largest palatable dose of MSG (60 mg/kg BW) was applied for the biological evaluation in the present study using both the adult and weaned rats. The partial replacement of MSG with 5´-IMP at the level of 1:1 (w/w) was also investigated.

Weight gain and reproductive organs weight

Body weight gain percentage (BWG%) and weight of the reproductive organs of adult and weaned rats after oral intake of MSG and/or 5´-IMP are presented in **(Table 1)**. There was significant differences ($p<0.05$) in BWG% between the studied groups. BWG % in the control group of the adult (G1) and weaned (G5) rats were 92.2 and 345.3%, respectively. The highest BWG% of the adult (111.2%) and weaned (480.7%) groups were recorded by G2 and G6 that received MSG. On the other hand, inside the classes of adult or weaned rats the descending order of BWG% was recorded by rats received MSG (G2 and G6), MSG combined with 5´-IMP (G3 and G7) then 5´-IMP (G4 and G8). This result indicated that MSG may cause obesity in rats. It could be explained by those mentioned by **Martins et al (2001)**. They suggested that the lesion in hypothalamus causes an impairment of sympathetic transmission in adrenal medulla and less catecholamines accumulation and secretion. Consequently, these defects might be involved in the onset of MSG obesity in rats. The same finding was stated also by **Elhariry and El-Dakak (2009 a, b)**.

No significant difference ($P>0.05$) was observed between weights of the seminal vesicles of all studied groups **(Table 1)**. In the adult rats cauda epididymes weights did not affect by intake of MSG and/or 5´-IMP. However, comparing with the control weaned-group (G5) significant decrease ($p<0.05$) was detected in cauda epididymes weight of the weaned rats received MSG (G6) or MSG combined with 5´-IMP (1:1 w/w) (G7). Also, significant reduction ($p<0.05$) in the weight of testes was noticed in weaned rats subjected to MSG **(Table 1)**.

These results indicated that the weaned rats were more sensitive to MSG compared with the adult rats. Previously, several studies demonstrated the negative effect of MSG on some reproductive organs including testes, prostate, and seminal vesicles weights of young rats due to oral intake of MSG; 6 g/kg BW for different periods; from 30 -to 120 days **(Villanúa et al 1992; Fernandes et al 2012b).** On the other hand, **França et al (2006)** showed unchanged sperm production, testes weight and seminiferous tubular diameter in obese adult rats after neonatal glutamate-induced obesity. In general, insignificant differences ($p>0.05$) were observed between the 5´-IMP groups (G4 and G8) and the control groups (G1 and G5). These results demonstrated the potential effect of 5´-IMP to reduce the changes in weight of reproductive organs induced by oral intake of MSG.

Testosterone and interstitial cell-stimulating hormone

Testosterone is an anabolic steroid hormone from the androgen-group and is found in mammals and other vertebrates **(Cox and John-Alder, 2005)**. It is primarily secreted in the testicles of males, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone that plays a key role in the development of male reproductive tissues such as the testes and prostate **(Bassil et al 2009).** Interstitial cell-stimulating hormone (ICSH) stimulates Leydig cell production of testosterone **(Louvet et al 1975)**. Therefore, the level of these two sexual hormones; testosterone (T) and ICSH were determined in blood serum of the investigated rats **(Table 2)**. The concentrations of testosterone in the control adult and weaned groups (G1 and G5, repectively) were in the normal range (1.50 and 1.40 ng/mL) that previously stated by **Fernandes et al (2012b)**. On the other hand, significant reduction ($p<0.05$) in the level of testosterone was observed in the blood serum of MSG- groups (G2 and G6). This finding is in agreement with those mentioned by **Villanúa et al (1992) and Fernandes et al (2012b)**. They stated that testosterone was reduced from ~ 1.59 ng/mL in control rats to ~ 0.57 ng/mL in MSG-treated animals. In the present study, severe reduction was noticed in testosterone level in blood

Table 1. Body weight gain and weight of reproductive organs of male rats after 90 days of oral administration with MSG and/or 5´-IMP.

Rat Class	Rat group	Daily intake (mg/kg BW)	Weight of organs (mg/ 100 g BW)				Body weight gain (%)
			t	p	c	sv	
Adult	G1	Control	$410^{a} \pm 10$	$580^{b} \pm 21$	$146^{a} \pm 21$	$280^{a} \pm 15$	92.2^{c}
	G2	MSG (60)	$415^{a} \pm 11$	$678^{a} \pm 24$	$149^{a} \pm 18$	$285^{a} \pm 16$	111.2^{a}
	G3	MSG (30) + 5´-IMP (30)	$410^{a} \pm 9$	$574^{b} \pm 30$	$139^{a} \pm 18$	$281^{a} \pm 16$	98.2^{b}
	G4	5´-IMP (30)	$412^{a} \pm 12$	$585^{b} \pm 29$	$158^{a} \pm 14$	$282^{a} \pm 19$	99.5^{b}
Weaned*	G5	Control	$435^{a} \pm 14$	$520^{c} \pm 22$	$125^{a} \pm 20$	$264^{a} \pm 20$	345.3^{c}
	G6	MSG (30)	$401^{b} \pm 12$	$618^{a} \pm 12$	$90^{b} \pm 17$	$260^{a} \pm 19$	480.7^{a}
	G7	MSG (15) + 5´-IMP (15)	$433^{b} \pm 12$	$585^{b} \pm 15$	$94^{b} \pm 11$	$265^{a} \pm 17$	398.2^{b}
	G8	5´-IMP (15)	$433^{a} \pm 16$	$525^{c} \pm 21$	$110^{a} \pm 19$	$256^{a} \pm 23$	358.5^{c}

* The doses of MSG and 5´-IMP were doubled when the weight of rat in weaned class reached to the weight of adult rats (7 weeks from the beginning of the experiment). Abbreviations; t = testes, p = prostate, c = cauda epididymes, sv = seminal vesicles. Values are means ± SD (*n=6*); within each class values with different superscripted letters in the same column are significantly different ($p < 0.05$).

serum of weaned rats (G6; 0.23 ng/mL) compared with that of adult rats (G2; 0.66 ng/mL). In both rat classes (adult and weaned rats), testosterone level did not significantly affected ($p>0.05$) when animals were subjected with 5´-IMP alone or combined to MSG **(Table 2)**. On the other hand, no significant changes ($p>0.05$) were observed in the ICSH concentration of both adult and weaned rats due to treatment with MSG, 5´-IMP or the mixture of them (1:1; w:w) **(Table 2)**. In agreement with this result, ICSH level in blood did not change due to MSG treatment at the level of 4.0 mg/g BW **(Fernandes et al 2012a)**. These results indicated that, MSG might have a direct effect on the secretion of testosterone or on its activity, where the regulator hormone (ICSH) did not be affected by the studied treatments. On contrary, previous studies demonstrated the negative effects of glutamate through a hypothalamic injury that disrupts the secretion of several hormones including the gonadotrophins (FSH and LH) **(Gong et al 1995)**. This might be due to the difference in dose treatment or duration of adminstration. Generally, results of the present study and the studies of **Elhariry and El-Dakak (2009a; 2009b)** demonstrated imbalance in hormone secretion and regulation due to treatment with MSG. Moreover, this hormonal imbalance was considerably enhanced due to replacement of MSG with 5´-IMP (1:1; w/w).

Fructose content and spermatozoal activity in the reproductive organs

Fructose content of the four tested reproductive organs were determined and represented in **(Fig. 1)**. Clearly, all treatments applied in the present study led to significant decrease in fructose concentration in the sexual studied organs. However, the severest treatment was MSG-treatment. Although, the use of 5´-IMP and its mixture with MSG led to reduce fructose content, these treatments had less effect compared with MSG-treatment **(Fig. 1)**. The reduction in fructose concentration in reproductive organs could be confirmed by the finding of decreases in testosterone levels. This explanation was supposed by **Melis (1999)**. It is well known that, fructose is a nutrient substance present in seminal plasma. Also, it's formation is initiated and controlled by testicular androgens **(Mann, 1948)**. In this respect, hormonal deficiency causes a decrease or even disappearance of seminal fructose, and a compensatory treatment with androgens restores the ability of the accessory glands to produce this sugar **(Kempinas and Lamano-Carvalho, 1988)**.

Moreover, fructose, if converted by spermatozoa to lactic acid, provides an important source of energy for sperm cells. Therefore, the spermatozoal activity was determined in the present study using resazurin reduction test (RRT) as a ratio

Table 2. Testosterone (T) and interstitial cell-stimulating hormone (ICSH) in blood serum of rats after 90 days of oral administration with MSG and/or 5´-IMP.

Rat Class	Rat group	Daily intake (mg/kg BW)	T ng/ mL	ICSH ng/ mL
Adult	G1	Control	1.50^{a} ± 0.19	3.07^{a} ± 0.15
	G2	MSG (60)	0.66^{b} ± 0.17	3.03^{a} ± 0.07
	G3	MSG (30) + 5´-IMP (30)	1.29^{a} ± 0.39	3.10^{a} ± 0.08
	G4	5´-IMP (30)	1.4^{a} ± 0.15	3.17^{a} ± 0.11
Weaned *	G5	Control	1.40^{a} ± 0.21	3.09^{a} ± 0.02
	G6	MSG (30)	0.23^{b} ± 0.08	3.49^{a} ± 0.08
	G7	MSG (15) + 5´-IMP (15)	1.43^{a} ± 0.25	3.11^{a} ± 0.09
	G8	5´-IMP (15)	1.44^{a} ± 0.04	3.03^{a} ± 0.11

* The doses of MSG and 5´-IMP were doubled when the weight of rat in weaned class reached to the weight of adult rats (7 weeks from the beginning of the experiment). Values are means ± SD (*n=6*); within each class values with different superscripted letters in the same column are significantly different ($p < 0.05$).

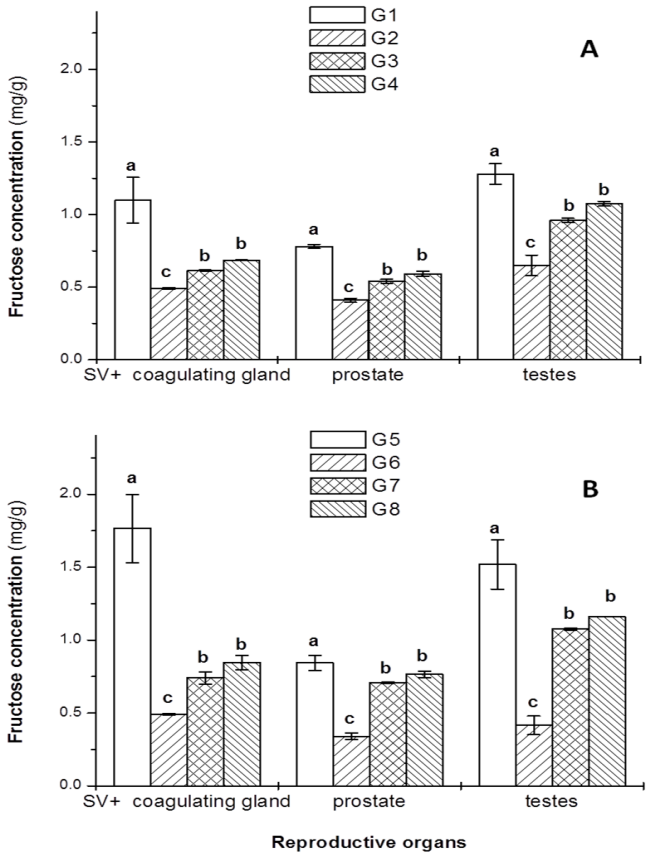

Fig. 1. Fructose content in different reproductive organs in adult (A) and weaned (B) male rats after 90 days of oral intake of MSG and/or 5´-IMP.

Rats were treated (per kg BW) with 60 mg MSG (G2), 30 mg MSG + 30 mg 5´-IMP (G3), 30 mg 5´-IMP (G4), 30 mg MSG (G6), 15 mg MSG + 15 mg 5´-IMP (G7) and 15 mg 5´-IMP (G8). The doses of MSG and 5´-IMP were doubled when the weight of rat in weaned class (G6, G7 and G8) reached to the weight of adult rats (7 weeks from the beginning of the experiment). G1 and G5 were represented as control groups. Results are the means ± SD (*n=6*). Columns with the same letter within each group are insignificantly differed ($p > 0.05$).

between A_{580} to A_{615} (RRTR). In adult rat, insignificant alterations ($p>0.05$) were recorded in spermatozoal activity after treatment with 5´-IMP and its mixture with MSG (**Fig. 2**; A). However, adult rats subjected to 60 mg MSG/kg BW showed significant reduction ($p<0.05$) in their spermatozoal activity. In the weaned rats, all treatments led to significant reduction ($p<0.05$) in spermatozoal activity of all tested reproductive organs, except the treatment with 5´-IMP alone (G8) that led to insignificant change in spermatozoal activity of cauda epididymes and testes **(Fig.2 B)**.

In general, the obtained data indicated that, the weaned rats are more sensitive to treatments with MSG and/or 5´-IMP compared with the adult rats. Moreover, the replacement with 5´-IMP led to attitude the hazard effect of MSG on the spermatozoal activity. The obtained results demonstrated the relationship between the reduction in spermatozoal activity and the low level of fructose recorded **(Fig. 1 and 2)**.

Histological changes

The microscopic investigation of testes revealed no histopathological changes in control groups of both adult and weaned rats; G1 and G5 **(Fig. 3)**. In adult rats, treatment with MSG led to degeneration of spermatogoneal cells liming the seminiferous tubules (G2), while the treatments with the MSG+5´-IMP mixture or 5´-IMP alone illustrated no histological changes (G3 and G4). On the other hand, histological examination of reproductive organs of the weaned rats revealed dramatic affection by the treatment with MSG **(Fig. 3)**. This group (G6) revealed vacuolation of spermatogoneal cells lining seminiferous tubules, interstitial edema and intraluminal desquamation of spermatogomeal cells associated with inflammation. In agreement with these results, **Ismail (2012)** stated that administrating MSG to young male rats led to different histological changes in their testes. These alterations included mainly seminiferous tubules, interstitial connective tissues. This author added also that, many spermatogenic cells have appeared with pyknotic nuclei, in addition to vacuolations between the inner cells of seminiferous tubules. In another study, rat treated with MSG have showed semniferous tubule with only few spermatids and interstitial space with inflammatory exudates **(Cemaluk et al 2013)**.

Microscopically, prostate of control adult and weaned rats (G1 and G5, respectively) and 5´-IMP-groups (G4 and G8) displayed normal histology of prostatic acini **(Fig. 4)**. MSG+5´-IMP-group of weaned rats revealed vacuolation of epithelial lining prostatic acini (G7), while the same treatment did not lead to visible changes in the adult rats (G3). On the other hand MSG-treatment led to vacuolation of epithelial lining prostatic acini accompanied with interstitial edema in weaned rats **(Fig. 4**; G6), and slight hyperplasia of epithelial lining prostatic with slight interstitial edema in adult rats (**Fig. 4**; G2).

Normal histopathological images of epididymes were observed in all groups, except MSG-groups **(Fig. 5)**. In adult rats, MSG-treatment led to marked interstitial edema associated with few leukocytic cells infiltration and spermatid giant cells in the lumen of epididymeal duct (**Fig. 5**; G2), while interstitial edema and congestion of blood vessels were clearly observed in weaned rats (**Fig. 5**; G6). In the adult rats, no histological changes in seminal vesicles were recorded in control-, MSG+5´-IMP- and 5´-IMP- groups (**Fig. 6**; G1,G3 and G4, respectively). However, hypoplasia of the acini was noticed when the adult rats were treated with MSG (G2). The seminal vesicles of weaned rats were more sensitive to all studied treatments, especially MSG-treatment **(Fig. 6)**. Rats of MSG-group (G6) revealed hyperplasia and vaculation of epithalial lining of the acini. Reducing the MSG due to partial replacement by 5´-IMP (1:1; w/w) led to attenuate the toxic effect of MSG. This could be indicated from the slight hyperplasia and accumulation of capicioas eosinophilic gladular section (**Fig. 6**; G7). Also, 5´-IMP led to slight hyperplasia of the acini (G8).

In general, it was clearly evident that, serum testosterone concentration was in agreement with fructose content that was confirmed with spermatozoal activity and histopatholgical examination findings.

CONCLUSION

This study spots light on the importance of rationalizing the consumption of MSG-fortified foods that may cause different risks in the reproductive organs of rats, especially in youngs. The partial replacement of MSG by IMP (1:1; w/w) could be suggested for attenuating MSG-induced hazards. More advanced studies on large animals are needed to demonstrate this finding. In general, the obtained results clearly provide a serious argument to withdraw MSG from human diets, especially that frequently consumed by children.

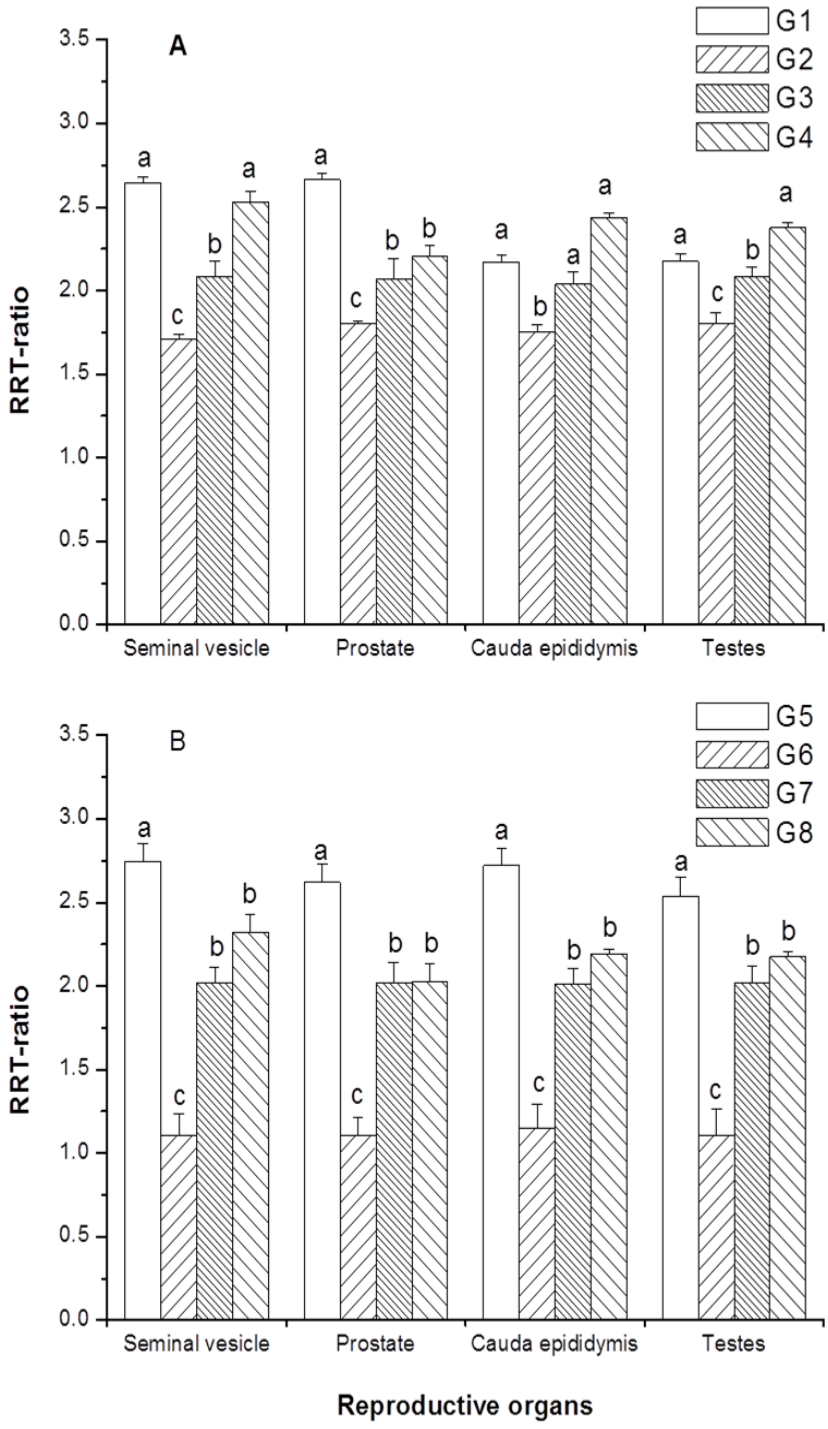

Fig. 2. Spermatozoal activity (Resazurin Reduction Test; RRTR) in different reproductive organs in adult (A) and weaned (B) male rats after 90 days of oral intake of MSG and/or 5´-IMP. Treatments are appended to **Fig. 1**.

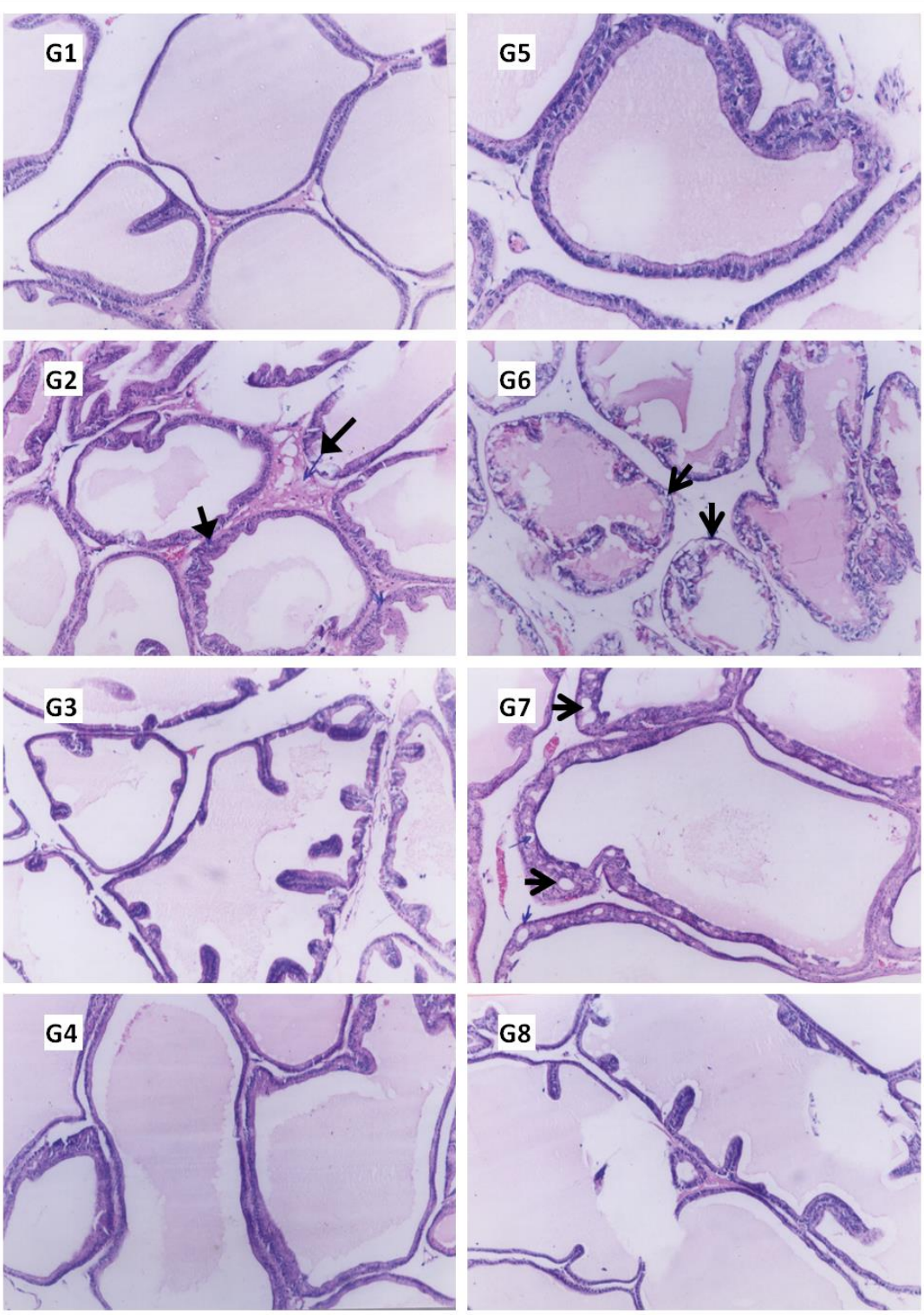

Fig. 4. Histopathological changes in prostate gland of the adult (G1-G4) and weaned (G5-G8) rats due to daily oral intake of MSG and/or 5´-IMP for 90 days. Treatments are appended to **Fig. 1**. (H&E, X200).Black open arrows indicate vaculation of epithilial lining prostatic acini; Black closed arrows indicate slight hyperplasia of epithelial lining protatic acini associated with slight interstital edema

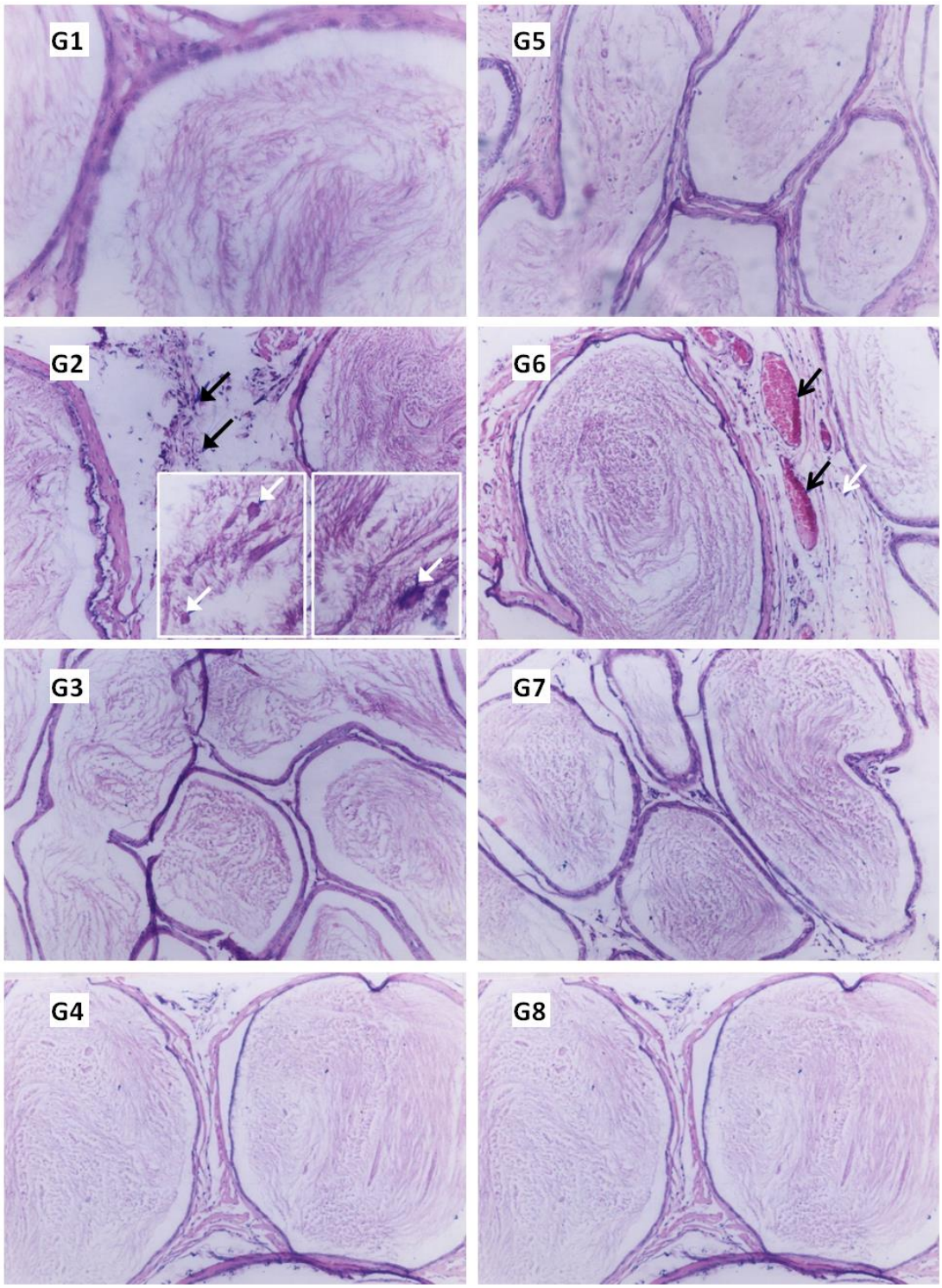

Fig.5. Histopathological changes in cauda epididymes of the adult (G1-G4) and weaned (G5-G8) rats due to daily oral intake of MSG and/or 5´-IMP for 90 days. Treatments are appended to **Fig. 1.** (H&E, X200). White open arrows indicate interstitial edema; White closed arrows indicate spermatid giant cell; Black open arrows indicate congestion of blood vessels; Black closed arrows indicate marked interstitial edema associated with forming leukocytic cells infiltration.

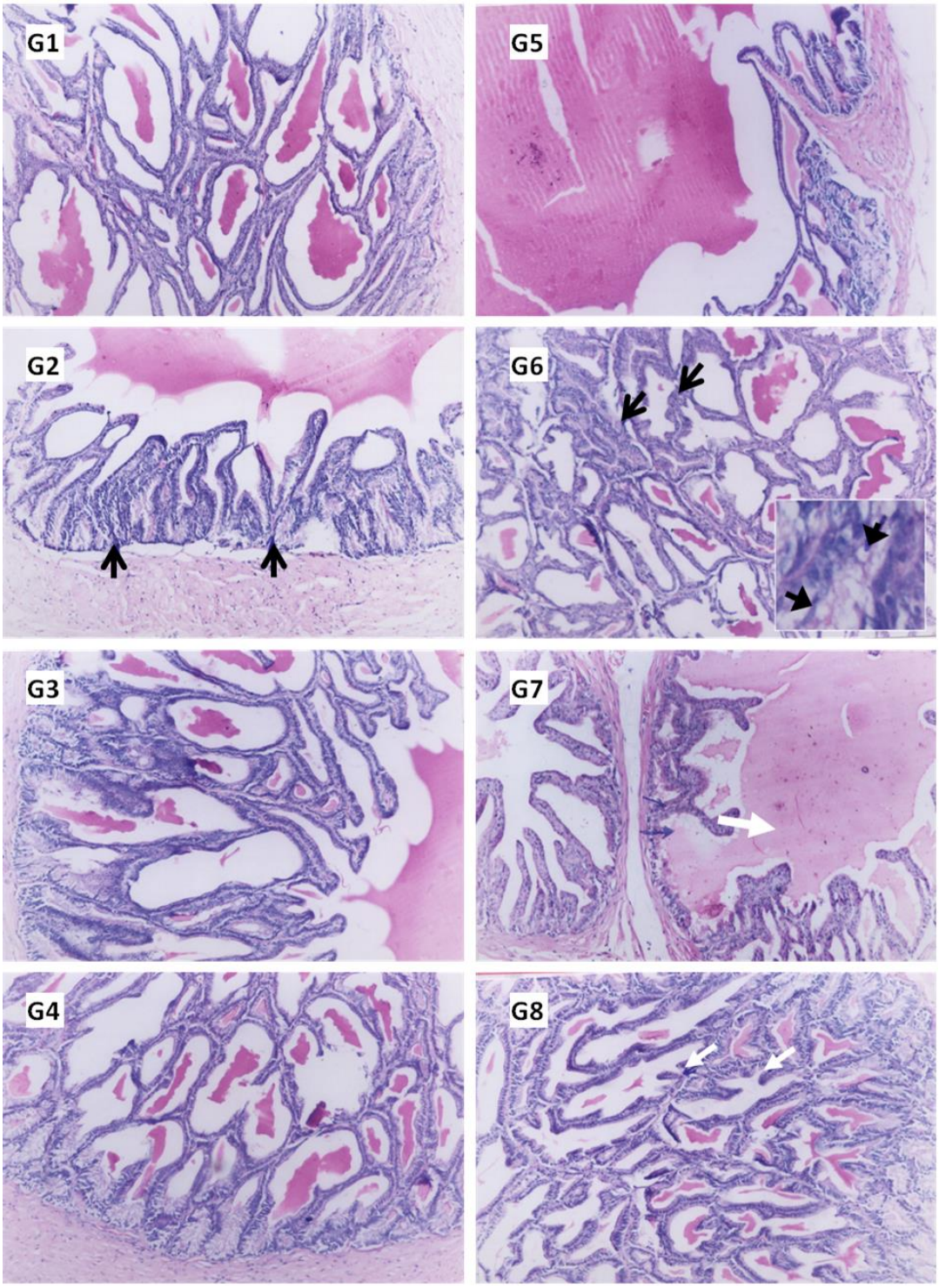

Fig.6. Histopathological changes in seminal vesicles of the adult (G1-G4) and weaned (G5-G8) rats due to daily oral intake of MSG and/or 5´-IMP for 90 days. Treatments are appended to **Fig. 1**. (H&E, X200). White closed arrows indicate slight hyperplasia and accumulation of capricious eosinophilic glandular secretion; Black open arrows indicate hyperplasia of acini; Black closed arrows indicate vaculation of epithelial lining the acini.

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