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RESEARCH ARTICLE

Antiapoptotic and antioxidant capacity of phytochemicals from Roselle (*Hibiscus sabdariffa*) and their potential effects on monosodium glutamate-induced testicular damage in rat

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Abstract

Monosodium glutamate (MSG), common flavor enhancer and feed additive, causes male reproductive dysfunction. However, Roselle tea, popular *Hibiscus sabdariffa* (HS) beverage, has a controversial effectiveness on male fertility. Therefore, the current study aimed to investigate either the adverse effect of aqueous HS extract (HSE) on the testicle or its potential ameliorative role including some stress markers, biochemical and immunohistochemical expressions in rats subjected to MSG. Here, the animals were divided into four groups that were given distilled water, HSE, MSG, and HSE + MSG respectively via gavage. After 6 weeks from the beginning of experiment, blood samples were collected for hormonal analysis. Additionally, testicular specimens were excised and processed for oxidative/antioxidant parameters determination, histological examination, and immunohistochemical evaluation of Bax and PCNA positive spermatogenic cells. Preliminary phytochemical analyses as well as antioxidant capacity of the HSE were tested. Our results revealed a strong inhibitory activity of the HSE phytochemical constituents against DPPH radical. MSG group revealed a significant decrease of testosterone, LH, FSH, and antioxidant parameters with elevated MDA compared with control and HSE groups. Additionally, an alteration of the testicular histo-architecture was observed among MSG group along with increased Bax and decreased PCNA positive cells. Meanwhile, the HSE showed a potent protective effect against testicular damage as well as oxidative stress induced by MSG. On the whole, our findings provide evidence that HSE can ameliorate MSG-induced testicular toxicity via induction of cell proliferation along with reduction of oxidative stress and cellular apoptosis in adult rat that attributed to the antioxidant and antiapoptotic effects of its phytochemical constituents.

Keywords Monosodium glutamate · Hibiscus · Phytochemical analysis · Immunohistochemistry · Oxidative damage · Testicular injury

Introduction

Monosodium glutamate (MSG) is the sodium salt of non-essential amino acid, glutamic acid (Eweka 2007). In general, MSG is accepted as a safe flavor enhancer and nutritional supplement that does not require an upper limit or a specified average daily intake (Samuels 1999). However, unintended use of these additives may occur due to their availability, often without labeling in many food ingredients (Egbuonu et al. 2009). Despite of being marketed as widely used feed additive and flavor enhancer (Hamzaa and AL-Harbi 2014), MSG induces testicular damage adversely affects male fertility (Das and Ghosh 2010; Igwebuike et al. 2011; Jubaidi et al. 2019). Some studies described the molecular mechanisms by which

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MSG induces damage of the different organs including testis. The induction of oxidative stress is a common mechanism of MSG-induced toxicity via increasing the level of intracellular reactive oxygen species (ROS) leading to an increase of lipid peroxidation and reduction of antioxidant enzymes which in turn disrupt cellular metabolism and damage lipids, proteins, carbohydrates and nucleic acids (Umukoro et al. 2015). Moreover, the increased ROS production activates intrinsic apoptosis pathway via increasing intracellular calcium and BAX with reduction of Bcl-2 which in turn increases cytochrome C that stimulates caspase 9 and 3 leading to cell death (Hajihasani et al. 2020).

Nowadays, some studies tend to use medicinal plants to prevent and treat many diseases induced by harmful elements (Mohamed et al. 2016; Abdellatief et al. 2017; AlBasher et al. 2020). One of the most popular beverages in Egypt and many other countries is Roselle tea which is a natural plant product prepared from the dried red calyces of *Hibiscus sabdariffa* (HS) and served either cold or hot. HS is an ideal but yet underutilized candidate for pharmaceutical applications (Patel 2014). Such plant has an international medical fame, where it has potent medicinal roles including anti-cholesterol and antihyperlipidemic (Chang et al. 2004; Ochani and D'Mello 2009), antihypertensive (Ajay et al. 2007; Hopkins et al. 2013), diuretic (Mojiminiyi et al. 2000), and laxative effect (Owulade et al. 2004). HS contains major compounds such as glucoside, and anthocyanin (Gaya et al. 2009; Beheshti et al. 2018). Pacôme et al. (2014) indicated that alkaloids, anthocyanins, flavonoids, saponins, and steroids that present in petals of the HS contribute to its antioxidative activity. The HS extract (HSE) antioxidant mechanism may be due to scavenging ROS and free radicals, xanthine oxidase inhibition, lipid peroxidation reduction and elevation of antioxidant enzymes activities (Costa-Rocha et al. 2014). It is interesting that HS has also antiapoptotic potential (Bakhtiari et al. 2015) via increasing cell viability and decreasing cell apoptosis of PC12 cells under serum/glucose deprivation condition. In spite of the medicinal benefits of HS, its effect on the male fertility is controversial.

It is conflicting that the administration of HSE at a dose of 1 g/kg b.w has a protective effect against cisplatin-induced testicular toxicity (Amin and Hamza 2006); meanwhile, the use of approximately the same dose (1.15 g/kg b.w) causes testicular toxicity (Orisakwe et al. 2004). It is also interesting that both Sirag et al. (2013) and Olusanya et al. (2018) used much lower doses of HSE (250 mg/kg b.w), and they recorded testicular damage however Suleiman et al. (2015) reported an ameliorative effect of HSE (200 mg/kg b.w) against testicular damage induced by carbamazepine.

Despite this inconsistency, Akindahunsi and Olaleye (2003) recommended a safe consumption of aqueous HSE at 150–180 mg/kg b.w/day but without evidence or results. Therefore, the current study was designed primarily to confirm or deny the recommendation of Akindahunsi and Olaleye (2003). Additionally, we tried to clarify if the HSE has an ameliorative role against MSG-induced testicular injury of adult rats. This work was done via hormonal analysis and oxidant/antioxidant parameters determination. Along with, histological examination and immunohistochemical expression of Bax and PCNA were evaluated for all rats' testes.

Materials and methods

Chemicals and other reagents

MSG was purchased from Al-Gumhoria Chemical Company, Egypt. For experimental use, working stock solution of MSG was prepared by diluting it in distilled water. Commercial bioassay kits were obtained from Biocheck Ltd. (UK) to estimate serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). All diagnostic kits for oxidative stress and antioxidant parameters were obtained from Biodiagnostic Company (Cairo, Egypt).

Preparation of HSE

Dried red calyces of HS were purchased from local market (Toukh, Kalubyia Governorate, Egypt). Aqueous HSE was prepared as outlined by Mahmoud (2012). Fifteen grams of dried calyces of HS was boiled with 500 ml distilled water for 15 min and allowed to cool to room temperature. Then, extract was filtered through filter paper (Whatman's No. 1).

Preliminary phytochemicals analysis

The aqueous HSE was subjected to qualitative phytochemical analysis for alkaloids, anthocyanins, flavonoids, glycosides, phenols, and steroids. Those tests were performed following the standard procedures adapted by Rajesh et al. (2014).

DPPH radical scavenging assay/capacity

It was determined using 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay according to Eshwarappa et al. (2014). Fresh DPPH solution was prepared then taken in test tubes and HSE were added in serial dilutions to every tube. After 30 min, incubation take place in dark place at RT, the discoloration was measured at 517 nm using an UV Spectrophotometer. Ascorbic acid was used as a positive control and dissolved in DW to bring the stock solution to the same concentration of HSE. The capacity to scavenge the DPPH radical (inhibition percentage) was calculated as follow:

Inhibition ratio (%) = [(absorbance of control − absorbance of sample) / absorbance of control] × 100.

Animals and experiment

Forty apparently healthy adult male albino rats, weighted 150–200 g, were housed in polypropylene cages, maintained in a pathogen-free environment as much as possible, at a controlled temperature 25 ± 2 °C with 12 h light and a 12 h light: dark cycle. All rats were fed standard pellet diet and water, given ad libitum. Prior to experimental use, rats were acclimated for 2 weeks.

For experiments, rats were randomly divided into four equal groups (10 rats each) and given a daily oral dose of different treatments by gavage method. Control group was given distilled water, HSE group was received HSE, MSG group was treated with MSG and finally, HSE+ MSG group was co-exposed to HSE + MSG. For each respective group, MSG and HSE groups were given at dose of 60 mg/kg and 180 mg/kg body weight according to Hamza and Al-Harbi (2014) and Akindahunsi and Olaleye (2003) respectively for consecutive 6 weeks. For HSE + MSG group, rats were pretreated with HSE for 7 days and subsequent co-treated with MSG for 6 weeks. The dose volumes were adjusted with rats' weight weekly. The rats were carefully monitored throughout the experiment period for any signs of toxicity, morbidity or even mortality.

Collection of blood samples

After the last dose, blood samples were collected from retro orbital venous plexus of all experimental groups in plain vacutainer tubes. Blood was allowed to coagulate at room temperature for 20 min and then centrifuged at 5000 rpm for 10 min. The separated sera were carefully collected and stored in deep freeze (−20 °C) till further analysis for biochemical assays.

Collection of tissue specimens

After blood collection, rats were than euthanized by decapitation under light anesthesia. Testicular specimens dissected from any adhering blood or surrounding tissue, were collected, and then washed and perfused with physiological saline solution to exclude the red blood cells and blotted on filter paper. The specimens were processed according to Badr El-Din and Abd-El Aty (2012). The obtained testicular samples were quickly fragmented and then homogenized in phosphate buffers (1 g tissue/ 5 ml PBS) using an electrical homogenizer. Tissue homogenates were centrifugated at 5000 rpm for 10 min; then, the resultant supernatant containing cell suspensions was isolated and then quickly preserved at − 20 °C until further use for assessment of oxidative and antioxidant parameters. Small pieces of the collected testes were rinsed in 10% buffered neutral formalin solution for histological and immunohistochemical examinations.

Hormonal analysis

The sera were biochemically analyzed to determine levels of testosterone, LH and FSH. The determination of serum testosterone and LH were done using ELISA kits as described by Tietz (1995); meanwhile, assessment of serum FSH was achieved according to Rose (1998).

Oxidative stress and antioxidant parameters

The stored tissue supernatant was used for determination of oxidative/antioxidant parameters, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). These parameters were measured as described by Ohkawa et al. (1979), Nishikimi et al. (1972), Aebi (1984), and Beutler et al. (1963) respectively.

Histological and histochemical examination

The formalin-fixed tissue samples were then embedded in paraffin blocks. Sections of 5 μm thickness were obtained using rotatory microtome and then stained with H&E and subjected to PAS technique. Fixation and staining were carried out as outlined by Bancroft and Gamble (2008).

Immunohistochemical examination

The paraffin sections were then deparaffinized in xylene then rehydrated through descending series of ethanol. The testicular sections were immersed in target retrieval solution (pH 6.0) and heated in water bath for 15 min at 98 °C to unmask antigens. Endogenous peroxidase and nonspecific background staining were blocked by incubating the sections in 3% H2O2 and 1% normal serum respectively for 10 min. The slides were incubated overnight at 4 °C with the following primary antibodies; mouse monoclonal PCNA antibody (anti-PCNA, dilution 1:100; DAKO, Glostrup, Denmark) and polyclonal rabbit antibody against Bax protein (anti-BAX, dilution 1:40; DAKO Corporation Carpinteria, CA, USA). A normal mouse and rabbit sera/Phosphate Buffer Saline (PBS) were parallel applied instead of the primary antibodies to represent as negative control sections. After incubation, sections were washed in PBS then incubated with biotinylated goat anti-mouse and goat anti-rabbit immunoglobulin G (IgG), respectively for 10 min at RT. The bindings of the primary antibodies were observed using a commercial avidin biotin-peroxidase detection system recommended by the manufacturer (Santa Cruz Biotech, CA, USA). Then the slides were then subjected to diaminobenzene (DAB) as the chromogen and counterstained with hematoxylin. The immunoreactions were visualized as brown-colored precipitates.

Semiquantitative assessment of the immunolabeling scores of PCNA and Bax were evaluated blindly as outlined by

Vermeirsch et al. (2002). Random five fields/slide ($n = 5$ slides) were examined by power field (× 40) for both antibodies. The spermatogenic cells were given proportional score (PS) reflecting the percentage of positive cells to the whole field (PS from 0 to 5 for no positive cells to > 65% positive cells respectively). Also, the cells were given intensity score (IS) reflecting the intensity of the brown positive staining (IS from 0 to 4 for no staining to very strong staining respectively). For each slide, the mean ± standard error (SE) was calculated. The total score (TS) was obtained by addition of IS to PS. The total immunostaining was scored at 1–3, 4–6, and 7–9 representing weak, moderate, and strong grades, respectively.

Statistical analysis

All data analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). Differences in mean ± SE were analyzed by one-way analysis of variance (ANOVA) (Petrie and Watson 1999).

Results

Preliminary phytochemical analysis

Different phytochemical constituents including anthocyanins, flavonoids, glycosides, and phenols were detected in the aqueous HSE. In contrary, this aqueous HSE neither contain alkaloids nor steroids (Table 1).

DPPH radical scavenging assay

All examined concentrations of HSE showed a good inhibitory activity against the DPPH radical indicating strong antioxidant activity. DPPH free radical scavenging activity of HSE exhibited a dose dependent manner as 30 mg/ml HSE showed the strongest scavenging potential of 74.19 ± 1.43% (Table 2).

Table 1 Summary of preliminary phytochemical analysis of aqueous HSE

Parameters	Aqueous HSE extract
Alkaloids	-
Anthocyanins	+
Flavonoids	+
Glycosides	+
Phenols	+
Steroids	-

HSE, *Hibiscus sabdariffa* extract; -, absent; +, present

Hormonal assessment following MSG and/or HSE exposure

As shown in Fig. 1, significant decreases in serum testosterone, LH, and FSH levels were noticed in rats treated with MSG compared with control group. On the other hand, HSE + MSG group ameliorated the level of these parameters near to the control group. Additionally, HSE treated group elicited observable non-significant changes in hormonal analysis as compared with control group.

Oxidative stress/antioxidant parameters findings

Testicular homogenate of rat exposed to MSG showed a significant increase in MDA level with significant decreases of SOD, CAT and GSH activities compared with control group. Meanwhile, HSE + MSG group revealed an observable decline in MDA level along with significant elevation in SOD, CAT, and GSH levels compared with MSG group. HSE-treated rats revealed non-significant changes in oxidative stress/antioxidant parameters close to control group (Fig. 2).

Histological findings

Microscopical examination of H&E stained testicular sections of both control and HSE groups revealed normal architecture of seminiferous tubules and interstitial connective tissue (Fig. 3A). Additionally, an intact PAS-positive basement membrane surrounding seminiferous tubule and normal stratification of the spermatogenic cells with evidence of spermatogenesis was clearly observed (Fig. 3B).

On contrary, several histological alterations were noted in the testicular structure of rats administrated with MSG. Evidence of damaged seminiferous tubules along with edematous interstitial tissues were clearly observed (Fig. 4A). Tubular degeneration was characterized by disruption of spermatogenic cells organization and vacuolated spermatogenic cells (Figs. 4B, C). Congested interstitial blood vessels (Fig. 4B) and thickened PAS-positive basement membrane were also identified among this group (Fig. 4D). Moreover, few spermatozoa with many sloughed and degenerated cells were noticed within the lumens of seminiferous tubules (Fig. 4C).

The co-exposed group to HSE and MSG showed significant enhancement of the testicular architecture with mildly damaged seminiferous tubules and the amount of edematous fluid dramatically decreased within the interstitial tissue (Fig. 5A). In addition, less vacuolated cytoplasm of spermatogonia only, reappearance of spermatid, and increased numbers of spermatozoa were characteristic for this group (Fig. 5B). PAS-positive tubular basement membrane became relatively thinner than that of MSG group (Fig. 5C).

Table 2 Mean ± SE of DPPH free radical scavenging activity of aqueous HSE at different doses

	DPPH radical scavenging activity (%)			
	30 mg/ml	15 mg/ml	7.5 mg/ml	3.75 mg/ml
HSE	74.19 ± 1.43	54.19 ± 0.73	39.51 ± 0.51	25.33 ± 0.23
Ascorbic acid	60.68 ± 1.19	46.08 ± 1.01	30.28 ± 0.99	19.68 ± 0.19

HSE, *Hibiscus sabdariffa* extract; DPPH, 1,1-diphenyl-1-picrylhydrazyl

Immunohistochemical findings

The immunoreactivity of spermatogenic cells against PCNA and Bax was demonstrated in Figs. 6 and 7. Nonspecific stains were confirmed by negative control sections (Figs. 6E and 7E).

The PCNA immunostaining appeared intranuclear of the labeled cells. In both control and HSE groups, strong PCNA reaction was identified in spermatogonia and spermatocytes and moderate labeling was seen in spermatids (Fig. 6A, B and Table 3). Meanwhile, moderate and weak immunoreactions were detected in the spermatogonia and spermatocytes, respectively of MSG group (Fig. 6C and Table 3). HSE + MSG group showed strong single layer of PCNA positive spermatogonia along the basement membrane of the seminiferous tubules in addition to weak immunoreaction in spermatocytes (Fig. 6D and Table 3).

Bax was localized intracytoplasmic of positive cells. Weak to moderate Bax immunoexpression was seen in the testes of both control and HSE groups. Moderate immunoreaction was observed in the spermatocytes and to a lesser extent, the spermatogonia and spermatids showed weak reaction (Fig. 7A, B and Table 4). However, the testis of MSG group showed overexpression of Bax and most of the spermatogenic cells showed strong immunoreaction (Fig. 7C and Table 4). HSE + MSG group showed moderate Bax immunoreaction in most of the spermatogenic cells in comparison to other groups (Fig. 7D and Table 4).

Discussion

The adversely toxic effect of MSG on the male fertility has been proved, as it caused a significant oligozoospermia and abnormal sperm morphology (Onakewhor et al. 1998). Also, it caused testicular hemorrhage, degeneration and alteration of sperm cell population (Das and Ghosh 2010; Igwebuike et al. 2011). In spite of the safe consumption of aqueous HSE (up to 180 mg/kg/day) is recommended with no any toxic effect on hematology, chemistry, and tissues structures (Akindahunsi and Olaleye 2003), other results confirmed a deleterious effect of HSE on male reproduction. A study confirmed that phenols and anthocyanins in HSE have estrogenic effect (Omotuyi et al. 2011). Moreover, Saeed et al. (2013) reported that HSE rich in phytoestrogenic compounds (quercetin and daidzein) that play key role in reducing reproductive hormones (Testosterone, LH and FSH) in addition to, the higher doses of HSE induced a reproductive toxic effect (Soliman and Mahmoud 2010; Mahmoud 2012; Olusanya et al. 2018). Therefore, the present study was aimed at assessing the safe/adverse effect of aqueous HSE at dose of 180 mg/kg b.w on the testis or its potential ameliorative role including some selective stress markers, biochemical and immunohistochemical expressions in rats subjected to MSG.

The oxidative damage is one of the most common mechanisms involved in MSG-induced testicular injury (Jubaidi et al. 2019). To better clarify the underlying mechanism of the testicular-protective effect of HSE against MSG toxicity, we estimated oxidative stress/antioxidant markers. MSG

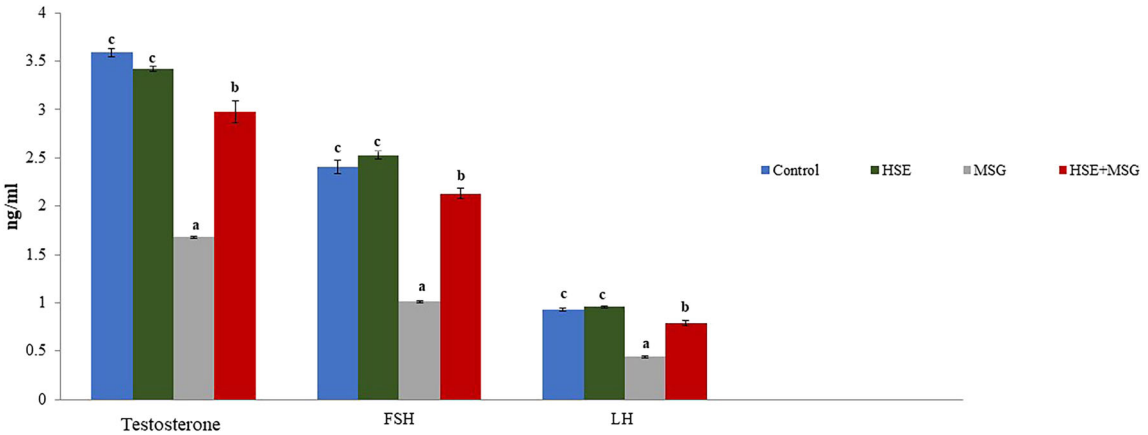

Fig. 1 Mean ± SE levels of sera testosterone, FSH, and LH from different experimental groups

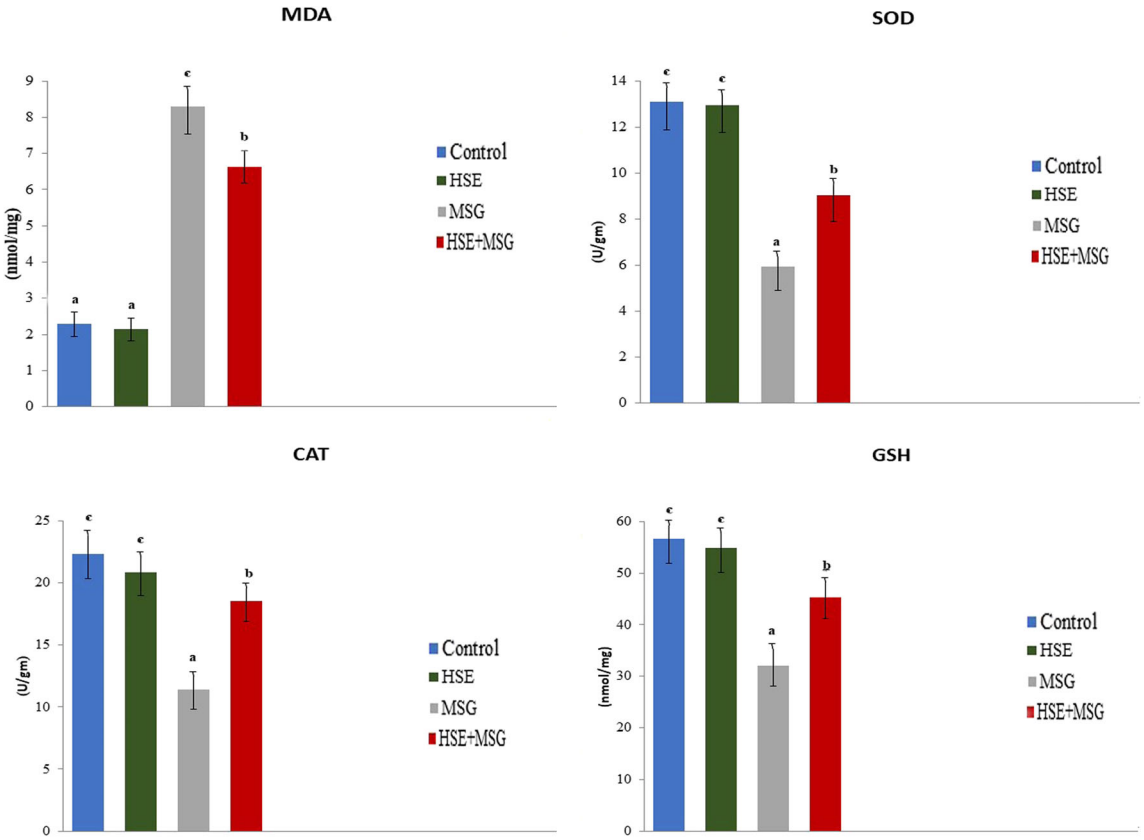

Fig. 2 Mean ± SE of MDA levels, CAT, SOD, and GSH activities in testicular tissue homogenates

caused a significant increase ($P < 0.05$) in MDA with significant decreases of SOD, CAT, and GSH activities indicating the development of oxidative stress that are in harmony with Fabio et al. (2012), Hamza and El-Harbi (2014), and Umukoro et al. (2015). This effect contributed to the production of excess ROS that depletes antioxidant leading to an increased lipid peroxidation (Hamza and El-Harbi 2014; Karaboduk et al. 2015).

Conversely, rats co-exposed to HSE plus MSG showed an observable decline in MDA level along with significant elevation in SOD, CAT, and GSH levels. Therefore, HS has significant protective effects against MSG-induced testicular toxicity and oxidative stress in rats that matched with reports of Gheller et al. (2017).

In cisplatin-treated rats, Amin and Hamza (2006) illustrated the ameliorative effect of HSE on antioxidant parameters. This improvement may be mediated by the strong antioxidant activity of HS which owed to its phenolic contents (Tsai and Huang 2004; Hopkins et al. 2013; Guardiola and Mach 2014; Patel 2014).

Our preliminary phytochemical analysis of HSE revealed presence of anthocyanins, phenols, flavonoids, and glycosides

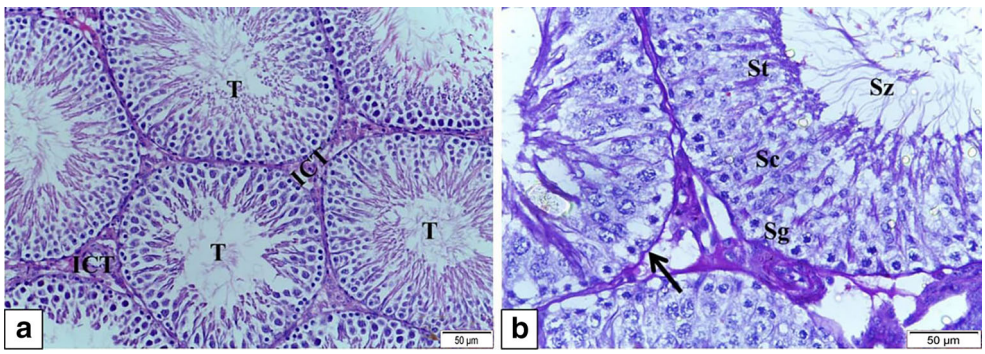

Fig. 3 Photomicrograph of testicular sections obtained from control and HSE-treated rats. A: showing normal architecture of seminiferous tubules (T) and interstitial connective tissue (ICT), H&E stain. B: showing intact PAS-positive basement membrane of seminiferous tubules (arrow), PAS technique. Notice, normal arrangement of spermatogenic cells ranging; spermatogonia (Sg), spermatocytes (Sc), spermatid (St) to spermatozoa (Sz)

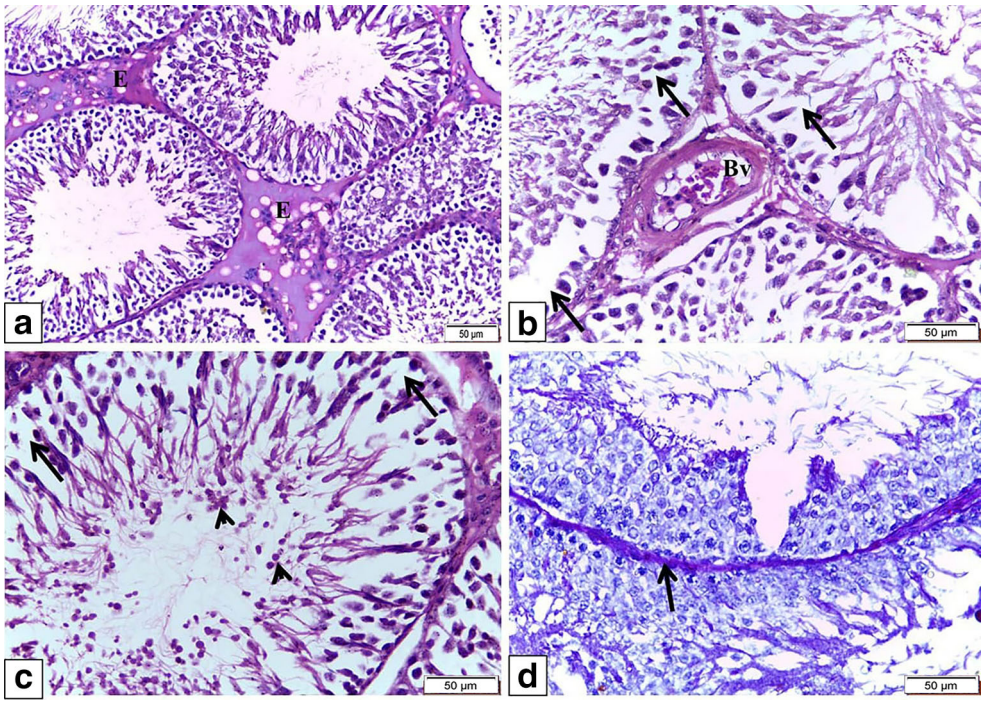

Fig. 4 Photomicrograph of testicular sections obtained from MSG-treated rats. A: showing damaged seminiferous tubules with edema of interstitial tissue (E), H&E stain. B: showing disruption of spermatogenic cells organization, vacuolated spermatogenic cells (arrows), and congestion of interstitial blood vessels (Bv), H&E stain. C: showing vacuolated spermatogenic cells (arrows) and many sloughed and degenerated cells within the lumens of seminiferous tubules (arrowheads), H&E. D: showing thick PAS-positive basement membrane of seminiferous tubules (arrow), PAS technique

as mentioned before by Pacôme et al. (2014) and Okereke et al. (2015). In respect of polyphenolic contents (anthocyanins, phenols and flavonoids), HS has been reported to have an antioxidant activity (Siriwoharn et al. 2004) as they can scavenge free radicals, quench singlet and triplet oxygen or decompose peroxides (Osawa 1994). Also, the glycoside derivatives have antioxidant activity (Azevedo et al. 2010; Zhang et al. 2011).

In the current study, the antioxidant capacity of HSE was confirmed using the DPPH scavenging assay. It has been proven that HSE is more effective than ascorbic acid (the reference standard antioxidant) indicating abundant antioxidative phytochemicals in the HSE that matched with Pacôme et al. (2014).

In the present study, MSG significantly decreased serum levels of reproductive hormones; testosterone, FSH and LH that was in accordance with Franc et al. (2006) and Sakr and Badawy (2013). The former owed the decrease in hormonal levels to the disruption of hypothalamic-hypophyseal-testicular axis that controls the steroidogenesis of Leydig cells (Mclachlan et al. 1996). Meanwhile, rat co-administrated with HSE plus MSG reversed the reduction in male sex hormones levels. These results agreed with Fadairo et al. (2008) who recorded the protective effect of HSE on serum reproductive

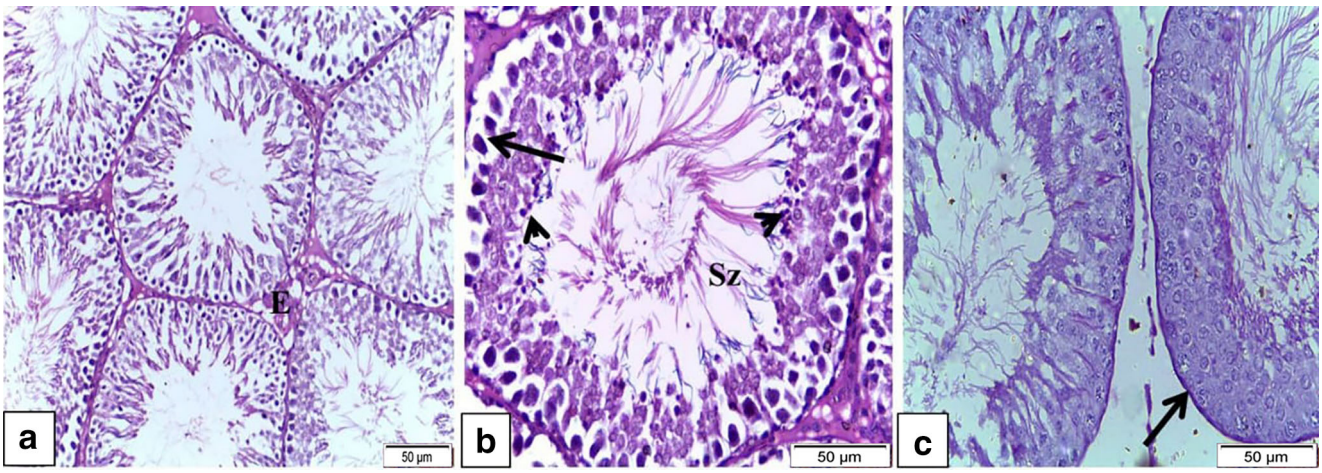

Fig. 5 Photomicrograph of testicular sections obtained from HSE + MSG group. A: showing less damaged seminiferous tubules with decreased amount of edematous fluid (E) within the interstitial tissue, H&E stain. B: showing less vacuolated spermatogonia only (arrow), reappearance of spermatid (arrowhead) and increased number of spermatozoa (Sz), H&E stain. C: showing relatively thin basement membrane of seminiferous tubules (arrow), PAS technique

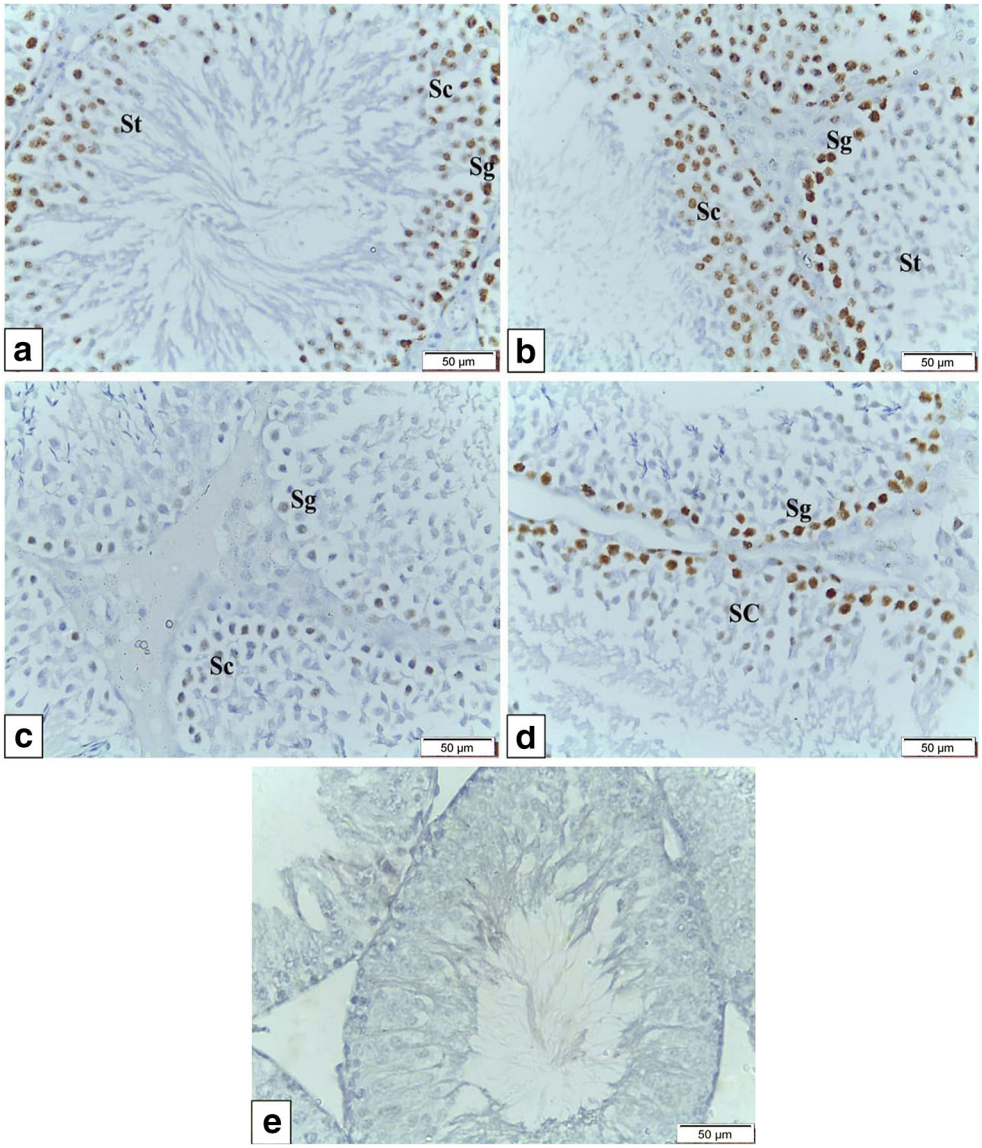

Fig. 6 Photomicrograph of immunohistochemical nuclear staining of PCNA in testicular tissue sections of different experimental groups. A: (control) and B: (HSE) groups: showing strong reactions in spermatogonia (Sg) and spermatocytes (Sc) and weak in spermatids (St). C: (MSG) group: showing moderate reaction in spermatogonia (Sg) and weak in spermatocytes (Sc). D: (HSE + MSG) group: showing strong reaction in spermatogonia and weak reaction in spermatocytes. E: Negative control of testicular sections for PCNA. A section from control rat treated with normal mouse serum instead of PCNA antibody

hormones in cadmium-treated rats, and this reversed effect of HSE attributed to its antioxidant properties (Ajiboye et al. 2011). On contrary to our results, Sirag et al. (2013) and Olusanya et al. (2018) used a high dose of HSE (250 mg/kg) and the experimental rats showed a significant decrease ($P \leq 0.05$) in the plasma levels of testosterone, LH, and FSH, compared with control group.

The changes in the hormonal and oxidant/antioxidant parameters could be attributed to testicular damage that were confirmed by the histological and immunohistochemical findings. The present study revealed several testicular histological alterations among MSG-treated rats. Damaged seminiferous tubules, disruption of spermatogenic cells organization, and degenerated cells in the tubular lumen were identified. Our results agreed with the previous findings, which owed these features to the oxidative stress induced by MSG (Alalwani 2014; Hamza and AL-Harbi, 2014). Storto et al. (2001) reported that there is a direct toxic effect of MSG via glutamate receptors on the spermatogenic cells as the testis is considered a target organ for MSG. As a sign of cellular toxicity, the cytoplasmic vacuolation of spermatogenic cells was detected that may be due to ROS production (El-Wakf et al. 2009), low plasma testosterone level (Ramzan and Qureshi 2011), or damaged mitochondria (Sarhan 2018). Also, edema of interstitial tissues and thickened basal lamina were observed that similar to finding of Orisakwe et al. (2004) and Elsabagh et al. (2014).

The present work clarified for the first time the effect of HSE and/or MSG on the profiles of apoptosis and cells proliferation in the rat testis. Since spermatogenesis is a complex and rapid cell cycle of proliferating cells, PCNA was used in this study to assess spermatogenesis. PCNA is an intra-nuclear polypeptide that is involved in DNA replication, excision and repair (Shivji et al. 1992). On other hand, apoptosis plays an important role in the maintenance of tissue homeostasis (Jin and El-Deiry 2005) through removing damaged, infected

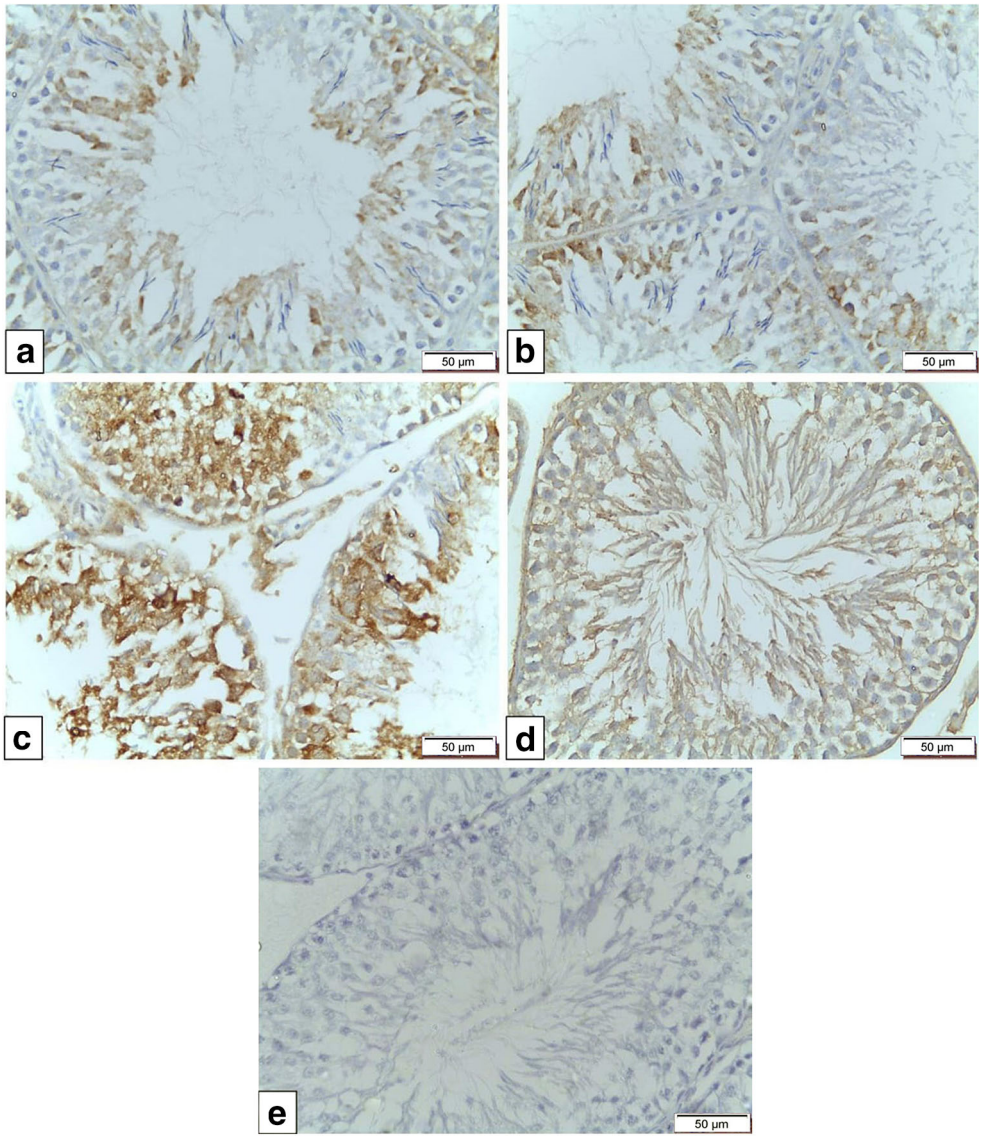

Fig. 7 Photomicrograph of immunohistochemical cytoplasmic staining of Bax in testicular tissue sections of different treatment groups. A: (control) and B: (HSE) groups: showing weak to moderate Bax immunoreaction. Notice, moderate reaction in spermatocytes and weak in spermatogonia and spermatids. C: (MSG) group: showing Bax over-expression in most of spermatogenic cells. D: (HSE + MSG) group: showing lower reaction in comparison to MSG group. E: Negative control of testicular sections BAX. A section from control rat treated with normal rabbit serum instead of Bax antibody

or neoplastic cells (Kannan and Jain 2000). However, excessive or inadequate apoptosis of the spermatogenic cells will result in abnormal spermatogenesis or testicular tumors (Lin et al. 1997).

Immunohistochemically, MSG exerted noticeable adverse effect on the testis in comparison to control and HSE groups. MSG increased the immunoreactivities of spermatogonia and spermatocytes for Bax and reduced them for PCNA revealing

Table 3 Mean ± SE immunostaining scores of PCNA in the testicular tissues of the different experimental groups

	Spermatogonia			Spermatocytes			Spermatids		
	PS	IS	TS	PS	IS	TS	PS	IS	TS
Control	4.8 ± o.2	3.4 ± 0.3	8.2 ± o.6	4.7 ± 0.4	3.0 ± 0.3	7.7 ± 0.3	4.2 ± 0.4	2.4 ± 0.3	6.6 ± 0.4
HSE	4.8 ± o.3	3.6 ± 0.5	8.4 ± 0.4	4.8 ± 0.3	3.0 ± 0.4	7.8 ± 0.5	4.2 ± 0.6	1.1 ± 0.4	5.3 ± 0.5
MSG	4.6 ± o.2	1.1 ± 0.4	5.7 ± 0.5	0.5 ± 0.2	0.7 ± 0.2	1.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HSE + MSG	4.8 ± o.4	3.2 ± 0.3	8.0 ± 0.4	2.1 ± 0.4	0.9 ± 0.4	3.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

PS, proportional score; *IS*, intensity score; *TS*, total score; *HSE*, rats received *Hibiscus sabdariffa* extract; *MSG*, rats received monosodium glutamate; *HSE + MSG*, rats co-administrated *Hibiscus sabdariffa* extract plus monosodium glutamate

Table 4 Mean ± SE immunostaining scores of Bax in the testicular tissues of the different experimental groups

	Spermatogonia			Spermatocytes			Spermatids		
	PS	IS	TS	PS	IS	TS	PS	IS	TS
Control	1.2 ± o.3	1.1 ± 0.2	2.3 ± o.4	3.2 ± 0.4	2.2 ± 0.3	5.4 ± 0.5	1.2 ± 0.2	1.3 ± 0.3	2.5 ± 0.3
HSE	1.3 ± o.2	1.2 ± 0.4	2.5 ± 0.3	3.4 ± 0.2	2.0 ± 0.4	5.4 ± 0.4	1.2 ± 0.2	1.2 ± 0.2	2.4 ± 0.4
MSG	4.4 ± o.2	3.6 ± 0.2	8.2 ± 0.3	4.9 ± 0.2	3.8 ± 0.2	8.7 ± 0.4	4.4 ± 0.3	3.4 ± 0.4	8.0 ± 0.5
HSE + MSG	3.2 ± o.3	1.2 ± 0.2	4.4 ± 0.4	4.2 ± 0.2	2.2 ± 0.2	6.4 ± 0.3	3.4 ± 0.3	2.2 ± 0.2	4.6 ± 0.3

PS, proportional score; *IS*, intensity score; *TS*, total score; *HSE*, rats received *Hibiscus sabdariffa* extract; *MSG*, rats received monosodium glutamate; *HSE + MSG*, rats co-administrated *Hibiscus sabdariffa* extract plus monosodium glutamate

increased apoptosis and decreased proliferation processes of the testicular germ cells, these changes were coincided with Sarhan (2018). The decreased cellular proliferation in the may due to the low levels of testosterone which causes stopping or reduction of spermatogenesis (D'Andrea et al. 2008; Alalwani 2014), meanwhile, the increased apoptosis may due to ROS and translocation of Bax (proapoptotic factor) from the cytoplasm to perinuclear position (Sinha Hikim et al. 2003; Zhang et al. 2007; Hajihasani et al. 2020). However, rats co-administrated HSE plus MSG showed a noticeable increase of PCNA and decrease of Bax immunoreactivities compared with MSG group. Of note, we can postulate that HSE could induce proliferation and reduce apoptosis of spermatogenic cells resulting in resuming of spermatogenesis consequently regeneration of seminiferous epithelial cells.

Conclusions

From the abovementioned data, we found that the consumption of aqueous HSE (180 mg/kg b.w) is safe on the testis and fertility of rats. HSE is an excellent source of phytochemical constituents including anthocyanins, flavonoids, glycosides, and phenols. Additionally, the improvement of biochemical, histological, and immunohistochemical findings in rats co-administrated HSE plus MSG suggests an ameliorative effect of aqueous HSE on MSG-induced testicular toxicity via reduction of oxidative stress and apoptosis, and induction of testicular germ cell proliferation which attributes to its phytochemical constituents and DPPH scavenging activity.

Authors' contribution F.A. Gad, S.M. Farouk, and M.A. Emam contributed to the design and implementation of the research, to the analysis and discussion of the results.

Data availability Not applicable.

Compliance with ethical standards

Animals were handled and maintained in accordance with the guidelines of National Institutes of Health for the Care and Use of Laboratory Animals, as well as the experiment was approved by Institutional Animal Cares and Use Committee Research Ethics Board (IACUCREB) at Faculty of Veterinary Medicine, Benha University, Egypt (Approval no; BUFVTM 01-04-20).

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations MSG, Monosodium glutamate; HS, *Hibiscus sabdariffa*; HSE, *Hibiscus sabdariffa* extract; PCNA, Proliferating cell nuclear antigen; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; MDA, Malondialdehyde; SOD, Superoxide dismutase; CAT, Catalase; GSH, Glutathione; PS, Proportional score; IS, Intensity score; TS, Total score; ROS, Reactive oxygen species; DPPH, 1,1-Diphenyl-1-picrylhydrazyl

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