

PROTECTIVE EFFECT OF TEMULAWAK (*Curcuma xanthorrhiza*) RHIZOME EXTRACT ON MONOSODIUM GLUTAMATE-INDUCED MICE SPERMAries Soewondo^{1)*}, Yuyun Indriani Kartiko Santi¹⁾, Agung Pramana Warih Marhendra¹⁾, Sri Rahayu¹⁾Submitted : January, 12 2023
Accepted : February, 20 2023**Authors affiliation:**¹⁾Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Indonesia.**Correspondence email:**

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How to cite:Soewondo, A, Santi YIK, Marhendra APW, Rahayu S. 2023. Protective effect of temulawak (*Curcuma xanthorrhiza*) rhizome extract on monosodium glutamate-induced mice sperm. *Journal of Tropical Biology* 11 (1): 9-16.**ABSTRACT**

The current study aimed to analyze the protective effect of the aqueous extract of temulawak (*Curcuma xanthorrhiza*) rhizome on the sperm quality of monosodium glutamate (MSG)-induced mice. This study used 30 male mice (aged 2.5-3 months old and weighing 25-30 g), which were then randomly divided into five groups: K- (healthy male mice received only aquadest), K+ (male mice exposed to 4 mg/kg BW MSG), MT1, MT2 and MT3 (MSG-induced male mice orally treated with 0.2, 0.4 and 0.6 mg/g BW temulawak (*C. xanthorrhiza*) rhizome extract for 14 days, respectively). At the end of treatment, all mice were sacrificed, and cauda epididymis was isolated. The obtained semen was analyzed for its quality, including motility, viability, concentration and spermatozoa abnormalities. MT2 group exhibited the highest sperm motility of MSG-induced mice (79.16±4.45%). The highest sperm viability was also observed in the MT2 (77.83±2.75%) followed by MT1 and MT3 groups (70.19±5.93 and 72.41±5.53%, respectively). MT2 and MT3 groups could increase ($p<0.05$) sperm concentration in MSG-induced mice by 14.03x10⁶ and 14.46x10⁶ cells/ml, respectively. While, sperm abnormalities of MSG-induced mice tend to decrease in all groups treatment by 38.10% (MT1), 36.32% (MT2) and 36.04% (MT3). In conclusion, the administration of 0.4 mg/g BW aqueous extract of *C. xanthorrhiza* rhizome could improve sperm quality by increasing the motility, viability and concentration of sperm and also altered the sperm abnormality of MSG-induced mice.

Keywords: sperm quality, MSG, temulawak, sperm abnormalities

INTRODUCTION

Monosodium glutamate (MSG) is frequently added to fast food and commercial meals. MSG is often applied to enhance "umami" flavour and increase appetite [1]. The body and the reproductive system may be negatively affected directly or indirectly by excessive MSG administration [2]. The testes are directly affected, and the hypothalamic-pituitary-adrenal (HPA) axis is involved in the indirect effect. When MSG is consumed orally, it first breaks down in the mouth and then digested in the small gut. Glutamate will be distributed throughout the body via circulation and attach to its receptors [1].

Glutamate receptors will be overstimulated by an excessive glutamate content, leading to increased intracellular Ca²⁺ ions. Because of the high intracellular Ca²⁺ ion concentration, the tricarboxylic acid cycle in mitochondria generates an excessive amount of Reactive Oxygen Species (ROS). Elevation of ROS levels causes lipid peroxidation, which damages membranes, damages Leydig cells' DNA, and interferes with the system that secretes the hormone that triggers spermatogenesis [3, 4]. MSG consumption has adverse effects on spermatozoa's motility, viability, and number of defective sperm, among other spermatozoa features. Furthermore, MSG causes oxidative stress in the testes, which reduces

glutathione reduction (GSH) and superoxide dismutase (SOD) levels. The administration of 6 mg/BB MSG to male rats daily for 45 days could induce ROS, as indicated by a significant increase in testicular malondialdehyde (MDA) levels [1].

Curcuma xanthorrhiza, commonly known as temulawak, is a native plant of Indonesia [5]. This plant has anti-microbial, anti-hepatitis, anti-inflammatory, and anti-carcinogenic effects. Curcumin is a vital compound of temulawak, which has antioxidant properties [6]. Previous research has shown that administering curcumin to MSG-exposed mice could enhance sperm counts and improve histological structure. Antioxidants can be obtained from *C. xanthorrhiza* because of its chemical components [7, 8]. There has not been much research done on the potential of temulawak aqueous extract to improve sperm quality due to MSG overexposure. This research is necessary due to the importance of sperm quality in reproductive health. Therefore, the study sought to analyze the effect of the aqueous extract of temulawak (*C. xanthorrhiza*) rhizome on the sperm quality of monosodium glutamate (MSG)-induced male mice (*Mus musculus*). The study expected that the aqueous extract of temulawak (*C. xanthorrhiza*) rhizome could protect the mice sperm from MSG induction.

METHODS

Preparation of temulawak extract. The simplicia of temulawak rhizome was purchased from UPT Balai Materia Medica Batu, Indonesia. A total of 1 kg powdered temulawak rhizome was macerated with 5 L of ethanol 70% for 24 h. The sample was filtered and then concentrated using a rotary evaporator to obtain a thick extract.

Experimental animals. This study used 35 male BALB/C mice (aged at 2.5-3 months, weighing 25-30 g), which were divided into five groups, including K- (healthy male mice received only aquadest), K+ (male mice exposed to 4 mg/kg body weight (BW) MSG), MT1, MT2 and MT3 (MSG-induced male mice orally treated with 0.2, 0.4 and 0.6 mg/g BW temulawak (*C. xanthorrhiza*) rhizome extract for 14 days, respectively).

All animals were acclimatized for seven days and housed in cages (32 cm × 28 cm) at 22°C, 50 % humidity, and under 12 h of light and 12 h of darkness). The animals were given free access to water and normal pellet food. MSG and temulawak extract were given orally for 30 days following acclimatization. Mice were sacrificed after the last treatment (day 15 after extract administration). The cauda epididymis was obtained and then crushed in 1.5 mL Phosphate Buffered Saline (PBS) at 34-37°C in a watch glass to collect semen.

Sperm motility. The object glass was filled with 10 µL of semen. A light microscope (magnification of 100x) and five fields of view was used to examine sperm movement (each repetition). The sperm motility can be determined using following category [9] (Table 1).

Table 1. The criteria of sperm motility and progression

Grade	Progressive motility
1	0-20%: Very poor
2	20-40%: Poor
3	40-60%: Good
4	60-80%: Very good
5	80-100%: Excellent

Sperm viability. Ten 10 µL semen was put and dropped into object glass. Semen was then stained with eosin-nigrosin and mixed slowly. A smear preparation was made at an angle of 45°C. A light microscope with a magnification of 400x was used to calculate live and dead sperm. The quantity of sperm in each group treatment was counted three times until 200 cells were collected. The viability of sperm was assessed according to the following formula [10]:

$$Viability (\%) = \frac{live\ sperm}{observed\ sperm} \times 100 \quad (1)$$

Sperm concentration. A total of 10 µL semen was dissolved in 100 µL physiological sodium chloride (NaCl). A hemocytometer chamber was filled with the sperm solution. For each treatment group, the number of sperm was assessed in 5 small boxes with three repetitions and then calculated using Equation 2 [11]:

$$Concentration = n \times k \times DF \times 10^4 \quad (2)$$

Note:

n = number of counted sperm in 5 small boxes

k = number of small boxes (5)

DF = diluted factor (10)

10⁴ = hemocytometer chamber volume

Sperm abnormalities. Sperm abnormalities were observed under a light microscope (400x magnification) and then calculated using Equation 3 [12]. There were three repetitions for abnormal sperm and total sperm (five fields of view for each repetition).

$$Abnormalities = \frac{abnormal\ sperm}{observed\ sperm} \times 100 \quad (3)$$

Data analysis. Data of motility, viability, and concentration of sperm were analyzed using One-Way Analysis of Variance (ANOVA) followed by the Tukey Test in SPSS for Windows (p ≤ 0.05).

RESULTS AND DISCUSSION

Sperm motility. The present study revealed that sperm motility significantly declined (p<0.05) in MSG-induced mice/K+ (42.80±1.80%) compared to healthy mice (70.68±3.65%). The administration of temulawak rhizome extract could enhance (p<0.05) the sperm motility of MSG-induced mice (Table 2). All temulawak extract groups (MT1, MT2, and MT3) showed no significant difference in improving sperm motility of MSG-induced mice (72.26±5.69, 79.16±4.45 and 74.06±4.79 %, respectively). However, the MT2 group has the highest percentage of sperm motility compared to healthy mice.

According to Montoto et al. [13], sperm motility should not be less than 70%. Previous research indicated that consumption of 4 mg/g BW MSG for 35 days [14], and 2 g/kg BW MSG for 15 days [15] could decline sperm motility by 50% and 15.59%. MSG at a dose of 17.5 mg/kg BW elevated MDA levels in mice testes by 46.37% [16]. Administration of 4 mg/g BW MSG for 21 days could enhance testicular MDA levels due to lipid peroxidation [17].

MSG has the potential to cause oxidative stress and a decrease in antioxidants in the body. Increased PARP-1 activation will accompany elevated ROS production. The hydrolysis of NAD⁺

to nicotinamide and PAR will be catalyzed by PARP-1. Sperm cells produce less ATP as an energy source due to the decreased supply of NAD⁺, resulting in cell death [19]. Sperm motility can be reduced if mitochondria do not have enough ATP [19].

Curcumin can act as an exogenous antioxidant and can stimulate endogenous antioxidant synthesis, as evidenced by an increase in glutathione peroxidase (GPx) and SOD [20, 21]. As a result, increasing the antioxidants could enhance sperm quality. Temulawak (*C. xanthorriza*) contains a similar active compound that promotes sexual behaviour or libido. Curcumin acts as an exogenous antioxidant, supported by previous research [22], that curcumin could increase spermatogenesis and reduce toxic effects on the testes of MSG-induced rats. Curcumin can also significantly increase LH and testosterone levels [23].

Sperm viability. The study also revealed that MSG exposure could reduce the sperm viability of

mice by 58.93±11.52%. After receiving temulawak rhizome extract, the sperm viability of MSG-induced mice significantly improved (p<0.05) (Table 2). MT2 group had the highest sperm viability (77.83±2.75%), followed by MT1 and MT3 groups (70.19±5.93% and 72.41±5.53%, respectively). The live and dead sperm are demonstrated in Figure 1.

Excess MSG in the body increases Ca²⁺ production in the mitochondria [24]. Elevated ROS production leads to lipid peroxidation in spermatogonia, spermatids, and sperm cells [25]. Previous research has found that consuming 2 g/kg BW MSG could reduce sperm viability by 37% [26]. Temulawak (*C. xanthorriza*) contains curcumin, which acts as an antioxidant and free radical scavenger [27]. Curcumin reduces oxidative stress and improves plasma membrane integrity, viability, and motility [28]. Curcumin has previously been shown to reduce MDA levels [29] and increase SOD levels [30].

Table 2. The sperm quality of mice in all treatment groups

Groups	Sperm quality		
	Motility (%) ± SD	Viability (%)± SD	Sperm concentration (x 10 ⁶ cell/mL)
K-	70.68 ± 3.65 ^b	71.26 ± 3.67 ^b	7.70 ± 5.71 ^a
K+	42.80 ± 1.80 ^a	58.93±11.52 ^a	7.13 ± 2.73 ^a
MT1	72.26 ± 5.69 ^b	70.19±5.93 ^b	14.03 ± 11.98 ^b
MT2	79.16 ± 4.45 ^b	77.83±2.75 ^b	14.46 ± 14.39 ^b
MT3	74.06 ± 4.79 ^b	72.41±5.53 ^b	8.06 ± 1.06 ^a

Note: K- (healthy male mice received only aquadest), K+ (male mice exposed to 4 mg/kg BW MSG), MT1, MT2, and MT3 (MSG-induced male mice orally treated with 0.2, 0.4, and 0.6 mg/g BW temulawak (*C. xanthorriza*) rhizome extract for 14 days, respectively). Different superscripts within the same column represented statistically significant differences (p<0.05).

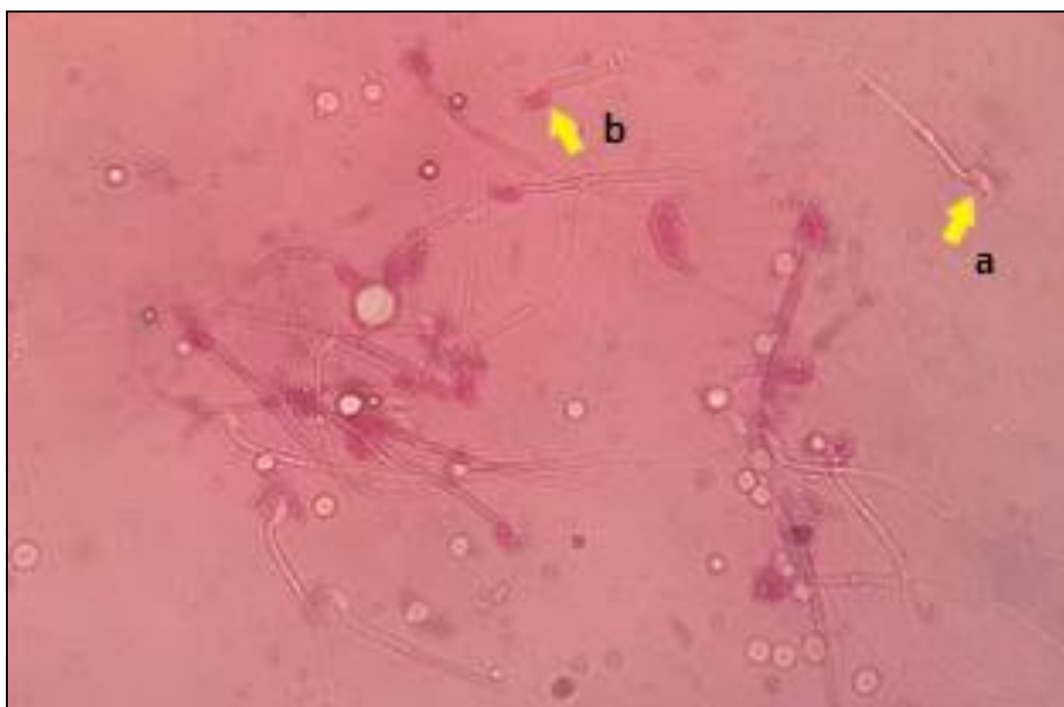


Figure 1. The morphological difference of a) live and b) dead sperm (100x magnification)

The present study revealed that temulawak (*C. xanthorriza*) rhizome extract had an important role ($p < 0.05$) in MSG-induced mice. Compounds with free radical scavengers could raise spermatozoa viability in MSG-induced mice. MSG has been linked to lipid peroxidation, which reduces the integrity of the spermatozoa membrane. Exogenous antioxidants can improve the spermatozoa membrane's fluidity and integrity [25]. This study observed a significant effect on sperm viability after treatment with temulawak (*C. xanthorriza*) rhizome extract. These findings are consistent with previous research, which demonstrated that temulawak extract effectively improves sperm quality by increasing testosterone, FSH, and LH, and decreasing testicular MDA [30]. Sperm maturation in the epididymis also increases spermatozoa's capabilities by elevating motility and viability [31].

Sperm concentration. The study also found that oral administration of MSG did not drastically reduce the sperm concentration of mice (7.13×10^6 cells/ml) compared to healthy mice (7.70×10^6 cells/ml) (Table 2). However, previous research indicated that MSG (4 mg/kg BW) administration for 28 days reduced sperm concentration in adult rats [32]. LH and testosterone levels are also decreased by MSG, which is harmful because they are both necessary for spermatogenesis and normal testicular function [23]. ROS can form when large amounts of MSG are consumed, affecting fluidity and inactivating cell membrane enzymes [33, 34]. Lipid peroxidation can occur in spermatogonia, spermatids, and sperm cells due to excess ROS [3]. Sperm concentrations may decrease due to spermatogenic cell death [35]. If the obstruction occurs while the proliferated spermatogonia, spermatocytes, spermatids, and sperm numbers will almost certainly decrease [36, 37].

The administration of temulawak (*C. xanthorriza*) rhizome extract in MT2 and MT3 groups could increase ($p < 0.05$) sperm concentration in MSG-induced mice by 14.03×10^6 and 14.46×10^6 cells/ml, respectively. Curcumin, essential oils, saponins, alkaloids, and flavonoids are the bioactive components found in temulawak (*C. xanthorriza*) rhizome extract [2]. Curcumin constitutes the most active component in *C. xanthorriza* [29]. The curcumin content of *C. xanthorriza* protects against oxidative stress [38]. The administration of temulawak (*C. xanthorriza*) extract has been reported to possess a protective role on the testes, as evidenced by higher spermatogonia, primary spermatocytes, and spermatids. Curcumin could enhance testosterone, SOD, and sperm concentration [27, 28]. Curcumin also increases the maximum capacity of the spermatogenesis process by facilitating the rise of

LH and testosterone hormones [23, 31]. Curcumin also serves as an antioxidant, reducing oxidative stress caused by MSG and protecting DNA from damage, resulting in increased sperm concentration. Curcumin is known to enhance sperm density, sperm motility, testosterone, and FSH level, which are both important in spermatogenesis [29].

Sperm abnormalities. The study indicated that all treatment groups had spermatozoa abnormalities (Table 3), including primary and secondary abnormalities. Compared to the healthy mice group ($26.16 \pm 2.91\%$), the oral treatment of 4 mg/g BW MSG increased sperm abnormalities by $46.30 \pm 1.79\%$. Abd [39] found that 14 days of MSG treatment at a dose of 4 mg/g BW could produce up to 62% increase in spermatozoa abnormalities. MSG also causes an abnormal sperm number from 27.97% to 37.83% [40]. Excess glutamate in the body enhances intracellular Ca^{2+} levels in mitochondria, disrupting mitochondrial ATP synthesis and increasing ROS concentrations [41]. ROS damages sperm DNA integrity through base modification, DNA strand breaking, DNA fragmentation, and changes in chromatin structure. Abnormal sperm in the testes is caused by DNA damage caused by changes in gene expression [42].

ROS produced by spermatozoa mitochondria can also directly affect spermatozoa abnormalities. Mitochondria, which are found in the centre of spermatozoa, generate energy for movement while producing by-products in the form of ROS. Excess ROS produced by spermatozoa mitochondria can cause oxidative stress, resulting in spermatozoa with abnormal morphology [43]. This research found several abnormalities of sperm, including banana-like heads, bent necks, coiled tails, circular midpieces, square heads, amorphous heads, no tail, asymmetric, bent tails, cytoplasmic droplets, round heads, no head, bent midpieces, folded tail, hookless heads, small heads, and tapped heads (Figure 2).

All treatment groups (MT1, MT2, and MT3) significantly ($p < 0.05$) diminished sperm abnormalities in MSG-induced mice by 38.10, 36.32, and 36.04%, compared to the K+ group (46.56%). Sharma & Singh [44] found that administering curcumin could reduce the rate of sperm abnormalities by 36.76% in the lindane-induced group. The administration of temulawak water extract greatly reduced abnormal sperm, which indicated that temulawak has antioxidant activity. Curcumin, an antioxidant compound in temulawak, can protect cells from free radicals [7]. Curcumin inhibits lipid peroxidation by activating endogenous antioxidants like SOD, CAT, glutathione peroxidase, and glutathione transferase [45, 46]. Curcumin can raise sperm

Table 3. The type of sperm abnormalities in all group treatment

Abnormality type		Abnormality (%)				
		K(-)	K(+)	MT1	MT2	MT3
Head	No tail	2.59	5.32	3.04	1.71	5.53
	Square	0.00	0.00	0.14	0.00	0.00
	Round	0.14	0.14	0.00	0.23	0.00
	Bent neck	1.09	2.16	1.24	1.03	0.94
	Amorphous	0.41	0.29	0.69	0.68	0.53
	Hookless	0.14	0.55	0.55	0.00	0.94
	Tapered	0.28	0.00	0.41	1.03	0.26
	Small	0.14	0.00	0.14	0.00	0.00
Midpiece	Asymmetric	0.14	0.43	0.14	0.11	0.13
	Looped	2.86	4.31	6.09	4.45	3.64
	Bent	3.54	5.46	4.43	4.26	3.23
	Cytoplasmic droplet	5.03	3.30	2.14	6.16	5.12
Tail	Folded	2.59	6.47	7.61	5.02	5.93
	Bent	2.18	6.18	5.26	5.02	3.77
	Coiled	2.04	7.33	3.18	4.11	2.38
	No head	2.99	4.62	3.04	2.51	3.64
Total of Sperm Abnormality		26.16 ^a	46.56 ^c	38.10 ^b	36.32 ^b	36.04 ^b

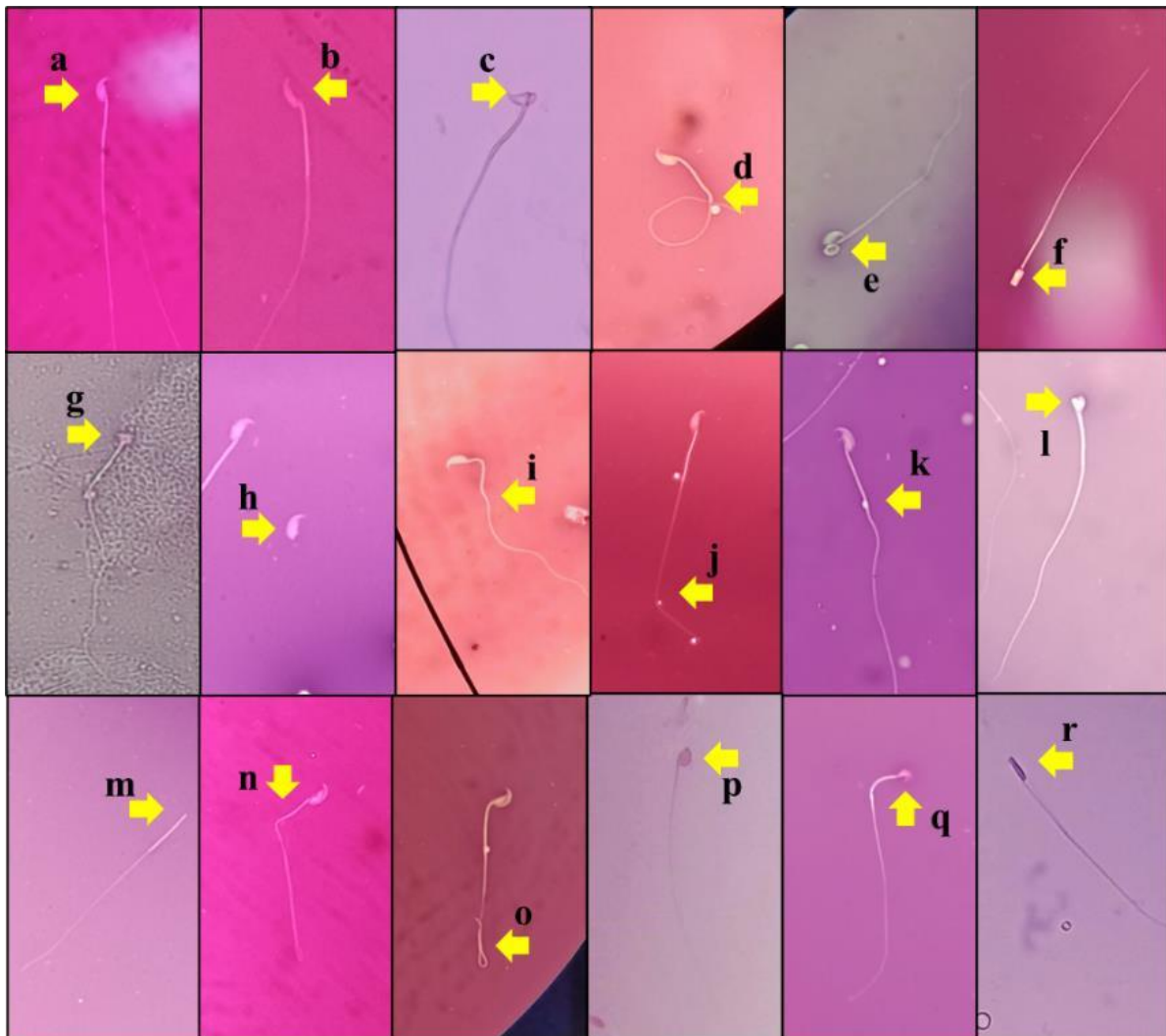


Figure 2. The morphological of several types of sperm abnormalities, including (a) normal sperm, (b) banana-like head sperm, (c) bent neck sperm, (d) coiled tail sperm, (e) circular midpiece sperm, (f) square head sperm, (g) amorphous head sperm, (h) tailless sperm, (i) asymmetric sperm, (j) bent tail sperm, (k) cytoplasmic droplet sperm, (l) round head sperm, (m) headless sperm, (n) bent midpiece sperm, (o) folded tail sperm, (p) hookless sperm, (q) small head sperm, (r) tapered sperm

counts and motility and prevent sperm chromatin condensation and apoptosis in rat testes [47].

CONCLUSION

The administration of 0.4 mg/g BW aqueous extract of *C. xanthorrhiza* rhizome could improve sperm quality by increasing the motility, viability, and concentration of sperm and also alter the sperm abnormality of MSG-induced mice.

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