

Full Length Research Paper

The protective role of honey against cytotoxicity of cadmium chloride in mice

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The present study aimed to investigate the honey (HY) protective role in opposition to cadmium (Cd) induced chromosomal aberrations of bone marrow and sperm abnormalities. Forty five (45) adult male albino mice were caged into six groups. Mice were injected, i.p, 300 mg HY/kg and/or 0.67 mg CdCl₂/kg b.w for 96 h, separately and alternated. The alternated trials were continued for consecutive eight days. Results show that mice injected with cadmium had significant increase in the frequency of aberrant chromosomes as fragment, centric fusion, gap, stickiness and aneuploidy and in sperm abnormality. The administration of HY improved the frequency of the chromosomal aberrations and sperm abnormality induced by Cd.

Key words: Cadmium, Honey, sperm, chromosome aberrations.

INTRODUCTION

The toxic adverse of cadmium (Cd) is known as environmental and industrial pollutant. Its physical and chemical properties constitute the industrial individuality for applications (Krichah et al., 2003). As a heavy metal, Cd causes severe injuries (Suzuki et al., 1989). People often develop health disorders starting from vomiting, stomach pain and diarrhea to bone fracture, lung and reproductive failure, particularly those who live in vicinity of factories that release cadmium or work in metal refinery industry (Nordberg, 2009). Amara et al. (2011) and Singh et al. (2007) mentioned that the damaged central nervous system and DNA or cancer progression appeared as consequences of Cd exposure. Cadmium

also causes severe soft tissues and bone damages (Cucu et al., 2011; Ercal et al., 2001; Ersan et al., 2008). Cadmium is known to enhance the production of reactive oxygen species (ROS) (Liu et al., 2001). The toxic effect of Cd is controlled by the oxidation of cellular organelles by generating ROS reactions which lead to lipid peroxidation, apoptosis, damage of DNA and altered gene expression (Stohs et al., 1999; Wu et al., 2002; Thévenod, 2003). Therefore, to relieve Cd adverse effect the antioxidants induction is considered as an important therapeutic approach (Renugadevi and Prabu, 2010; Sinha et al., 2009).

At least 181 substances are included in the composition

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of honey (Wang and Li, 2011). This composition varies primarily depending on the floral source rather than seasonal and environmental factors. Glucose (31%) and fructose (38%) represents the main contributors of honey solution. Minor constituents of honey have antioxidant properties as phenolic acids, enzymes, ascorbic acid, carotenoid-like substances and flavonoids (Andrade et al., 1997; Ferreres et al., 1992; Wang and Li, 2011; Cherchi et al., 1994). The present study aimed to investigate the protective role of honey (HY) opposing the chromosomal and sperm abnormalities induced by cadmium.

MATERIALS AND METHODS

Cadmium was used as cadmium chloride (CdCl_2) (Oxford Laboratory, Mumbai, India). The concentrations used was 0.67 mg/kg or 1/10 LD_{50} according to Bench et al. (1999). Honey (HY) clover flower was purchased from Isis Company, Cairo, Egypt. 300 mg HY/kg b. w. was used as an optimal dose according to El-Raby (2010).

Animals

In this study, 30 adult Swiss albino mice (*MUS musculus*) were used, varying from 20 to 25 g in weight and aged three month old. These mice were obtained from the National Research Center (N.R.C.), Dukki, Cairo, Egypt, were caged individually under standard conditions of light, temperature, humidity and fed with standard pellet diet and water *ad libitum*.

Experiment

The experiment was categorized in six groups of five animals each. Group I was the control group, group II: mice were i.p injected with 0.5 ml saline solution daily for 96 h, as positive control, group III: mice were i.p injected, as a single dose, with 0.67 mg CdCl_2 /kg b.w. dissolved in 0.5 ml saline solution, for 96 h, group IV: mice were injected i.p, with 300 mg honey (HY)/kg b.w. dissolved in 0.5 ml saline solution daily for 96 h, group V (HYCd): mice were injected i.p with HY daily for 96 h, then single dose of CdCl_2 for 96 h (as a protective trial), and group VI (CdHY): mice were i.p injected, single dose, CdCl_2 for 96 h then followed by four consecutive honey doses, for 96 h (as a treatment trial).

Cytogenetical study

Colchicine was injected intraperitoneally 2 to 3 h before sacrificing. Bone-marrow was extracted from femur bone and metaphases according to the method of Yosida and Amano (1965). The preparations of mitotic chromosome were made according to Ford and Hamerton (1956). Giemsa stain (7%) in phosphate buffer (pH 6.8) was used for slides. Hundred spreads metaphases per animal were investigated for chromosomal aberration analysis.

Sperm head morphology assay

Cauda epididymides were excised and both *epididymides* were

minced together in isotonic medium then filtered to exclude large fragments. The cells' smears were prepared and stained with 5% Eosin Y. Light microscope (100x) green filter was used to examine smears. Thousand sperms were assessed for each animal to investigate the morphology of sperm abnormality according to the criteria of Wyrobek and Bruce (1975). Any overlay or contact sperms or heads without tails were ignored.

Statistical analysis

Mean \pm SE was expressed to all values where five animals were evaluated, $n=5$, in each group. Statistical analysis of cytogenetic was performed on SPSS software (version 18) using one-way analysis of variance (ANOVA) test. Significance was considered when P values less than 0.05.

RESULTS

Various chromosomal aberrations were observed in the bone marrow cells of albino mice injected with cadmium. These were of structural and numerical type, were identified and quantitated relative to non-treated control (Figure 1A).

Structural aberration included chromatid deletions (Figure 1B); fragments (Figure 1C); centric fusion (Figure 1D); centromeric attenuation (Figure 1E); end to end (Figure 1F); rings; gap (Figure 1G) (Figure 1H); polyploidy (Figure 1I); stickiness (Figure 1J). A chromatid was considered to have a gap when it had an unstained area shorter than its diameter or equal to it (Figure 1G). End to end association was scored when two chromatids of different chromosomes appeared attached this could result from reciprocal translocation or stickiness (Figure 1F). The stickiness is considered as assort of chromosomal agglutination of unknown nature which resulted in a pycnotic or sticky appearance of chromosome (Figure 1J).

As shown in Table 1, the mean number nuclei with chromosomal aberration in mice treated with cadmium appear high significant of chromosomal aberrations in all animals compared with control. The more types of aberration appeared with chromosomal fragments, centric fusion, gaps, stickiness and aneuploidy. Also the mitotic activity shows decrease in the treated groups with cadmium chloride when compared to control. Honey administration has the same results as control of chromosomes abnormalities and the rate of cell division. Honey as a protective or treatment dose lead to decrease of chromosome aberration with increase in cell division that shows Cd HY and HY Cd groups could protect the body from cytotoxicity arising from cadmium.

Figure 2 shows normal sperm (a), hummer sperm (b), without hook (c) and banana shape (d). Honey recorded increased in number normal sperm head (Figure 3). Honey as protective or treatment group (Cd HY or HY Cd) could protect the body from cytotoxicity arising from

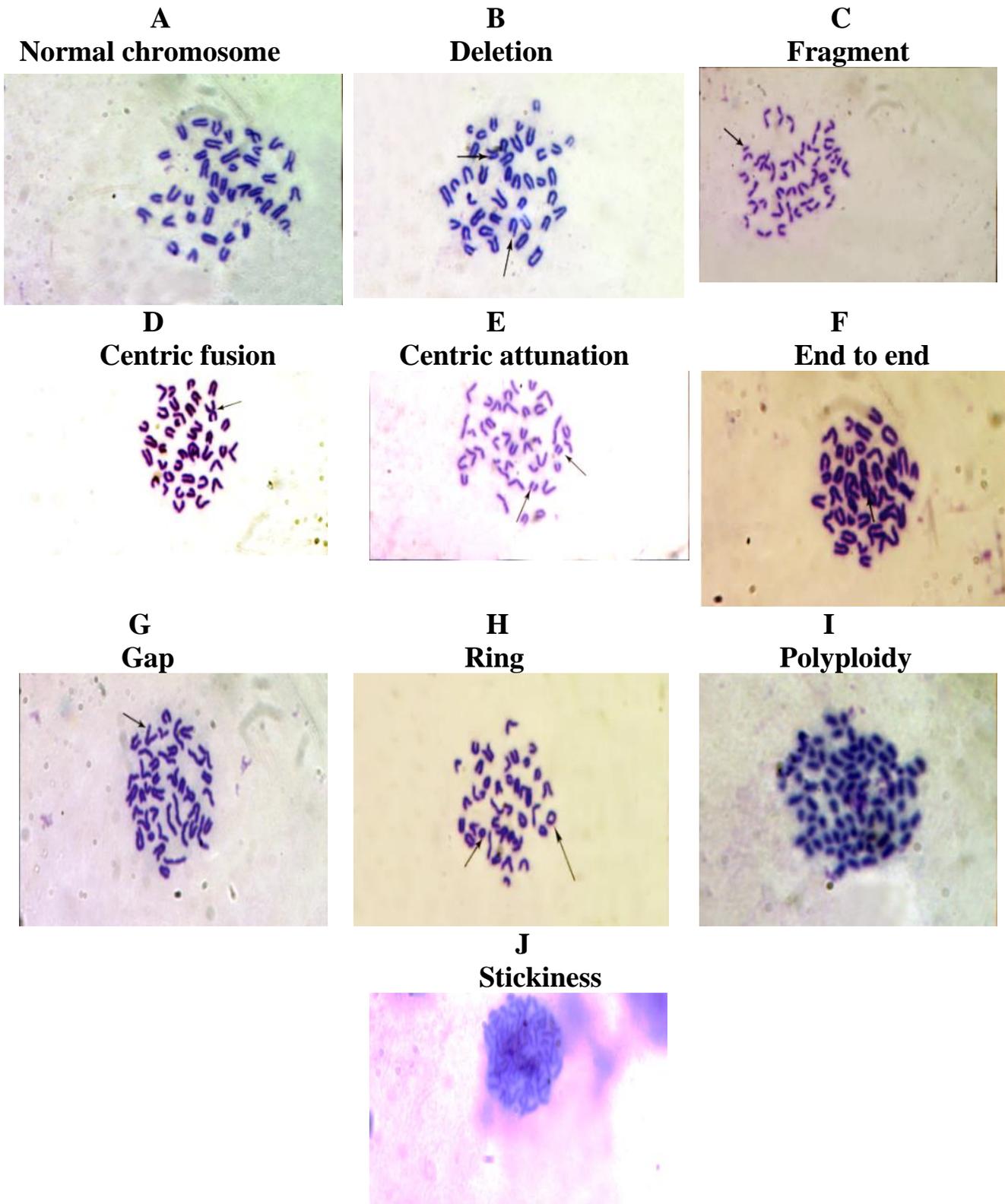


Figure 1. Metaphase spread from mouse bone marrow cells showing: (a) normal chromosomes spread, (b) deletion, (c) fragment, (d) centric fusion, (e) centromeric attenuation, (f) centric attenuation, (g) end to end, (h) ring, (i) polyploidy, (j) stickiness. All aberrations are indicated by arrows.

Table 1. Average of chromosomal aberration observed in bone marrow cells of male mice treated with Cd and HY.

Group	Fragment	Centric fusion	Gap	Stickiness	Trisomy	Total	%
Control ^a	3.0±0.5 ^{b,d}	8.2±0.7 ^d	0.0±0.0 ^d	0.0±0.0 ^{d,e}	0.2±0.8 ^d	12.4±2 ^{dd}	1.24
+Control ^b	5.8±0.7 ^{a,c,e,f}	8.6±1.0 ^{c,d}	0.0±0.0 ^d	0.0±0.0 ^{d,e}	0.2±0.6 ^d	14.6±2.3	1.46
HY ^c	4.8±0.6 ^b	5.4±0.7 ^{b,d}	0.2±0.2	0.0±0.0 ^{d,e}	0.2±0.7 ^d	10.6±2.2	1.1
Cd ^d	6.8±0.4 ^a	11.8±1.5 ^{a,b,c,e,f}	0.4±0.2 ^{a,b,e,f}	1.0±0.3 ^{a,b,c}	1.4±0.3 ^{a,b,c}	21.4±2.7 ^{a,b,c,e,f}	2.1
CdHY ^e	5.0±1.0 ^b	8.0±1.1 ^d	0.0±0.0 ^d	0.8±0.4 ^{a,b,c}	0.8±0.3	7.4±2.8 ^d	0.74
HYCd ^f	5.0±0.7 ^b	9.0±1.2 ^d	0.0±0.0 ^d	0.6±0.2	0.6±0.3	15.2±2.4 ^d	1.52

Average was expressed as mean±standard error. The significances were indicated as follow: ^asignificant with control, ^bsignificant with +control, ^csignificant with HY, ^dsignificant with Cd, ^esignificant with Cd Hy, ^fsignificant with HY Cd. Significant means P<0.05.

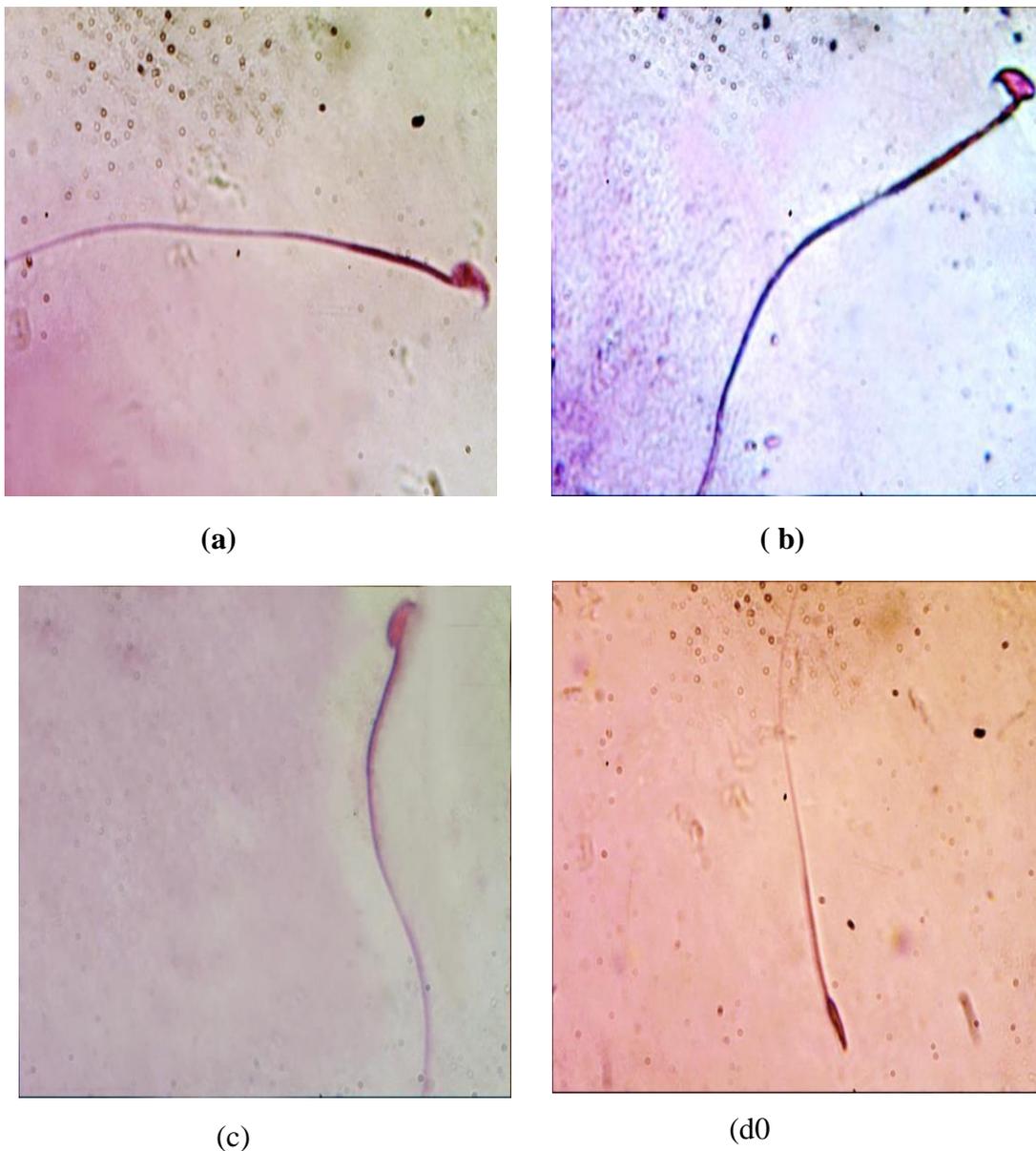


Figure 2. (a) Normal sperm, (b) hummer sperm, (c) without hook, (d) Banana shape.

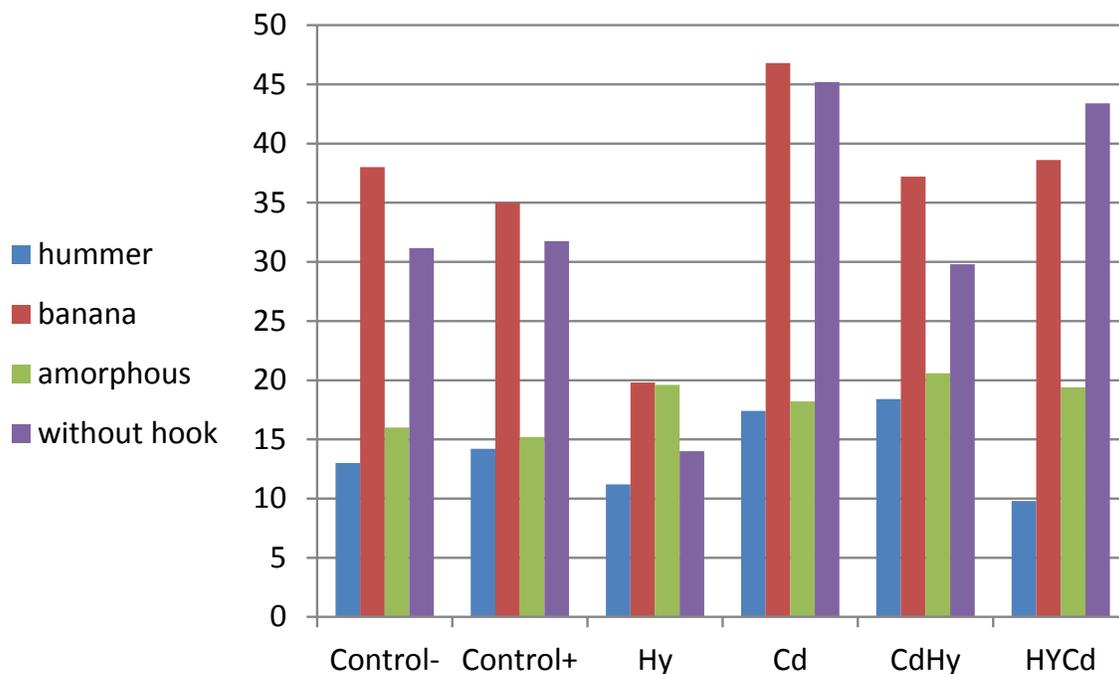


Figure 3. Incidence of total abnormally shaped sperms per thousand after injection of HY and Cd.

cadmium. The same values of normal sperm were recorded (Figure 3).

DISCUSSION

Currently, chromosomal fragment, centric fusion, gap, stickiness and aneuploidy were produced by Cd injection. Other study revealed that Cd induced chromosomal break, centric fusion, terminal association and C-mitosis in *Oreochromis mossambica* (Chandra and Khuda-Bukhszh, 2004). Cd burden has been correlated with chromosomal aberrations (IARC 1993). Singh et al. (2007) showed that low dose of Cd (1 mg/kg/day) for 30 days resulted in chromosomal aneuploidy, breaks, gaps and centromeric fusion. However, dose of 25 mg Cd/kg/day for 20 days and 200 mg/kg/day for five days resulted in severe damage of chromosome. Singh and Sankhla (2010) have assured that Cd increased the number of chromosomal aberrations and declined the mitotic index. On the other hand, the Cd exposure has been shown to stimulate free radical production, resulting in various pathological conditions in humans and animals (El-Demerdash et al., 2004; Shaikh et al., 1999). Acute or chronic exposure of Cd has been associated with increased lipid peroxidation in testes, erythrocytes, and other soft tissues (Manca et al., 1991; Sarkar et al., 1997). Free-radicals can originate from exposure to cadmium, resulting in elevated chromosomal aberrations

and declined mitotic index. Abnormal sperm population was observed in the present study. Due to the effect of Cd-induced ROS on specific gene loci of germ cell chromosomes, it may dysfunction the maintenance of normal sperm structure. Most of the germ cells have been destroyed in Cd-treated mice due to either membranous or macromolecular damage incurred by formation of ROS leading to declined sperm count and ultimately testicular weight loss (Acharya et al., 2003; Oldereid et al., 1994); as well as the decreased sperm count and alterations in motility have been associated with cigarette smoking (Kulikauskas et al., 1985).

Honey administration has the same results as control of chromosomes abnormalities and the rate of cell division. Honey as protective or treatment dose lead to decrease in chromosome aberration with increase in cell division that shows Cd HY and HY Cd groups could protect the body from cytotoxicity arising from cadmium. Honey could be used to prevent and eliminate mutation caused by aflatoxins (Sharmanov et al., 1986). This was in accordance with reducing the chromosomal aberrations induced by aflatoxins (Sharmanov et al., 1986). The chromosomal aberrations induced by mycotoxins could be minimized by honey administration (Ezz El-Arab et al., 2006). Using 300 mg HY/kg against the *brodifacoum* pesticide, *mitomycin-c* anticancer drug and zinc phosphide pesticides lead to chromosome aberration, micronucleus formation and sperm head abnormalities (El-Raby, 2006, 2007, 2010). The association of honey

with cyclophosphamide (CPM) ameliorates the evaluated reduced glutathione (GSH), malondialdehyde (MDA) and chromosomal aberrations. Zoheir et al. (2015) also stated that honey inhibited the cytotoxic and genotoxic risks associated with the treatment by CPM in mice. Royal jelly has shown a protective effect in opposition to chromosomal abnormalities in bone marrow and histological alterations in kidney produced by valproic acid in male mice (Galaly et al., 2014). The treatment with flavonoids significantly reduced the chromosome abnormalities and delayed tumorigenesis in adult mice exposed to fetal irradiation (Uma Devi and Satyamitra, 2004). Honey sugar displayed complex behavior toward the enhanced or inhibited mutagenic activity in a model by cooked food systems (Skog, 1993). Royal jelly (RJ) had shown highly efficient antioxidant where it scavenges the produced free radicals (Cemek et al., 2010; El-Nekeety et al., 2007; Türkmen, 2009). El-Monem (2011) also revealed that RJ caused a significant recovery in antioxidant status. However RJ reduced glutathione (GSH) and inhibited malondialdehyde production which ameliorated DNA damage and genotoxicity induced by malathion in rat cells. Inoue et al. (2003) and Narita et al. (2006) previously reported the protection effect of RJ to DNA and the stimulation of bone marrow formation (Narita et al., 2006).

Honey recorded increased in the number of normal sperm head. Honey as protective or treatment group (Cd HY or HY Cd) could protect the body from cytotoxicity arising from cadmium. The same values of normal sperm were recorded. Syazana et al. (2011) observed the increased sperm count, percentage of normal sperm and reduced percentage of sperm abnormalities as a result of the Malaysian Gelam honey administration in male rats. Interperitoneal injection of nicotine could adversely affect sperm qualities, and Gelam honey was useful to increase the sperm motility and number that might increase the fertility of juvenile male rats (Asiyah et al., 2011). On the other hand, flavonoids and phenolic acids were found to improve semen quality and quantity, as well as decrease the sperm-shape abnormalities and histological damage in reproductive tissues of different animals exposed to several toxicants (Hala et al., 2010; Purdy et al., 2004; Türk et al., 2008). Moreover, the sexual behavior and fertility have been increased in male rats by honey administration against the toxic effect of cigarette smoke (Mohamed et al., 2012). Haron and Mohamed (2015) observed increased testis and epididymis weights as well as the sperm motility and percentages of normal spermatozoa in male rat offspring after honey supplementation during prenatal restraint. The honey supplementation seemed to reduce the adverse effects of restraint stress on reproductive organs weight and sperm parameters in male rat offspring (Haron and Mohamed, 2015). Also Mohamed et al. (2012) added that honey at dose of 1.2 g/kg may enhance spermatogenesis in adult

rats.

Conclusion

From the current study, we can conclude that both the treatment and protection with honey ameliorated the adverse effects of Cd on chromosome and sperm. The cover honey as monofloral honey was the type of honey collected by most beekeepers and consumed by Egyptians.

Conflict of Interests

The authors have not declared any conflict of interests.

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