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Consolidating probiotic with dandelion, coriander and date palm seeds extracts against mercury neurotoxicity and for maintaining normal testosterone levels in male rats

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ABSTRACT

Objective: Heavy metals are major elements polluting our universe. The inhalation, ingestion or even contacting human body with these elements results in huge health problems. The most common pollutant in our surrounding is mercury. Therefore, the present study aimed to elucidating the protective ability of hot water extracts of dandelion (DA), coriander (CO), date palm seeds (DS), probiotic supernatant (PS) and their combined mixture against mercury-induced neurotoxicity and altered testosterone levels in male rats.

Methods: Fifty six male rats were randomly allotted into seven groups (n = 8 rats/group). Group1 (negative control; NC) animals were fed on the basal diet only, group2 (positive controls; PC) animals were fed on the basal diet and given an aqueous solution of mercuric chloride (25 ppm mercuric) in drinking water. Animals of the antioxidant-treated groups (3–7) were fed on the basal diet and given an aqueous solution of mercuric chloride (25 ppm mercuric) in drinking water together with the herbal antioxidant extracts and probiotics (25 ml/rat/day) throughout the experimental period. Where, group3 (Hg/CO) given coriander extract, group4 (Hg/DA) given dandelion extract, group5 (Hg/DS) given date palm seeds extract, group6 (Hg/PS) given probiotic supernatant, and group7 (Hg/Mix) given mixture of equal quantities of probiotic supernatant together with the three herbal extracts. The treatment lasted for 6 weeks, animals were sacrificed and blood samples were collected. Blood testosterone, enzyme activity and histopathological sections were performed.

Results: The obtained data exhibited that mercury intoxication revealed increases of lactic dehydrogenase and decreases of glutathione-s-transferase and testosterone. Light microscopic investigations of the brain cortex and cerebellum were suggestive of multiple foci of inflammation, cellular infiltration, gliosis and degeneration. Moreover, decreased glial fibrillary acidic protein (GFAP)-immunoreactivity and potential astrocyte toxicity both reflected impaired neuro-protective function of astrocytes necessary for maintaining the brain structure and function.

Conclusion: Administration of the herbal extracts and their mixture with probiotics enhance the body defense and contain protective factor against mercury neurotoxicity and for maintaining normal testosterone levels in male rats. Also, treatment restored the normal control levels of biochemical attributes and histological architecture.

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1. Introduction

Generally, mercury and its derivatives exist in most environmental media including ambient and indoor air, surface and drinking water, soil and sediments, microorganisms, food and the occupational environment. Mercury has been recognized as a neurotoxic and as well as immune-toxin and designated by the World Health Organization as one of the ten most dangerous chemicals to public health [1,2].

Mercury exists mainly in three forms: metallic elements, inorganic salts and organic compounds, each of which possesses different toxicity and bioavailability. The brain remains the target organ for mercury, yet it can impair any organ and lead to malfunctioning of nerves, kidneys and muscles. It can cause disruption to the membrane potential and interrupt with intracellular calcium homeostasis [3]. Vapors of Mercury may cause bronchitis, asthma and some respiratory problems. Mercury plays a key role in damaging the tertiary and quaternary protein structure and alters the cellular function by attaching to the selenohydryl and sulfhydryl groups which undergo reaction with methyl mercury and hamper the cellular structure [4]. People who chronically consumed mercury-contaminated foods and water revealed increased incidence of severe hearing loss, mental retardation, epilepsy, and other brain damage [5]. McAlpine and Araki [6] were the first to report the presence of seizures and epilepsy due to contamination with mercury. According to Ballatori and Clarkson [7] glutathione is an intracellular antioxidant that reacts with Methylmercury (MeHg) to form the MeHg-glutathione complex, an essential step in mercury detoxification. Then, Glutathione S-Transferase (GST) catalyzes the MeHg-glutathione complex conjugation reaction. Functional food and its micronutrients play a functional role as a protective agents in human health. It have been attributed to prevent the oxidative stress and reduce the risk of diseases [3,7,8]. Dietary phytochemicals from Coriander, date palm seeds and dandelion are rich in bio-active components and anti-oxidants capacity. It also, used traditionally in folk medicine as a herbal medicine due to its antidiabetic, choleretic, antirhematic, diuretic properties, inflammation, gastrointestinal complaints and tumors [9–11]. Therefore, the goal of this study aimed at elucidating the beneficial effects of combining some usual herbs with probiotic to alleviate or even reduce the detrimental effects of mercury.

2. Materials and methods

2.1. Experimental materials

Coriander, date palm seeds and dried dandelion (*Taraxacum officinalis*) leaves and roots were purchased from the local market in Al-Qassim, Saudi Arabia. Also, milk samples were donated from healthy lactating cows. Starter cultures of *Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium bifidum* were obtained from Chr. Hansen`s Laboratory, Copenhagen, Denmark. Chemicals and pure reagents were purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA).

2.2. Preparation of hot aqueous extracts of coriander, dandelion and date palm seeds

Coriander, dandelion and date palm seeds were dried and milled. The hot water extracts were prepared according to the method described by Abdel-Salam et al. [12,13]. Briefly, coriander, dandelion and date palm seeds materials were cut into small pieces, melded and placed in a flask (2 l) and then extracted with 1000 ml hot distilled water in an electric blender for 15 min. The clear aqueous extract was preserved in sterile dark bottles (500 ml) at −20 °C until further used. Afterwards, the suspension were left at room temperature for 1 h. The suspension was filtered twice, first through cheese-cloth (50% cotton/50% polyester) and then through filter paper (Whatman No.2). The water extract was then preserved in sterile dark bottles (500 ml) in a cool environment (4 °C) until further use.

Table 1
Total antioxidant capacity and Total polyphenol in formulated beverages to reduce the risks of mercury induced neurotoxicity.

	Antioxidant capacity*			Total polyphenol**		
	Mean	SD	% CV	Mean	SD	% CV
DA	349.00^{ab}	1.41	0.41	58.96^{ab}	0.23	0.38
CO	46.98^{c}	0.11	0.23	23.85^{c}	0.35	1.48
DS	3009.1^{a}	1.56	0.05	63.75^{a}	0.64	1.00
PS	9.61^{d}	0.69	7.21	7.70^{d}	0.71	9.18
Mix.	299.46 ^{ab}	0.93	0.31	46.65^{ab}	0.78	1.67

Antioxidant capacity * = μmol Trolox equivalents/dL sample.
Total phenolic compound ** = mg galic acid/dL sample.Means in the same column with different superscripts significantly differ ($P < 0.05$).
Means in the same column with different superscripts significantly differ ($P < 0.05$).

Table 2
Daily body weight gain (g/day) and brain weight ratio of rat fed on formulated beverages against mercury-induced neurotoxicity.

	Daily body weight gain (g/day)			Brain weight ratio to body weight		
	Mean	SD	% CV	Mean	SD	% CV
NC	2.54^{ab}	0.33	12.89	0.67^{a}	0.02	2.77
PC	1.97^{c}	0.37	19.03	0.71^{a}	0.03	4.07
DA	2.81^{b}	0.62	22.00	0.65^{a}	0.03	5.27
CO	3.04^{bd}	0.51	16.82	0.66^{a}	0.02	3.54
DS	2.85^{b}	0.45	15.94	0.65^{a}	0.02	3.84
PS	2.93^{b}	0.14	4.87	0.70^{a}	0.01	2.02
Mix.	2.96^{bd}	0.33	11.00	0.69^{a}	0.01	2.15

Means in the same column with different superscripts significantly differ ($P < 0.05$).

Table 3
LDH and GST activities in sera of rats fed on formulated beverages against mercury-induced neurotoxicity.

	LDH (U/L)			GST (U/mg protein)		
	Mean	SD	% CV	Mean	SD	% CV
NC	5.64^{c}	1.19	21.10	0.88^{bc}	0.07	7.95
PC	14.15^{a}	1.84	13.00	0.63^{d}	0.02	3.17
DA	8.16^{b}	1.10	13.48	0.91^{b}	0.06	6.59
CO	8.87^{b}	1.92	21.65	0.83^{bcd}	0.07	8.43
DS	8.74^{b}	1.36	15.56	1.54^{a}	0.08	5.19
PS	6.54^{bc}	1.13	17.28	0.71^{cd}	0.04	5.63
Mix.	5.74^{c}	1.08	18.82	1.56^{a}	0.08	5.13

LDH: Lactate dehydrogenase GST: Glutathione-S-transferase.
*Means in the same column with different superscripts significantly differ ($P < 0.05$).

2.3. Preparation of probiotic supernatant

Fresh cow milk was standardize to be 12% total solid, then heated at 85 °C for 15 min, cooled to 5 °C to kill pathogens. Probiotic fermented milk containing *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* (10^7–10^9 cfu/g) was prepared according to the method described by Tamime and Robinson [14]. After coagulation, the curd was tested for pH, stirred in an electric blender, then filtered twice through sterilized cheese-cloth (50% cotton/50% polyester) and centrifuged at 3500 rpm for 15 min. The supernatants were decanted and stored at −20 °C until further used.

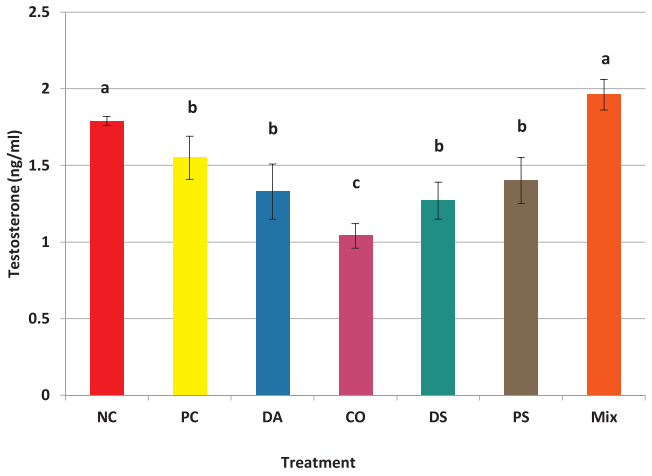

Fig. 1. Effect of various nutritive preparations on the peripheral testosterone of mercury-intoxicated rats (The statistical analysis of each column was performed. Similar letters mean that there are no significant differences at $P < 0.05$, the different letters mean that there are significant differences between the group tests).

2.4. Animals and experimental design

Fifty-six mature male Wister albino rats weighing 121–152 g were purchased from the animal unit, Faculty of Pharmacy, King Saud University, Saudi Arabia. The rats were housed in controlled housing unit and were kept under standard conditions of temperature and humidity (temperature at 25 °C, 55% humidity (40–70%) and in a 12-h: 12-h light: dark cycle) in an experimental animal house. The animals were fed on basal diet according to AIN-93 guidelines and were provided with water *adlib* during the experimental period. The dose were carried out according Johns Hopkins Animal Care and Use Program who reported that Rats are usually supplied feed free choice and they eat 10–30 g a day and they usually drink 20–50 ml a day. The experimental animals were treated in conformity according the European Union Directive for animal use in scientific research [15] during the course of the experiment (42 days). Animal procedures were performed in accordance with the approved procedures by Institutional Ethical Committee. Rats were randomly allotted into seven groups (n = 8 rats/group). Group 1 (negative control; NC) animals were fed on the basal diet only, group 2 (positive controls; PC) animals were fed on the basal diet and given an aqueous solution of mercuric chloride (25 ppm mercuric) in drinking water, group 3 (coriander extract; CO) animals were fed on the basal diet plus aqueous solution of mercuric chloride (25 ppm mercuric) in drinking water and coriander extract (25 ml/rat/day) throughout the experimental period, group 4 (dandelion; DA) animals were fed similar to group 3, except that the coriander was replaced by dandelion extract (25 ml/rat/day), group 5 (date palm seeds; DS) animals were fed similar to group 3 except that the coriander was replaced by date palm seeds extract (25 ml/rat/day), group 6 (probiotic supernatant; PS) animals were fed similar to group 3 except coriander was replaced by probiotic supernatant (25 ml/rat/day) and group 7 (mixture; Mix) animals were fed similar to group 3 except the coriander was replaced by a mixture of equal quantities of the three herbs.

2.5. Animals sacrificing, blood collection and serum harvesting

At the end of the experiment (i.e. after 42 days) and after an overnight fasting, rats were anesthetized with an anesthesia mixture (1:2:3, ethanol: chloroform: diethyl ether) [16], sacrificed and blood samples for serum were collected by retro-orbital puncture using blood capillary tubes. Blood samples were then centrifuged at 3500 rpm for 15 min at 5 °C. Sera were harvested, carefully transferred into clean tubes and stored frozen (−20 °C) for analysis.

2.6. Enzymes activity determinations and brain histopathology

Glutathione-S-transferase (GST) activity was determined according to Habig et al., [17] Lactate dehydrogenase (LDH) activity was determined according to Bergmeyer and Bernt [18].

2.7. Histopathological study

For light microscopic study, samples from cortex and cerebellum were taken; after fixation in 10% neutral buffered formalin, they were dehydrated through alcohols, cleared in xylene and embedded in paraplast plus. Sections 4 to 5 µm thick were cut with a microtome and stained with haematoxylin and eosin for histopathological studies according to Bancroft and Gamble [19].

2.8. Immunohistochemical staining for GFAP of astrocytes

For immunohistochemical study, staining was performed for GFAP as an indicator for glial reactivity. Primary mouse monoclonal antibodies anti-human GFAP was purchased from Dako, Carpenteria, CA. Paraffin sections of 5 µm thickness were deparaffinized and dehydrated, including positive control sections from the rat cortex and cerebellum. The endogenous peroxidase activity was blocked with 0.05% hydrogen peroxide in absolute alcohol for 30 min. The slides were washed for 5 min in phosphate buffered saline (PBS) at pH 7.4. To unmask the antigenic sites, sections were placed in 0.01 mol/L citrate buffer (pH 6) in a microwave for 5 min. The slides were incubated in 1% BSA dissolved in PBS for 30 min at 37 °C in order to prevent nonspecific background staining. Slides were incubated with the primary antibody (1:500 mouse monoclonal anti-GFAP) at 4 °C for 18–20 h and after washing, they were incubated with biotinylated secondary antibodies and then with avidin–biotin complex. Finally, sections were developed with 0.05% 3,3-diaminobenzidine for 15 min. Slides were counterstained with Mayer's hematoxylin, dehydration, clearing and mounting were done by DPX [20].

2.9. Testosterone determination in serum

Testosterone determination in serum was carried out using an ELISA commercial kit (Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany) and according to the method of Rassaie et al. [21]. The procedure was applied according to the manfacturer guideline. All samples were assayed in one assay in duplicates. The intra-assay CV was 3.8%, and the precision was 0.1 ng/mL.

2.10. Statistical analysis

Mean, standard deviation and coefficient of variation of the data from each different experimental group were calculated and conducted using Excel program [22] and according to the method described by Miller and Miller [23].

3. Results

As shown in Table 1 the highest antioxidant capacity was found in date seeds extract; however, the lowest antioxidant value was found in probiotic supernatant. When probiotic was mixed with the three herbal extracts at equal volumes it elevated its antioxidant capacity reaching about 30 folds of its initial capacity. Total antioxidant capacity in date seed's extract surpassed other beverages by about 9, 64 and 313 folds of its counterparts in dandelion, coriander and probiotic, respectively.

Daily body weight gains throughout the experimental period show a significant ($P < 0.05$) decrease when animals were intoxicated with mercury by 22% of the control negative animals. Giving the tested

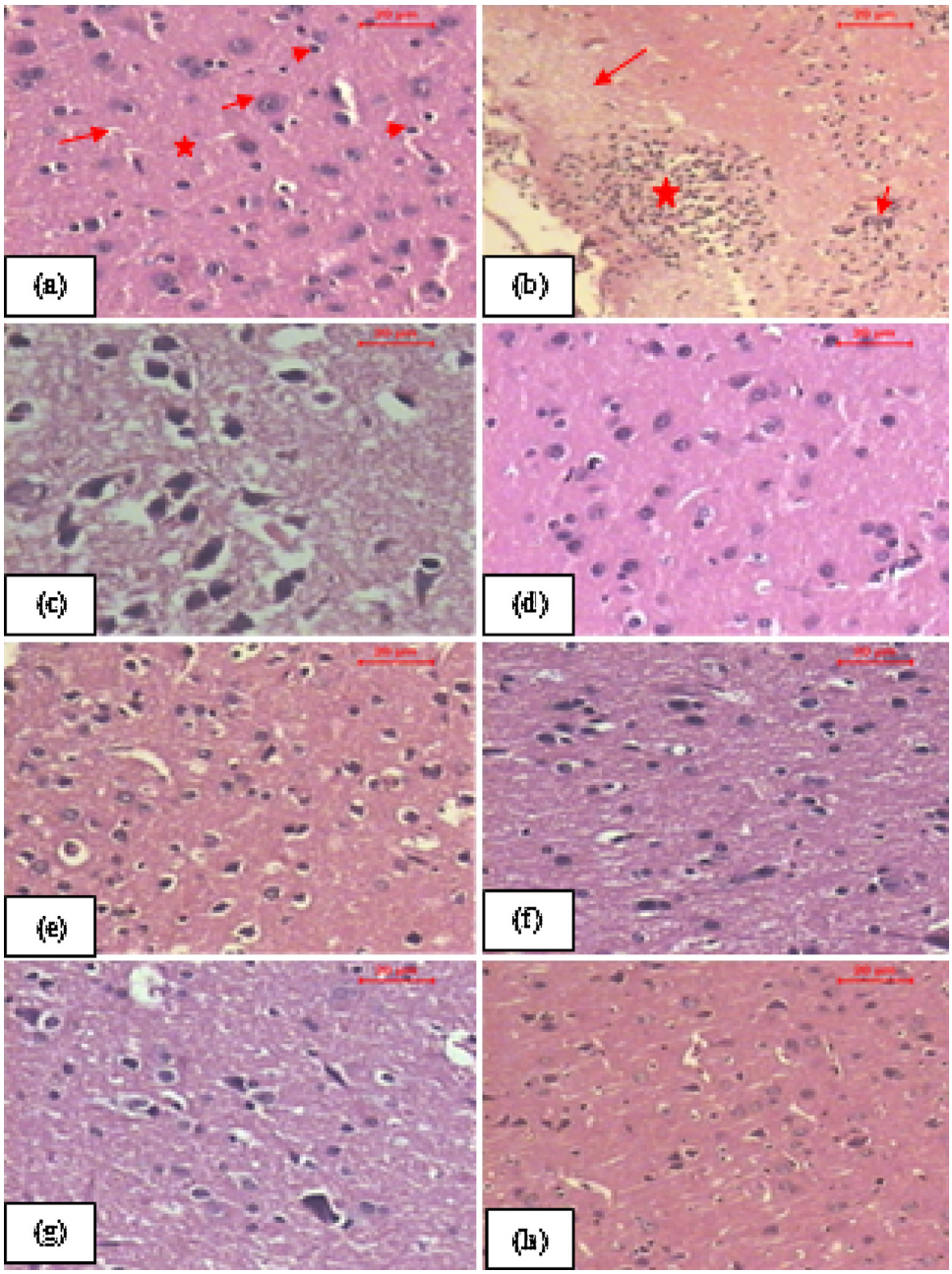

Fig. 2. Photomicrographs of rat cortex: (a) control group (NC) showing normal neural architecture of neuron (short arrow), blood vessel (arrow), glial cell (arrowhead) and neuropil (asterisk), (b) Hg-group (PC) shows severe neural injury in the form of spongiosis (arrow), focal inflammatory cell infiltration (asterisk), focal gliosis around the degenerating neurons (arrowhead), (c) Higher magnification of (b) showing multiple vacuolated areas and pyknosis, (d) Hg/CO group showing nearly normal cortical tissue, © Hg/DA group showing recovered many neurons, (f) Hg/PS group showing more potential ameliorative effects as many neurons are recovered,(g) Hg/DS group showing ameliorative effects except for few dark neurons, and (h) Hg/mix group showing neural architecture more or less similar to the normal (H & E, Scale bar: 20 μm).

beverages to intoxicated animals alleviated the body daily weight gain to be recovered to the normal case or even increased than the normal control (Table 2). There were no significant differences in daily body gain or relative brain/body weight among all tested beverages (Table 2). As for the antioxidant enzymes (Table 3), exposing rats to mercury resulted in significant ($P < 0.05$) increase in lactic dehydrogenase activity, but decreased ($P < 0.05$) the activity of glutathione-s-transferase. All beverages alleviated the normal levels of these enzymes activities, with the mixture of probiotic containing the three herbal extracts showing the best remediation. Fig. 1 depicts the effects of various nutritive preparations on the blood testosterone of mercury-intoxicated rats. Mercury intoxication caused a significant ($P < 0.05$) reduction in peripheral testosterone. Among the given beverages it is obvious that the sole beverage restoring the normal level (i.e. in control negative animals) of testosterone was the mixture containing the 4 preparations. Other beverages were not effective to restore the original testosterone level.

As shown in Fig. 2, brain cortex histological examination of negative control (NC) rats exhibits normal neural architecture with intact cells and normal neurons, glial cells, blood vessels and neuropils (Fig. 2a). Histopathological investigation of Hg-group (PC) indicates severe neural injuries in the form of spongiosis, focal inflammatory cell infiltration, focal gliosis around the degenerating neurons (Fig. 2b). Multiple vacuolated areas and pyknosis were found in the Hg-intoxicated animals (Fig. 2c, high magnification).

Photomicrographs of cortex of coriander-given group (Hg/CO) showed nearly normal cortical tissue (Fig. 2d). Photomicrographs of brain cortex of Hg/DA-animals showed recovery of several neurons (Fig. 2e). In rats given Hg/PS, cortex showed more potential ameliorative effects as many neurons are recovered (Fig. 2f). However, brain cortex of the Hg/DS-given animals showed ameliorative effects with slightly few dark neurons (Fig. 2g). The cortex of the rats given Hg/mixture showed neural architecture with probably more or less similarity to the normal control animals (Fig. 2h).

Histological examination of rat control rat cerebellum (NC; Fig. 3) showed normal neural architecture with its different layers (i.e.;

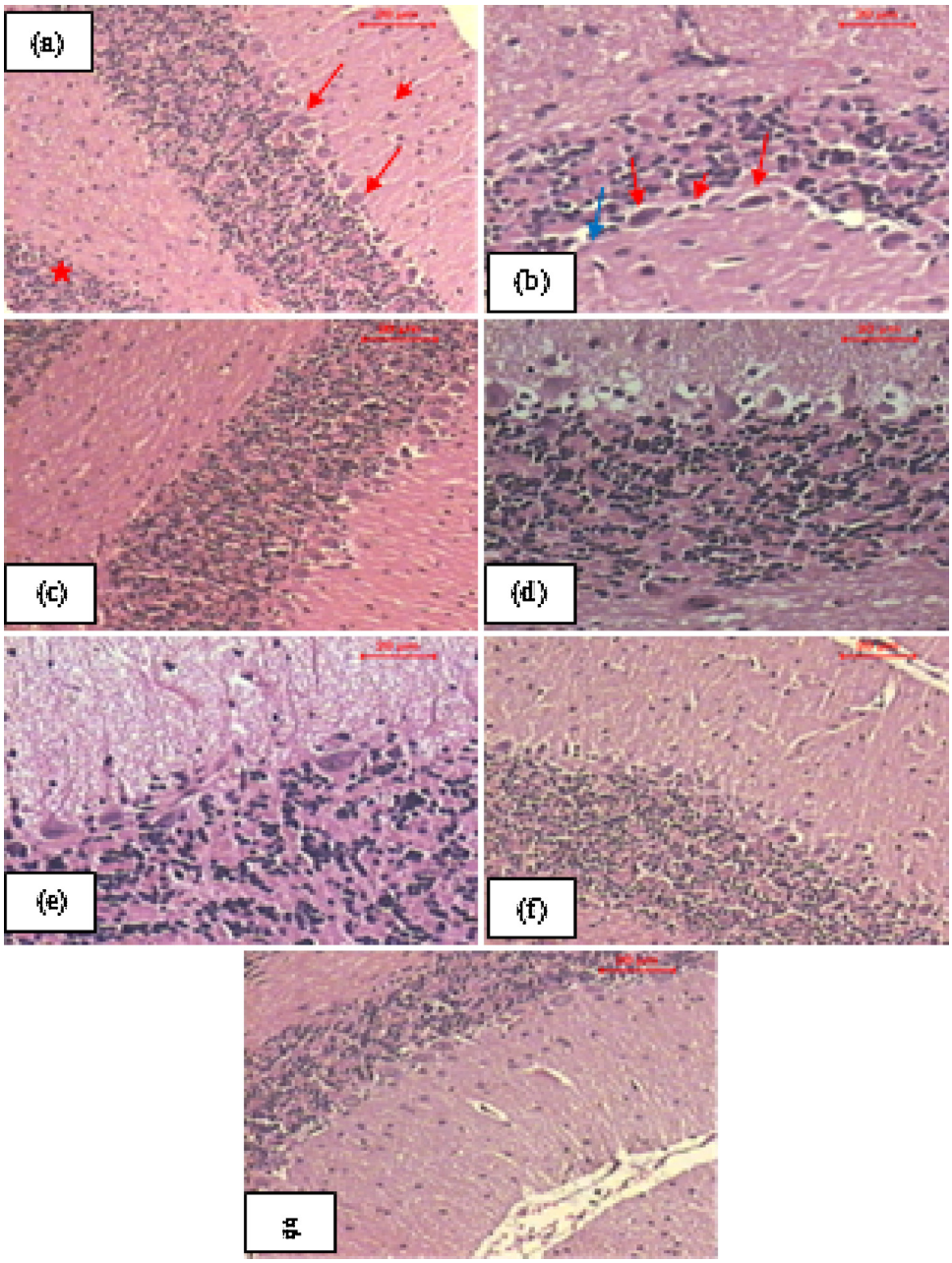

Fig. 3. Photomicrographs of rat cerebellum: (a)control group (NC)showing normal neural architecture with its different layers: molecular layer (arrowhead), Purkinje cell layer (arrow), granular layer (asterisk), (b) Hg-group (PC) showing mild degenerative change as purkinje cells appeared pyknotic (arrowhead), irregular (arrows) or distorted (blue arrow), (c) Hg/DA group showing ameliorative effects as many neurons are recovered, (d) Hg/CO group showing amelioration effects except for few dark neurons, (e & f) Hg/PS and Hg/DS groups, respectively, showing many neurons are recovered, (g) Hg/mix group showing neural architecture more or less similar to the normal (H & E, Scale bar: 20 μm).

ML = molecular layer, PK = Purkinje cell layer and GL = granular layer) (Fig. 3a). Histopathological investigation of Hg-group (PC) showed mild degenerative changes appeared in the form of pyknotic, irregular, distorted and shrunken purkinje cells, dilated blood vessel filled with polymorphs and hyperchromasia (Fig. 3b). In Hg/DA rats, cerebellum showed ameliorative effects marked by the several recovered neurons (Fig. 3c). Whereas, Hg/CO rats cerebellar sections showed ameliorative effects with slightly few dark neurons (Fig. 3d). In case of Hg/PS and Hg/DS groups, several neurons were totally recovered (Fig.3e & f). On the other hand, Hg/Mix-animals cerebellar sections showed that the neural architecture appeared more or less similar to the normal animals (Fig. 3g).

Immunohistochemical investigation of glial fibrillary acidic protein (GFAP) in rat's cortex of negative control group showed normal elongated brownish astrocytes with their multi-processes (Fig. 4a). In case of Hg-intoxicated rats, microscopic examination showed moderate increase in number of astrocytes with thin fragmented processes (Fig.4b). Conversely, antioxidant-treated rats showed well-organized astrocytes with extended multi-processes (Fig. 4c–g).

In case of GFAP immunostaining in rat's cerebellum of control group, examination exhibited normal elongated brownish astrocytes with their multi-processes appeared in the granular layer and molecular layer (Fig. 5a). Immunohistochemical investigation of GFAP immunostaining in Hg-intoxicated group illustrated reduced immunoreactive astrocytes with their thin fragmented processes (Fig. 5b). Antioxidant treatments revealed an increase in immunoreactivity of well-organized astrocytes with extended multi-processes (Fig. 5c–g).

4. Discussion

The recent lifestyle of the people inhabiting the industrialized countries imposes great risk of the exposure to the heavy metals, through food contaminations, vapor, sewage, aquatic microorganisms and many others. Due to its high toxicity and its several usages in the daily life, mercury was the focus of the present research. The presence of this metal in the environment is in the forms of organic or non-

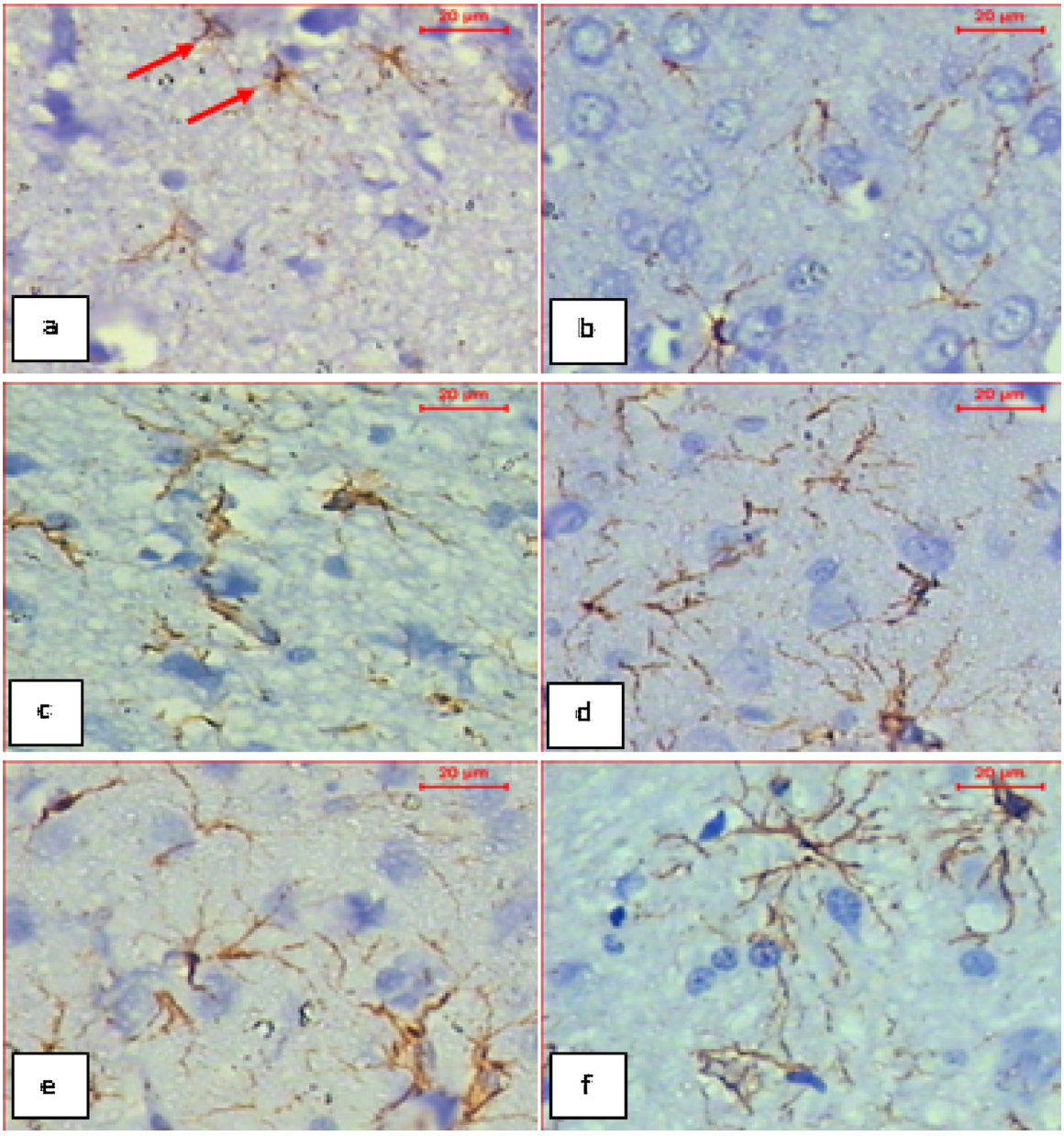

Fig. 4. Photomicrographs of rat cortex: (a) control group (NC) showing normal brownish astrocytes with their multi-processes (arrows);(b) Hg-group (PC) showing moderate increase in number of astrocytes with faint,thin fragmented processes (arrows); (c& d) Hg/CO and Hg/PS groups, respectively, showing well-organized astrocytes with their multi-processes; (e & f) Hg/DS and Hg/mix groups, respectively, showing many elongated brownish astrocyteswith their extended processes; (GFAP immunostaining, Scale bar: 20 µm).

organic with the organic being the most toxic as it is fat-soluble and readily absorbed in the intestinal epithelium.

Peripheral neuropathy from mercury exposure most commonly involves distal latency sensory slowing for short-term exposures, followed by motor slowing for more long-term exposures. Central nervous system effects are more common with organic mercury exposures. Exposure to mercury revealed increases in LDH activity and decreases in the activity of GST, a result was reported in a human population living in low amazon and consumed fish polluted with mercury [24]. Probiotics using lactic acid bacteria have shown beneficial roles in restoring the normal activity of various metabolic and antioxidant enzymes [13]. Additionally, dietary supplements especially of the plant (i.e., high-fibers source) origin have shown alleviation against heavy metals toxicity [25]. The presence of probiotics which are rich in indigestible fibers and to some extent contain simple sugars or fructo-oligosaccharides (FOS) such as dandelion in fermented milk have shown anti-toxicity effects against heavy metals such as arsenic [26]. Augmenting the intestinal microbiota by providing fermented milk with probiotics have shown health effects in individuals exposed to heavy metals [27]. In a recent study on the toxic effect of mercury upon the domestic animals it revealed a decreased immune function, altered neuronal function and decreased reproductive capabilities [28].

The main target organ subject to the vapor inhalation of mercury is the brain. Thus in this study, the cortex of mercury-intoxicated animals revealed severe neural injury in the form of spongiosis, focal inflammation with cellular infiltration and focal gliosis around the degenerated dark neurons. Examination of rat cerebellum visualized mild degenerative changes appeared in distorted Purkinje cells with pyknotic and shrunken nuclei. Similarly, it was suggested that chronic intake of mercury can induce various neurological disorders on different parts of the brain such as multiple foci of necrosis, gliosis and marked congestion of vessels with perivascular necrosis [29]. Moreover, effects of sub-

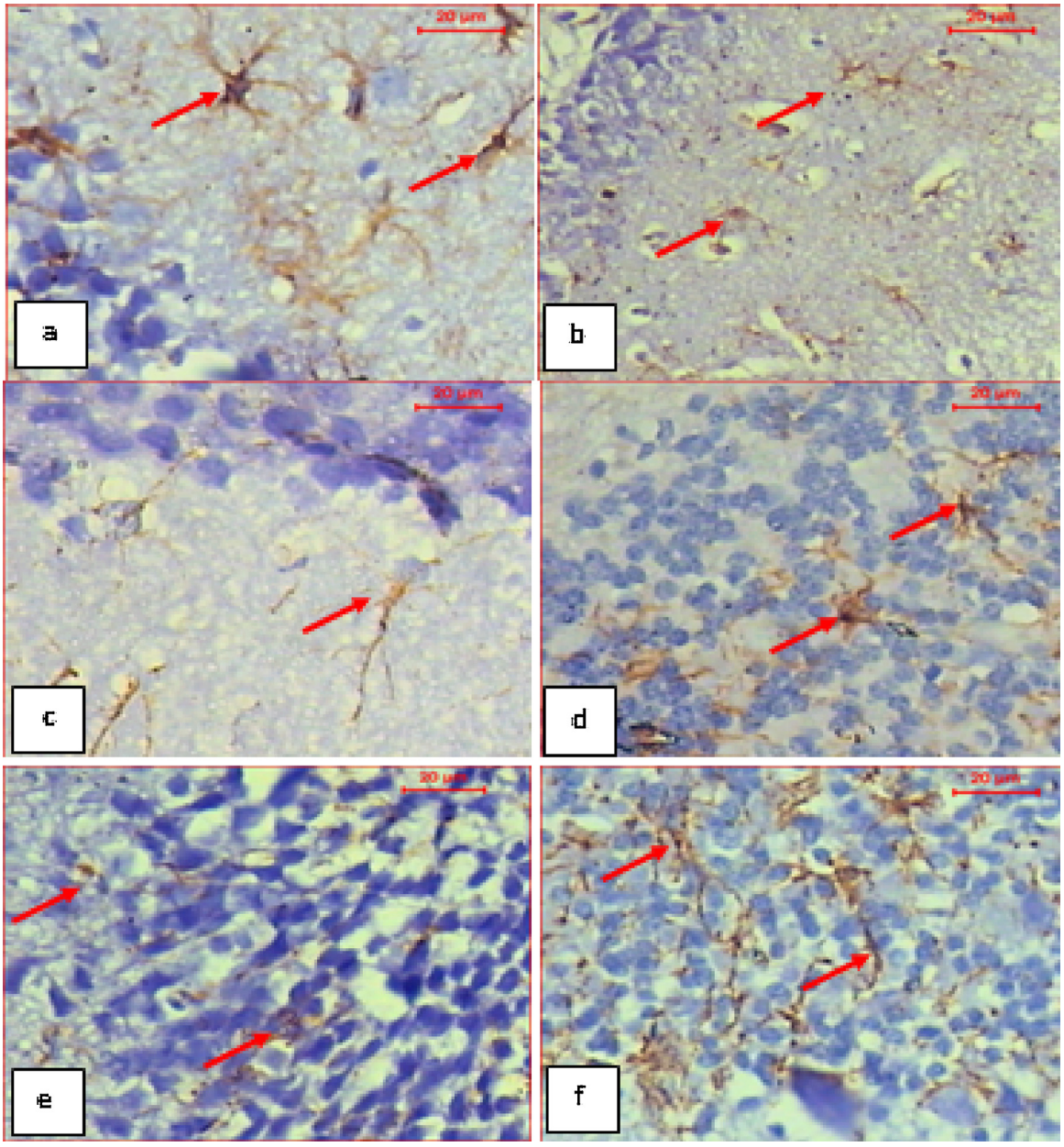

Fig. 5. Photomicrographs of rat cerebellum: (a) control group (NC) showing normal elongated brownish astrocytes with their multi-processes (arrows), (GL) the granular layer,(ML)the molecular layer; (b) Hg-group (PC) showing decreased immune-positive and fragmented astrocytes; (c & d) Hg/DA and Hg/PS groups, respectively, showing well-organized immunoreactiveastrocytes; (e & f) Hg/DS and Hg/mix groups, respectively, showing many elongated brownish astrocytes with their extended processes; (GFAP immunostaining, Scale bar = 20 µm).

chronic exposure via drinking water to a mixture of eight water-contaminating metals, including mercury revealed vascular and degenerative lesions in brain sections [30]. Studies also demonstrate that mercury, even at low doses, has the ability to reduce the number of neuron and cyto-architecture in individuals with prenatal exposure to mercury [31–33]. In addition, it was believed that changes caused by mercury poisoning may be inhibits the cell division and the organization of microtubules that are important in CNS development [34–36].

The present immunohistochemical investigation observed marked decreased in size and intensity of brownish immuno-reactive GFAP-positive astrocytes in the cortex and cerebellum of mercury intoxicated rats. In accordance with our results, several reports demonstrated that mercury has the ability to reduce the number of neurons and cyto-architecture [31,32]. On the other hand, it was reported that the intermediate filament protein, GFAP is highly correlated with brain injury [9,37–39]. In chick embryo, Kim [40] suggested that inorganic lead and mercury mediated cytoskeletal reorganization of the structural proteins that resulting in neurotoxicity. The toxic effects of mercury may attributed, at least in part, to elevated levels of reactive oxygen species (ROS) that reflected in the present work by depletion in the level of GST in vitro, Allen et al. [41] reported inhibition of cystine uptake in astrocytes by methyl mercury, hence decreased production of intracellular glutathione, a vital intracellular defense against oxidative damage induced by increased production of ROS. The elevated level of ROS may be responsible for fragmentation of astrocytic multi-processes and reduced GFAP-immunorectivity observed in this study.

The antioxidant treatment with the three herbal extracts, probiotics and their mixture fully reversed the cytotoxicity induced by $HgCl_2$ (with variable degrees). This significant attenuation of the biochemical, immunohistochemical and histopathological deteriorations may be

attributed to decreased oxidative stress together with concomitant production of ROS [42]. Thus, the protective effects elicited by these antioxidant strengthen the idea that probiotic-herbal mixture represent promising approaches to neutralize mercury induced neurotoxicity.

Furthermore, the sharp decline of the male sex hormone (testosterone) and the reduced male libido as a consequence of drinking mercury was shown elsewhere [43,44]. There appears to exist cellular receptors for heavy metals in the testicular interstitial cells, modulating the secretory mechanism of these cells to androgens. Adding a source of fiber-rich ingredient to the fermented milk enhances the usefulness of the probiotics to alleviate the toxic effects due to exposure to heavy metals. Coriander was found to be effective in normalization of the adverse effects of lead on renal functions [45]. Dandelion is a herb historically used a hepatogenic to cure the jaundice in human. It is rich in the FOS which are easily used by the lactic acid bacteria to do their useful effects in the hindgut of the host. In a study by Zeitoun et al. [46] using dandelion roots and leaves aqueous extract in fermented cow's milk on prepuberal lambs, they concluded that at low levels of dandelion combined with probiotic enhanced the histological structure of testicles and testosterone production. Date palm seeds are rich in fibers and phenolic compounds which mask the deteriorating effects of mercury. This useful function was recently pointed to in carp fish exposed to mercury and lead [47]. The supplementation of carp diet with date palm seeds at 1–4% of the diet significantly reduced muscle and liver content of mercury compared to positive control. The brain represents the major target organ of organic mercury species exposure [48]. In the current study, the use of inorganic mercury, $HgCl_2$, has shown a weak blood brain barrier which resulted in mild toxic effects on the brain architecture confirming that the organic mercury shows more toxic effects due to its ability to overcome the blood brain barrier [49].

5. Conclusion

In conclusion, the uptake of probiotic fermented milk fortified with a rich source or combined source of natural fibers (i.e. dandelion, coriander and date palm seeds) could be a beneficial approach to counteract the adverse effects of the toxicity of the mercury, the metal that the people compelled to inhale in their surroundings.

Availability of data and materials

All necessary data supporting our findings can be found in the repository.

Conflict of interest statement

The authors declare that there are no conflict of interest.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors have read and approved the paper for publication.

Ethics approval and consent to participate

Animal procedures were performed in accordance with the ethics committee of Qassim University and were treated in conformity according the European Union Directive for Animal use in Scientific Research.

The Transparency document associated with this article can be found in the online version.

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