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ORIGINAL ARTICLE

# Drinking desalinated seawater for a long time induces anomalies in the development of new-born albino rats



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## KEYWORDS

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Embryotoxicity

**Abstract** The present study aimed to elucidate the abnormalities in the development of rat brains, livers, kidney and behaviours after drinking desalinated seawater prenatally. Three types of drinking water were employed as an experimental probe (bottled water, filtered desalinated seawater and tap desalinated seawater) to investigate neurobehavioral and morphological changes in the development of pup rats. Female rats from each group were administered water from their birth until gestation and lactation. The 1st and 2nd generation pups were divided into three groups: Group C, mothers and pups administered with bottled drinking water (the control group); Group F, mothers and pups administered with filtered drinking water; Group T, mothers and pups administered with unfiltered desalinated seawater (tap water). Morphological changes (CNS aberration) and neurobehavioral changes were studied. The aberrations recorded in the tissues (brain, liver, kidney and spinal cord) of rats from groups T and F may be due to oxidative stress in these tissues such as reduced glutathione, lipid peroxidation, peroxidase and super oxide dismutase. In conclusion, drinking desalinated seawater for a long time may cause teratogenic effects in the development of New-born rats.

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## 1. Introduction

The salinity of water is the major factor determining the suitability of a particular water source for use by livestock. Certain gases and salts in water make it more palatable, while an excess of certain salts may reduce water palatability and/or lead it to become toxic (Sandford, 1996). Scarcity of fresh water has been estimated to affect one in three people globally, especially

in the Gulf area (Cotruvo et al., 2010), and, in environments where fresh water is scarce, desalination has been used to provide drinking-water (Qar and Abdel-Monem, 2014).

Although the primary intention of the desalination process is to remove natural ionic contaminants from seawater, some substances are not as well removed as others, such as boron ions, since the process of reverse osmosis is not efficient in removing them. In addition, many chemical treatments have been used to control the desalination process to ensure that the final water does not include unacceptable concentrations of microbial pathogens and compounds (Cotruvo et al., 2010). Desalinated water should, therefore, not be considered as a source of drinking-water production (WHO, 2011).

While the literature on the process and technology of desalination is huge, data on teratogenic and neurobehavioral consequences during the postnatal development of albino rats of drinking desalinated water for a long time are much less extensive. With respect to rats, it is known that water toxins may be transferred to the pups postnatally through breast milk and prenatally through the placenta. Signs of toxicity include decreased body weight and decreased food and water consumption (Frieda and William, 1999). Indeed, the development of pups' body weights has been used as a sensitive indicator of developmental toxicity (Wise et al., 1995).

Furthermore, the sensorimotor reflexes may be useful references to show the changes in neurobehavioral development that may be produced by toxicological and pharmacological factors in exposed rodents (Ten et al., 2003). Excess elements in drinking water may behave as toxins, affecting the CNS, sensorimotor reflex maturation, and behavioural measures such as open field activity in animals; they may also produce noticeable effects on balance and rota rod performance (Garey et al., 2005), since these activities require the involvement of central and peripheral nervous system components (Bouet et al., 2004).

The delicate balance between the production and removal of oxidants is critical for maintaining cells' biological function (Allam et al., 2010), but the ingestion of metals has been shown to cause dose-dependent oxidative stress disturbance (Abu-Taweel et al., 2013). In this regard, Visavadiya et al. (2015) reported that the induction of lipid peroxidation in different tissues increases the thiobarbituric acid-reactive substances (TBARS) due to the depletion of glutathione (GSH). Thiols are vital cellular antioxidants and protect animals' cells from free radicals (Allam et al., 2016), and GSH is recognized as the principal endogenous thiol that acts as a redox buffer in our body (Visavadiya et al., 2015). Metals polluting water include well-documented neurotoxins that produce pathology both in the peripheral and central developed nervous system, as well as in the liver and kidney (Maodaa et al., 2016).

Most studies on water toxins have been performed using adult rodents, and not much information is available on their effects on the development of pups. During maturation of the animal, there are extensive reorganizations of cellular structures, and exposure to toxins such is likely to affect the expression of proteins, lipids, nucleic acids and enzymes (Allam et al., 2011).

The present study aimed to study the effects of drinking desalinated water for a long time and its relation to the teratogenic and neurobehavioral changes as reflected in the development of new-born rats. Our hypothesis is that the desalinated drinking water (whether filtered or tap) may induce imbalance

in the TBARS/GSH system causing oxidative stress disturbance on long-term exposure that, in turn, leads to morphological changes in the brain regions (cerebrum, cerebellum and medulla oblongata), liver, kidney and spinal cord. The rats have been used as a model to explore potential risks of direct using the desalinated seawater to other species (including humans).

## 2. Materials and methods

### 2.1. Water

#### 2.1.1. Types of water used in the experiment

[describe here, precisely, the three types of water used; for the bottled water – what brand; the desalinated water was sourced from where; what filter system was used for the filtered water, etc. you need to be detailed enough for others to be able to directly replicate your work].

#### 2.1.2. Element estimation assay (water screening)

The analytical estimation of 14 periodic table elements (Table 1) in the three types water investigated was carried out every 15 days during the experimental period using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) following the operating conditions described by Shah et al. (2013).

### 2.2. Animals

#### 2.2.1. Experimental animal schedule

This study was performed on three generations of albino rats (*Rattus norvegicus*). The first generation mothers were three mature virgin females in each group (the 1st generation), weighing 120–140 g and purchased from the animal house laboratory, College of Pharmacy, King Saud University. Proestrous females were mated overnight by housing a female with a male (Also sourced from the College of Pharmacy) in a steel wire mating cage. The detection of a vaginal bulge or the pres-

**Table 1** The elements concentrations in the three water types.

	Drinking bottle water (Given to group C)	Filtered water (Given to group F)	Tapped water (Given to group T)
Li	0.51 ± 0.21	1.20 ± 0.53	4.21 ± 0.62**
B	51.64 ± 2.5	76.77 ± 9.2*	84.26 ± 02.2*
Al	0.03 ± 0.00	0.08 ± 0.01	1.10 ± 0.05*
Si	135.19 ± 5.2	198.23 ± 8.69*	761.34 ± 10.25**
S	14,596 ± 168	17,224 ± 120*	33,333 ± 121*
Cl	889 ± 25	3830 ± 12.1**	18,974 ± 98***
Ti	0.06 ± 0.01	0.11 ± 0.04*	0.30 ± 0.02**
V	0.18 ± 0.05	0.20 ± 0.01	0.87 ± 0.06**
Co	0.00 ± 0.0	0.03 ± 0.0*	0.11 ± 0.02**
Br	9.39 ± 2.6	17.30 ± 1.23*	149.3 ± 10.2***
I	0.75 ± 0.10	27.211 ± 2.1***	24.63 ± 5.1***
Ba	0.04 ± 0.01	0.18 ± 0.03	3.10 ± 0.20**
Pb	0.00 ± 0.0	0.01 ± 0.0	0.05 ± 0.0*
Cd	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0

Data are expressed as mean ± SE ( $N = 6$ ),  $P > 0.05$  insignificant; \* $P < 0.05$  significant; \*\* $P < 0.01$  highly significant; \*\*\* $P < 0.001$  very highly significant.

ence of sperm in a vaginal smear determined the first day of gestation. Each of the three pregnant females was assigned to a group and administered one of the three types of drinking water. In the second generation there were five mothers in each group. Forty pups in each group were produced by the second generation mothers. Male and female pups were labelled and each housed in a cage and fed on a standard diet (rodent pellets) manufactured by the Saudi Company for Animal Foods. Drinking water was given *ad libitum*, according to the group. The mothers and pups of each group were given bottled water, filtered or tapped desalinated seawater as drinking water from the first day of the first generation mothers' gestation through the maturation and mating of the second generation and until the subsequent pups were investigated during the lactation period. The experimental examination and investigations, however, were performed only on the pups of the second generation mothers. The first and second generation pregnant females were observed daily to record the date of birth, which would be postnatal day zero (D0).

The animals were labelled and assigned to one of the three groups as follows:

Group C: first and second generation mothers and pups given bottled drinking water (*Control group*).

Group F: first and second generation mothers and pups given filtered desalinated seawater.

Group T: first and second generation mothers and pups given unfiltered desalinated seawater.

The number of pups of each mother from each group was limited to eight pups at D1 by transferring excess pups from one mother to another within the same group if the pups numbered more than eight.

### 2.3. Basic developmental measures

The pups born to the second generation mothers were observed every day and the following notes were recorded.

- a. The body weights of ten pups from each group.
- b. Time at which fur appeared.
- c. Time at which ears opened.
- d. Time at which eyes opened.

### 2.4. Behavioural tests

Forty pups from the second generation of mothers in each group were examined from D1 to D21 (i.e. during the lactation period). The examination was conducted in a special room reserved for that purpose (maintained at 25 °C, with a red light). The animals were examined on a 90 × 60 cm plastic cart with an 8-cm lip around the edge. The cart was covered with a plastic sheet wiped with an ethanol sweep between the testing of the animals. Each test was conducted every day from postnatal D1 until the reflex was expressed at its adult level for three consecutive days. Six pups from each group were tested daily for each reflex. Each of the six subjects was tested five times for a given reflex at a given age and the positive responses were recorded (scores of 0/5 to 5/5). The average score was then calculated, converted to percentage and a reflex

was described as being present or absent depending on what is the percentage. A reflex was considered stable when it was present at the adult level for three consecutive days. The period between the appearance of a reflex and its stabilization was considered as the period of maturation. All tests were conducted blindly by the same experimenter.

#### 2.4.1. Sensorimotor reflex assays

2.4.1.1. *Grasp*. The pups were held in the air, and the soles of the hands or feet being gently touched with the tip of a fine brush. The left and right sides have been tested equally. The reflex was considered present when the pup closed the stimulated hand or foot around the brush.

2.4.1.2. *Hopping*. The pups were held so that only one hand or foot was touching the test surface, and then moved forward so that the tested limb dragged on the surface. The left and right sides were tested equally. The reflex was considered to be present when the animal lifted its limb and hopped in the direction of the movement.

2.4.1.3. *Body righting on a surface*. The pups were held in a supine position so that the dorsum of the head and of the trunk were in contact with the test surface. The reflex was considered to be present when the pup turned on its ventrum or limbs within 15 s of the experimenter releasing his hold.

2.4.1.4. *Body righting in the air*. The pups have been held supine in the air at a distance of 30 cm from the soft surface. The reflex has been considered present when the pup turns in the air and landed on its ventrum or limbs. The reflex testing surface consists of a slightly rough piece of Carton (60 cm × 80 cm) of a light brown colour.

2.4.1.5. *Chin tactile placing*. The pups were held by the posterior half of the body and the skin of the chin was gently rubbed on the edge of the test surface. The reflex was considered to be present when the animal lifted one (or both) fore-limb and placed it on the surface.

#### 2.4.2. Rota-rod assay

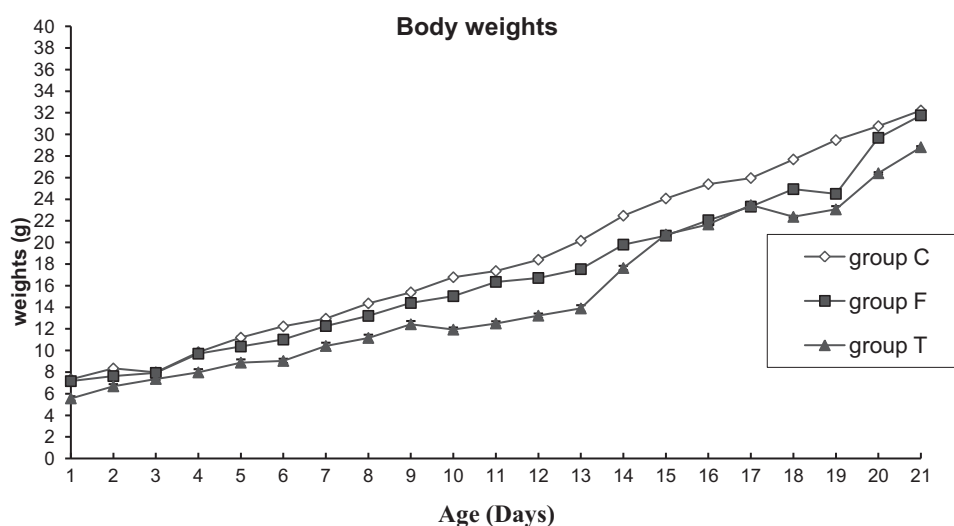
In this examination, the young rat was placed on a horizontally oriented rod rotating at 10g. The rod was suspended above a cage floor, which was low enough not to injure the pups, but also high enough to induce avoidance of fall. Pups will naturally try to stay on the rotating rod, and avoid falling to the ground. The length of time that the pups spend on this rotating rod was measured to show their balance, coordination, physical condition and motor-activity.

#### 2.4.3. Cage activity test

The Ugo Basile 47420-Activity Cage was used to record spontaneous co-ordination activities of the pups and the variations in this activity in a time. This test was performed for 3 min for each pup.

#### 2.4.4. Grip-strength metre assay

The Ugo Basile 47200-Grip-Strength Meter suitable for rat pups automatically measures grip-strength (i.e. peak force and time resistance) of the pups' forelimbs. The aim was to assess the forelimb muscle strength. Each pup was tested three



**Figure 1** Shows the newborns body weight development from postnatal day 1 to day 21 in the groups C, F and T. Data are expressed as mean  $\pm$  SE ( $N = 6$ ).

**Table 2** Shows the litter size and age of eye opening and fur appearing in the group's newborns.

	Group C	Group F	Group T
Litter size ( $N$ )	11 $\pm$ 1.0	10 $\pm$ 1.1	9.75 $\pm$ 0.8
Days of fur appearing	9–11	10–11	10–12
Days of eye opening	14–16	15–16	15–16

times and the peak force of each pup was recorded. The mean of three values for each animal was recorded.

### 2.5. Biochemical assays

Eight Pups from each group were anaesthetized by light ether and sacrificed by decapitation at D7, D14 and D21. The liver, kidney, cerebrum, cerebellum and medulla oblongata were then dissected. A 0.2 g sample of each tissue was homogenized in 3 ml of cold saline under ice. The homogenate was centrifuged at 8000g for 10 min at 4 °C and the clear supernatant was collected in a microfuge tube (0.5 ml each) and stored at  $-80$  °C until use. The following biochemical assays were then performed:

Lipid peroxidation was determined by estimation of thio-barbituric acid-reactive substances (TBARS) according to the procedure of [Preuss et al. \(1998\)](#). Glutathione content was estimated according to the method of [Beutler et al. \(1963\)](#) with some modification as reported by [Allam et al. \(2011\)](#). Superoxide dismutase activity was determined according to the method of [Marklund and Marklund \(1974\)](#); and, peroxidase activity was assayed according to the method of [Kar and Mishra \(1976\)](#).

### 2.6. Histological preparations

Three Pups from each group were anaesthetized by light ether and sacrificed by decapitation for the histological preparations at D7, D14 and D21. The left lobe of the liver, cerebral cortex,

cerebellum, medulla oblongata, kidney and the brachial region of the spinal cord were immediately cut into small pieces and fixed in 20% formalin saline for 24 h for nervous system regions and 10% neutral buffer formalin for liver and kidney pieces. The tissues were washed to remove the excess of the fixative and then dehydrated in ascending grades (70%, 80%, 90% and 95%) of ethyl alcohol for 45 min each, then in two changes of absolute ethyl alcohol for 30 min each. This was followed by two changes of xylene for 30 min each. The tissues were then impregnated with Paraplast Plus (three changes) at 60 °C for three hours and then embedded in Paraplast Plus. Sections (4–5  $\mu$ m) were prepared with a microtome, dewaxed, hydrated and stained in Mayer's haemalum solution for 3 min. Staining with haematoxylin and eosin was according to the method of [Mallory \(1988\)](#) and by periodic acid Schiff's (PAS) for polysaccharides according to [McManus \(1946\)](#).

All chemicals used throughout were of analytical grade and purchased from Sigma Chemical Company (USA).

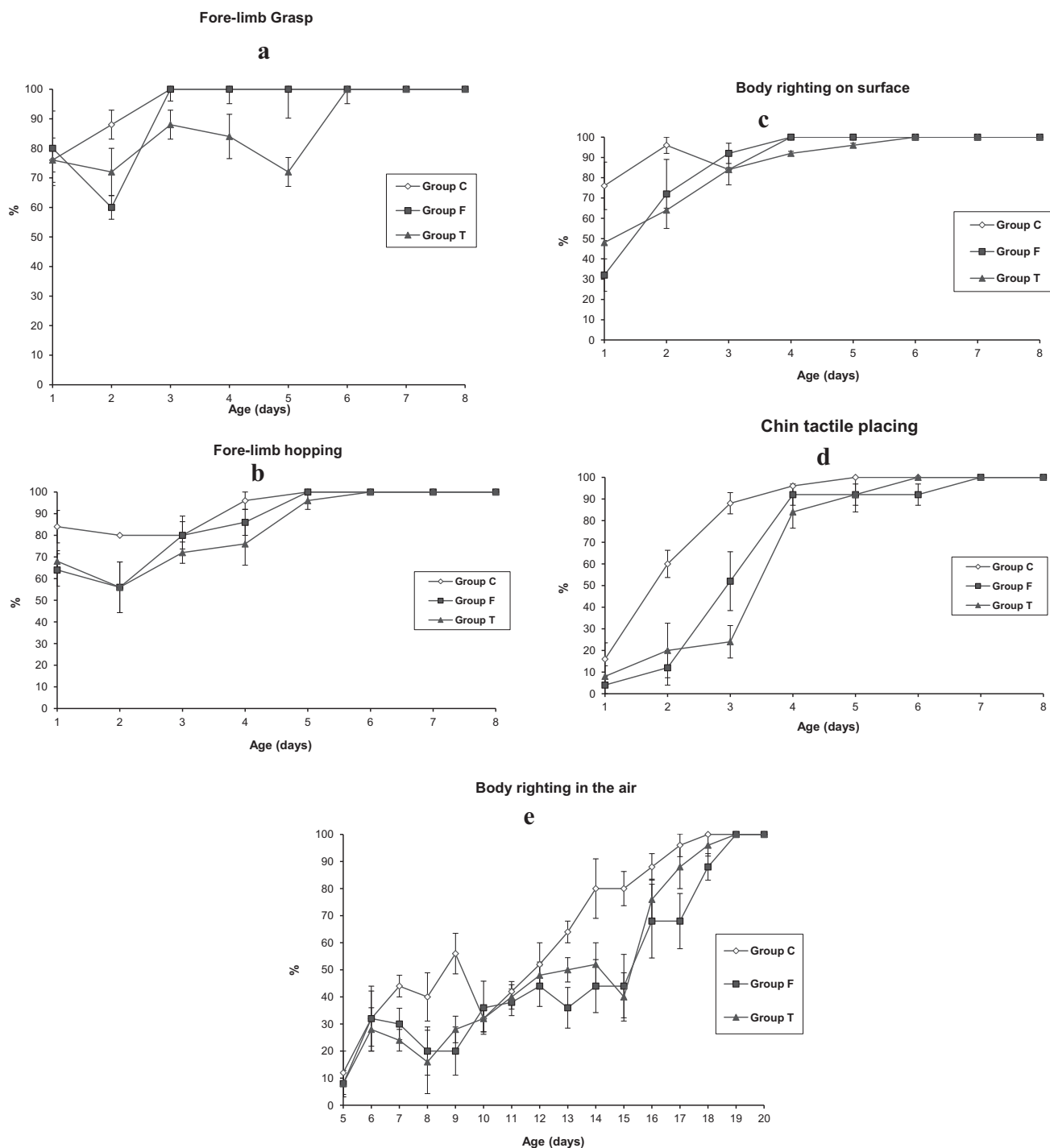
### 2.7. Statistical analysis

The present data were analysed using SPSS (Statistical Package for the Social Science) for Windows, version 7. Comparative analysis of results was undertaken using the linear models procedure. The data were examined by a paired sample t-test followed by LSD computation to compare the treated groups with the control one. Results are expressed as mean  $\pm$  Standard error and  $N = 6$ . The level of significance was expressed as very highly significant at  $***P \leq 0.001$ , highly significant at  $**P \leq 0.01$  and significant at  $*P \leq 0.05$ .

## 3. Results

### 3.1. General developmental observations

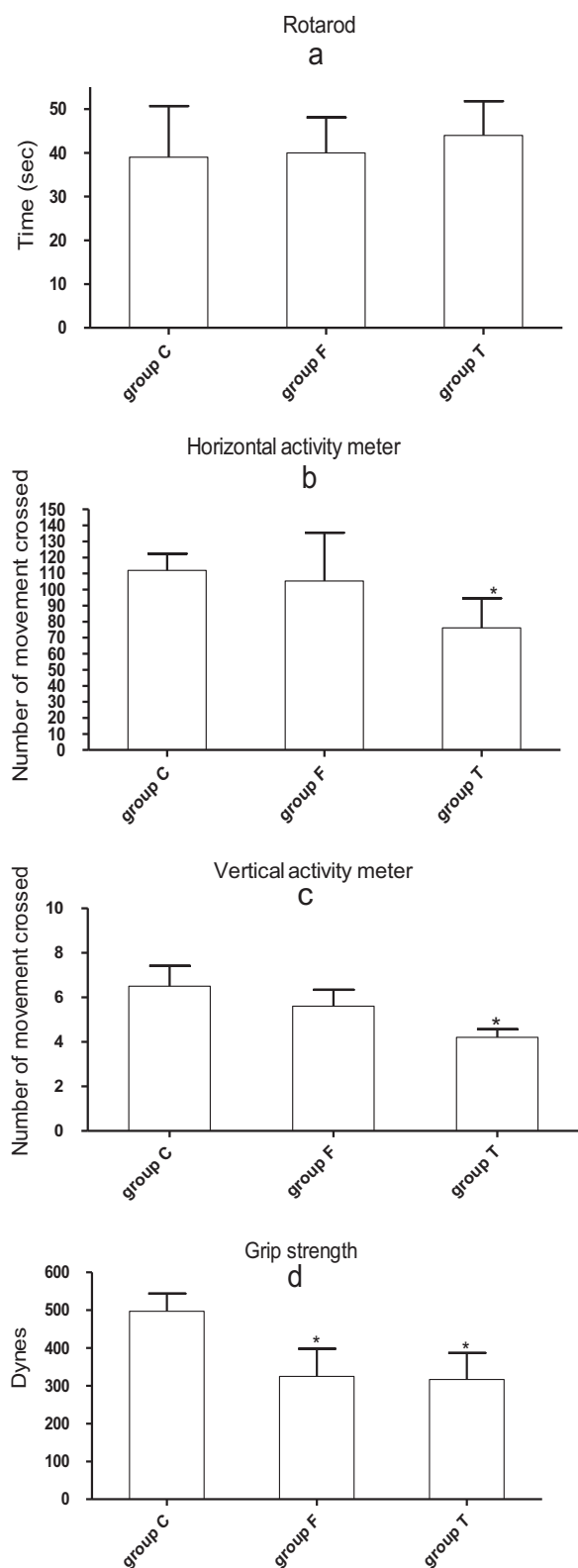
The females (mothers) of each group drank the specified water for that group from birth through maturity, pregnancy and motherhood. The new-born rats depend on their mother's milk



**Figure 2** The percentage of sensorimotor reflex development relative to the age of developing newborns in groups C, F and T. (a) fore-limb grasp reflex, (b) fore-limb hopping reflex, (c) surface body righting reflex, (d) chin tactile placing reflex and (e) air body righting reflex. Data are expressed as mean  $\pm$  SE ( $N = 6$ ).

until postnatal day 21 and hence we investigated the pups from D0 (day of birth) to D21. Each mother's daily mean consumption of water was nearly 5 ml after maturation. Group C rats drank bottled water, which is ideal drinking water (Table 1). Group F rats drank filtered desalinated seawater and Group T drank unfiltered desalinated seawater direct from the tap. These water types were screened by ICP-MS to estimate the con-

centration of hazardous elements (Table 1). The most prevalent hazardous elements in the filtered and unfiltered water were lithium (Li), aluminium (AL), silicon (Si), chloride (Cl), vanadium (V), cobalt (Co) and bromide (Br), the last of which reacts with oxygen to form hazardous bromate ions (Table 1). High concentrations of these elements accumulating in animal tissues may behave as toxins.



**Figure 3** Behavioral investigations of the newborns of each group at D21. (a) Rota-rod records, (b) vertical movements, (c) horizontal movements and (d) grip strength records for the forelimb. Data are expressed as mean  $\pm$  SE ( $N = 6$ ; \* $P < 0.05$ , significantly different from the control group).

Group T and F pups showed retardation in body weight development compared to the pups from group C from D1 to D21 (Fig. 1). The mean litter size of the present groups is varied (Table 2). Although a smaller litter size was evident in group T, the differences were insignificant ( $P > 0.05$ ) between groups. Also, there was a delay in the time of fur appearing and eye opening in groups T and F (Table 2).

### 3.2. Sensorimotor and behavioural reflexes

The forelimb grasp reflex appeared in all groups at D1 and increased rapidly to reach 100% by D3 in groups C and F (Fig. 2a). In group T pups, however, the development of this reflex was delayed to D6. In group C, the expression of the linear reflex expression appeared in a curve pattern that shown regular pup development. There were fluctuations in the development of this reflex in groups F and T, however. Fig. 2a shows the level of significance between group C and groups T or F.

Forelimb hopping appeared by D1 in all groups and increased to 100% by D5 in both groups C and F. In group T, however, the reflex development increased slowly at a regular rate, only attaining 100% by D6. Fig. 2b shows the forelimb hopping reflex development and the level of significance between the experimental groups.

Body righting on a surface was expressed by D1 in all the groups. In groups C and F, the reflex expression increased fastest to record 100% at D4, while in group T, this reflex reached maturation by D6 (Fig. 2c). The chin tactile placing reflex was also detected by D1 in all the groups; its development was more regular and faster in group C compared to groups T and F, however: the reflex achieved 100% by D5, 6 and 7 in groups C, F and T, respectively (Fig. 2d). The reflex expression differences between group C and groups T and F were highly significant ( $P < 0.01$ ) at D2 and 3 (Fig. 2d). Finally, the body righting in the air reflex appeared later (by D5), with its expression increasing irregularly to attain 100% at D16 (Fig. 2e). In group C, the expression of this reflex was higher than in groups F and T on most days.

In the rota rod test, the time consumed by pups from groups F and T on the rod was insignificantly elevated compared to group C (Fig. 3a). In the activity cage, the pups from group C showed a significant ( $P < 0.05$ ) elevation in their vertical and horizontal movements compared to the pups from group T (Fig. 3b and c), while this elevation was insignificant ( $P > 0.05$ ) compared to the pups from group F. The forelimb muscles of the pups from group C recorded a relatively highly significant ( $P < 0.05$ ) peak in the grip strength examination scores compared to groups T and F (Fig. 2d). The peaks recorded by the pups of groups T and F were relatively similar to each other.

### 3.3. Oxidative stress

In the pups' cerebra, at D7, 14 and 21, the lipid peroxidation (TBARS), peroxidase and SOD differences between groups C and F were insignificant ( $P > 0.05$ ) but were highly significant ( $P < 0.01$ ) between groups C and T. The detected depletion in the cerebral GSH of groups F and T was very highly significant ( $P < 0.001$ ) at all ages compared to group C (Table 3).

**Table 3** Shows the oxidative stress parameters in the newborns cerebrum at D7, D14 and D21.

Parameter	Group/Time	D7	D14	D21
TBARS (nmol/100 mg)	Group C	23.66 ± 1.20	19.66 ± 1.20	18.83 ± 1.24
	Group F	32.33 ± 1.58	30.5 ± 1.54	23.5 ± 1.92
	Group T	33.66 ± 1.74**	31.33 ± 1.83**	26.0 ± 1.54**
GSH (nmol/g)	Group C	53.5 ± 1.6	51.66 ± 1.22	50.5 ± 1.25
	Group F	43.0 ± 1.2***	42.5 ± 1.88***	40.83 ± 1.4***
	Group T	38.16 ± 1.16***	39.83 ± 1.22***	36.33 ± 1.22***
Peroxidase (U/g)	Group C	41.33 ± 1.43	44.0 ± 1.93	47.0 ± 1.69
	Group F	47.83 ± 2.01	50.66 ± 2.1	53.0 ± 1.61
	Group T	54.33 ± 1.89**	57.16 ± 1.66**	58.66 ± 3.08**
SOD (U/g)	Group C	9.0 ± 0.85	9.83 ± 0.7	11.0 ± 0.85
	Group F	13.16 ± 0.79	13.16 ± 1.07	14.17 ± 0.95
	Group T	14.16 ± 1.07**	15.33 ± 1.47**	16.83 ± 1.19**

Data are expressed as mean ± SE (N = 6), P > 0.05 insignificant; \*P < 0.05 significant; \*\*P < 0.01 highly significant; \*\*\*P < 0.001 very highly significant.

**Table 4** Shows the oxidative stress parameters in the newborns cerebellum at D7, D14 and D21.

Parameter	Group/Time	D7	D14	D21
TBARS (nmol/100 mg)	Group C	21.83 ± 1.19	24.00 ± 1.36	26.83 ± 1.81
	Group F	25.5 ± 1.60	30.33 ± 1.45	36.0 ± 1.98
	Group T	38.16 ± 1.62**	35.33 ± 2.07**	37.16 ± 2.72**
GSH (nmol/g)	Group C	46.33 ± 2.12	46.66 ± 1.43	51.33 ± 1.35
	Group F	32.0 ± 1.82***	34.5 ± 1.72***	39.16 ± 2.21**
	Group T	31.33 ± 1.49***	31.33 ± 1.20***	36.16 ± 1.51**
Peroxidase (U/g)	Group C	107.16 ± 2.7	116.16 ± 2.88	121.66 ± 2.64
	Group F	118.66 ± 2.64*	128.0 ± 3.05*	136.16 ± 2.73
	Group T	128.83 ± 3.86***	137.5 ± 3.9***	143.33 ± 2.14***
SOD (U/g)	Group C	10.5 ± 0.76	11.5 ± 0.92	13.33 ± 0.91
	Group F	13.33 ± 0.84*	17.0 ± 1.06*	20.66 ± 1.22
	Group T	16.5 ± 0.76***	21.0 ± 0.89***	22.33 ± 1.70***

Data are expressed as mean ± SE (N = 6), P > 0.05 insignificant; \*P < 0.05 significant; \*\*P < 0.01 highly significant; \*\*\*P < 0.001 very highly significant.

**Table 5** Shows the oxidative stress parameters in the newborns medulla oblongata at D7, D14 and D21.

Parameter	Group/Time	D7	D14	D21
TBARS (nmol/100 mg)	Group C	21.16 ± 1.99	23.0 ± 1.31	22.66 ± 1.22
	Group F	25.5 ± 2.12	25.33 ± 1.72**	27.66 ± 0.88
	Group T	29.0 ± 2.08	32.0 ± 1.23**	31.5 ± 1.17***
GSH (nmol/g)	Group C	85.33 ± 1.89	85.16 ± 1.92	90.0 ± 2.68
	Group F	73.0 ± 1.98**	78 ± 1.46**	74.83 ± 1.86***
	Group T	65.66 ± 2.01***	66 ± 1.48**	73.5 ± 1.89***
Peroxidase (U/g)	Group C	69.83 ± 2.04	68.83 ± 1.53	70.83 ± 2.08
	Group F	77.33 ± 1.70	77.16 ± 1.35**	84.16 ± 1.32
	Group T	80.33 ± 2.36**	84.83 ± 2.35***	87.5 ± 2.93***
SOD (U/g)	Group C	22.0 ± 1.43	21.33 ± 1.05	21.83 ± 1.35
	Group F	30.83 ± 1.24	26.33 ± 1.74	25.83 ± 2.05*
	Group T	32.0 ± 1.12***	29.66 ± 1.20**	31.83 ± 1.23**

Data are expressed as mean ± SE (N = 6), P > 0.05 insignificant; \*P < 0.05 significant; \*\*P < 0.01 highly significant; \*\*\*P < 0.001 very highly significant.

At D7, 14 and 21, compared to group C, the cerebellar TBARS elevation in group F was insignificant (P > 0.05) but was highly significant (P < 0.01) in group T. The cerebel-

lar GSH depletion, meanwhile, was similar to the cerebral GSH except the difference at D21 between Groups was highly significant (P < 0.01). SOD and peroxidase elevation in group

**Table 6** Shows the oxidative stress parameters in the newborns liver at D7, D14 and D21.

Parameter	Group/Time	D7	D14	D21
TBARS (nmol/100 mg)	Group C	44.83 ± 1.77	39.5 ± 0.99	34.33 ± 1.49
	Group F	55.33 ± 1.33	48.33 ± 1.86	41.16 ± 1.57
	Group T	57.16 ± 5.38	52.0 ± 1.92***	43.38 ± 1.53***
GSH (nmol/g)	Group C	73.22 ± 2.20	7.33 ± 2.07	66.33 ± 1.45
	Group F	56.5 ± 1.4***	55 ± 1.65***	54.0 ± 1.36***
	Group T	54.83 ± 1.88***	49.33 ± 2.17***	50.83 ± 2.45***
Peroxidase (U/g)	Group C	62.0 ± 2.55	52.5 ± 1.31	49.83 ± 0.94
	Group F	72.86 ± 2.48**	62.16 ± 2.35**	55.83 ± 1.57**
	Group T	83.0 ± 1.57***	73.33 ± 1.68***	70.83 ± 1.77***
SOD (U/g)	Group C	23.66 ± 1.2	20.33 ± 1.43	20.16 ± 1.77
	Group F	34.66 ± 1.45	30.16 ± 2.19	25.16 ± 1.47
	Group T	35.16 ± 2.04***	33.0 ± 1.93***	32.33 ± 2.33**

Data are expressed as mean ± SE (N = 6),  $P > 0.05$  insignificant; \* $P < 0.05$  significant; \*\* $P < 0.01$  highly significant; \*\*\* $P < 0.001$  very highly significant.

**Table 7** Shows the oxidative stress parameters in the newborns kidney at D7, D14 and D21.

Parameter	Group/Time	D7	D14	D21
TBARS (nmol/100 mg)	Group C	28.83 ± 1.07	27.0 ± 1.48	26.83 ± 1.77
	Group F	32.66 ± 1.33	31.83 ± 1.42	33.16 ± 1.01
	Group T	34.0 ± 1.46***	34.83 ± 1.49***	36.0 ± 1.82***
GSH (nmol/g)	Group C	50.93 ± 2.02	52.5 ± 1.33	52.66 ± 1.08
	Group F	38.5 ± 2.01**	45.38 ± 1.81	41.83 ± 2.54***
	Group T	37.0 ± 1.69***	40.5 ± 2.27***	36.33 ± 1.22***
Peroxidase (U/g)	Group C	52.0 ± 1.46	46.16 ± 1.92	42.83 ± 1.70
	Group F	62.16 ± 1.99	55.16 ± 1.47**	45.0 ± 1.39*
	Group T	66.66 ± 1.7***	66.83 ± 2.08**	60.33 ± 1.33***
SOD (U/g)	Group C	20.5 ± 1.47	18.16 ± 1.64	18.5 ± 1.23
	Group F	34.16 ± 1.32	31.33 ± 0.88	23.5 ± 1.82
	Group T	34.0 ± 2.3***	32.0 ± 1.69***	27.5 ± 1.76**

Data are expressed as mean ± SE (N = 6),  $P > 0.05$  insignificant; \* $P < 0.05$  significant; \*\* $P < 0.01$  highly significant; \*\*\* $P < 0.001$  very highly significant.

F was significant at D7 as well as D14 and insignificant at D21, while in group T, it was very highly significant ( $P < 0.001$ ) at all ages compared to group C (Table 4).

Table 5 displays the disruption in the medulla oblongata oxidative stress parameters, with TBARS, peroxidase and SOD showing significant elevations ( $P < 0.05$ ) at different ages in the pups from groups F and T while GSH showed highly significant depletion ( $P < 0.01$ ) at all ages in these groups compared to group C pups.

The liver TBARS and SOD showed similar elevation conditions; the extent of the increase was insignificant ( $P > 0.05$ ) between groups C and F but was very highly significant ( $P < 0.001$ ) between groups C and T at all ages; except lipid peroxidation at D7 was insignificantly different ( $P > 0.05$ ) between groups C and T (Table 6). At D7, 14 and 21, the liver GSH decreased in groups F and T, was very highly significant ( $P < 0.001$ ). At all ages, the peroxidase enzyme levels in the pups' livers were highly significantly ( $P < 0.01$ ) increased in group F and very highly significantly ( $P < 0.001$ ) increased in group T (Table 6) compared to group C.

Table 7 reports the kidney oxidative stress values, with all the parameters showing very highly significant changes in group T (elevations in TABRS, peroxidase and SOD but

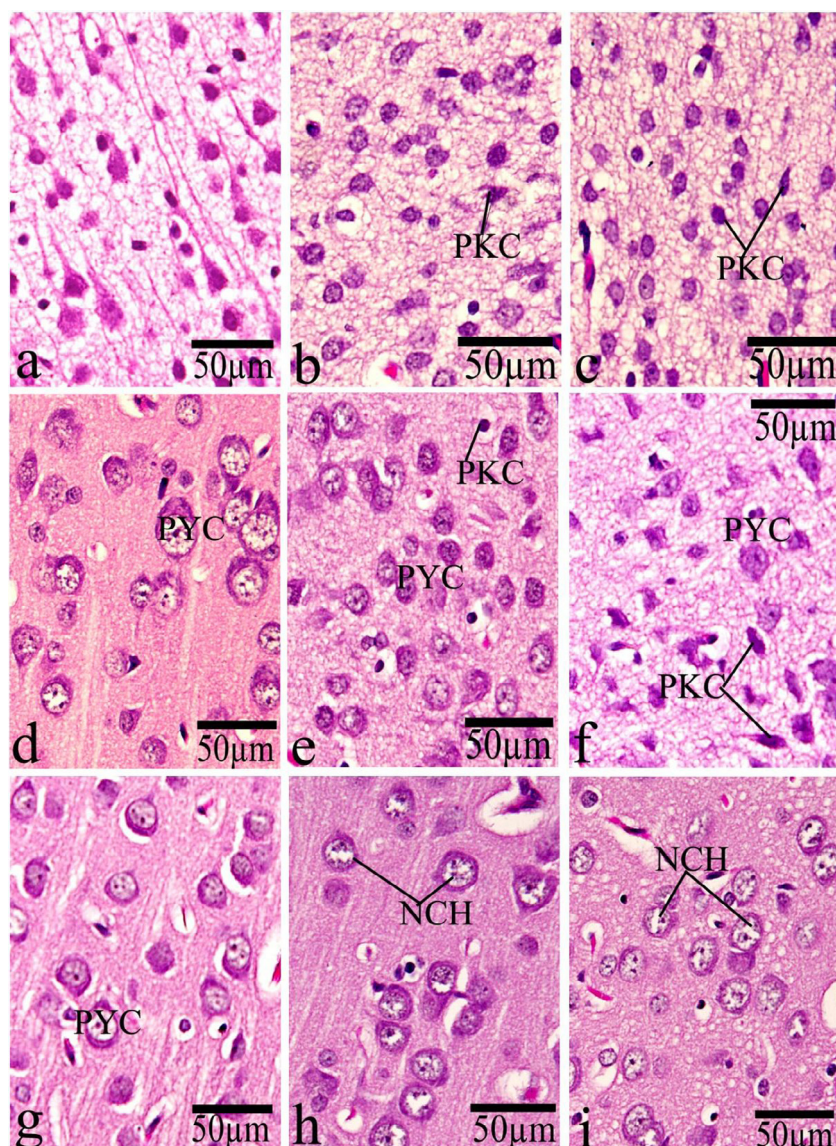
depletion in GSH), while, in group F, these changes were highly significant ( $P < 0.01$ ) at some ages and insignificant at others ages ( $P > 0.05$ ). In group F, kidney GSH at D14 showed insignificant ( $P > 0.05$ ) depletion compared to the GSH of other organs of group F pups (Table 7).

#### 3.4. Histological Studies

At D7, the cerebral histological sections of the groups' pups showed undifferentiated pyramidal neurons. At D14 and 21 well-developed pyramidal neurons (PYC) were detected in the three groups but the size of the neurons in group C was larger than those in groups F and T. Although fully matured pyramidal neurons appeared at D21, the differentiation of cerebral cortex into layers was undetectable (Fig. 4). The pathological changes evident in groups F and T were the neuronal loss by pyknosis (PKC) at D7 and 14 and chromatolysis (NCH) at D21. The severity of the chromatolysis in group T was more than in group F (Fig. 4e, f, h and i).

From D7 to D21, the cerebellum in the pups consisted of white and grey matter. The cerebellum developed as folds and the grey matter in each fold appeared in four layers. These layers are, from inside to outside, the deep stratum granulosum





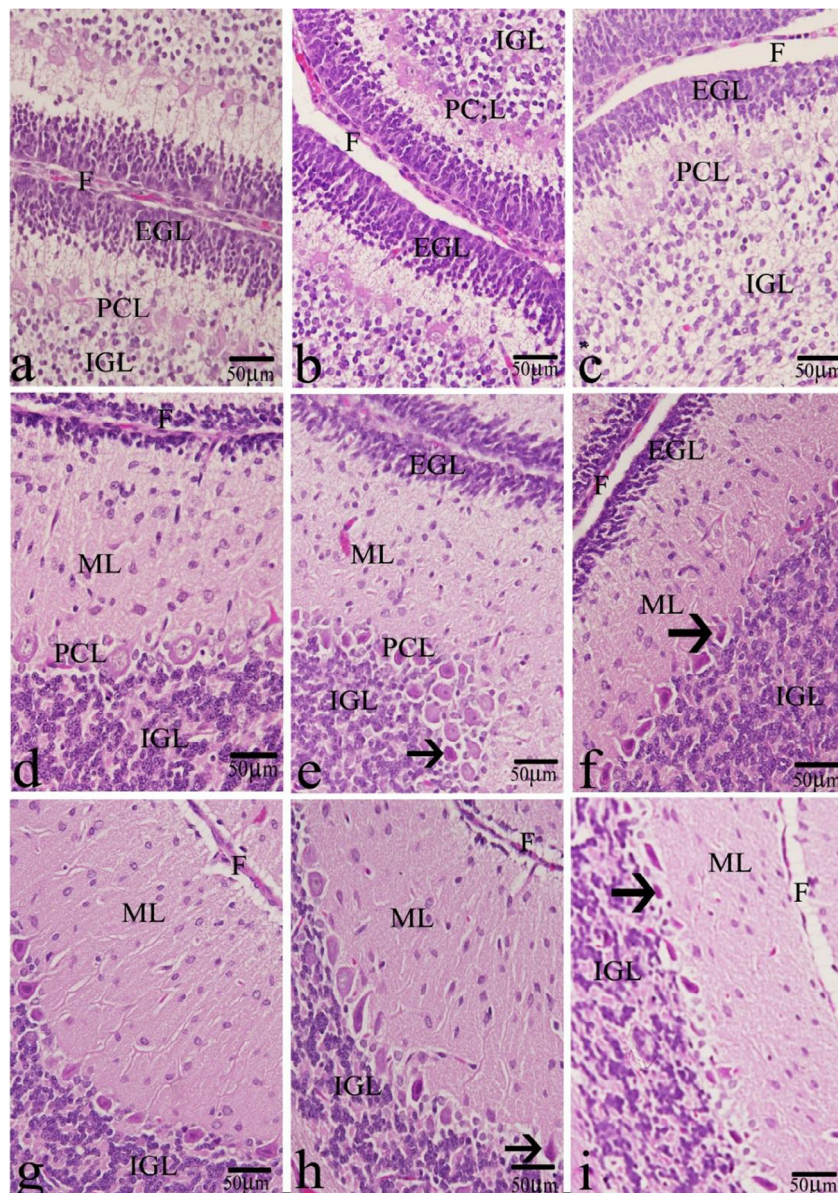
**Figure 4** Sagittal sections in the cerebral cortex show the neurocyte chromatolysis (NCH), pyramidal cells distribution (PYC) and pyknosis (PKC). (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50 µm. (H&E).

(the deep internal granular layer), the Purkinje (ganglionic) cell layer, the stratum moleculare with a few neurons (molecular layer) and the temporary superficial external granular layer, which disappeared by D21. The cerebellar folds were separated by transverse fissures which moved vertically on the anterior and posterior axes of the animal brain (Fig. 5). The detected abnormalities in the pups' cerebella in groups F and T were wide fissures, with the Purkinje cells arranged in more than one row, degenerated Purkinje cells and a narrow molecular layer (Fig. 5b, c, e, f, h and i). In groups F and T, many of the living Purkinje cell appeared to possess an abnormal spindle shape.

At D7, the medulla oblongata neurons appeared to be well developed and large in group C but were small and abnormal in shape in groups F and T (Fig. 6a–c). At D14 and 21, in group C, the medulla neurons appeared more mature and developed while in groups F and T, the rate of neuronal loss

by pyknosis (PKC) and chromatolysis (NCH) had increased (Fig. 6d–i). At D7, the brachial spinal cord was examined because the current spinal mediated sensorimotor reflexes for the pups' fore-limbs developed in the first week of age. In group C, the histological sections of the brachial spinal cord showed well-matured motor neurons which mediate the reflex expressions to maturation (Fig. 7A). In groups F and T, however, motor neuronal loss by pyknosis (PKC) was observed (Fig. 7b and c).

Fig. 8 illustrates the haematoxylin and eosin (H&E) stained sections showing the hepatic lobules, hepatic sinusoids (HS) and central vein (CV) in the pups from the three groups between D7 and D21. The sections from groups F and T showed fatty droplet deposits (F), congested central veins (CCV), dilated central veins (DCV) and central chromatolysis (Fig. 8b, c, e, f, h and i). In group C, Fig. 8a, d and g displays well-developed normal hepatic lobules. Fig. 9 shows the pups'



**Figure 5** Sagittal sections in the cerebellar cortex showing the Purkinje cell layer (PCL), degenerated Purkinje cell (arrow), external granular layer (EGL), fissure (F), internal granular layer (IGL) and molecular layer (ML). (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50  $\mu$ m. (H&E).

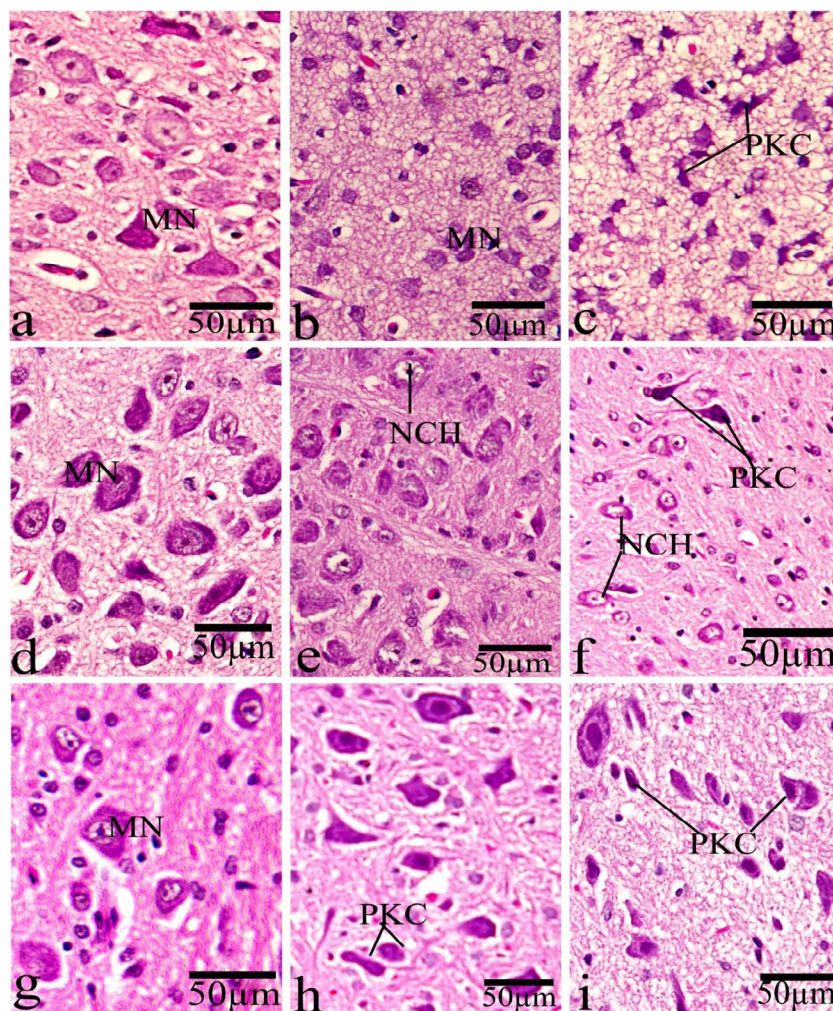
hepatic sections stained by PAS in order to stain carbohydrates flakes in the hepatic sinusoids. The intensity of the purple colour was high in group C, moderate in group F and low in group T, which indicates the relatively high rate of metabolism in group C. The sections in [Figs. 8 and 9](#) show that the rate of hepatic anomalies in group T was more than in group F ([Fig. 9](#)).

The kidney sections in [Fig. 10](#) show the developed pups' glomeruli. At D7, many well-formed glomeruli (G) were observed in groups C and F while there was retardation in the glomeruli development in group T ([Fig. 10a-c](#)). At D14 and 21, many developed glomeruli ( $\rightarrow$ ) and abnormally formed glomeruli ( $\blacktriangleright$ ) were observed in groups F and T ([Fig. 10e, f, h and i](#)).

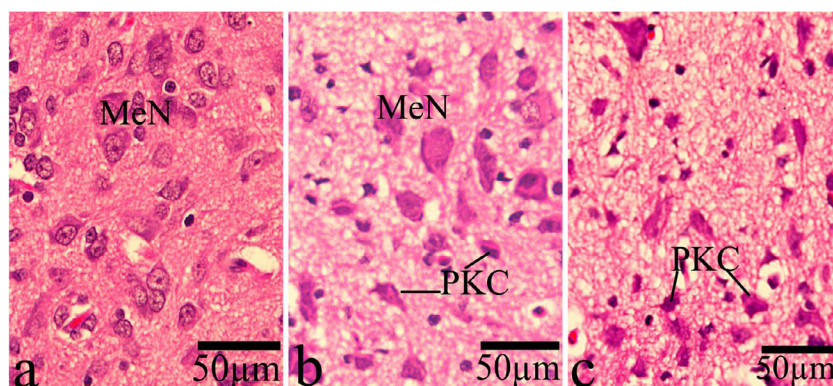
#### 4. Discussion

The current study was designed to show the teratogenic effects of drinking desalinated sea water, whether unfiltered (directly from the tap) or after filtration using an electrochemical water filter. Two consecutive generations were exposed to the water type with the investigations being undertaken on the pups from the 3rd generation. The investigation included the appearance of some external features, body weight development from D1 to D21, ontogenesis of sensorimotor reflexes, behavioural activities at D21, oxidative stress and histological changes in the brain regions of the new-born rats.

The rats were assigned to three groups to show the effect of drinking desalinated seawater for two generations whether



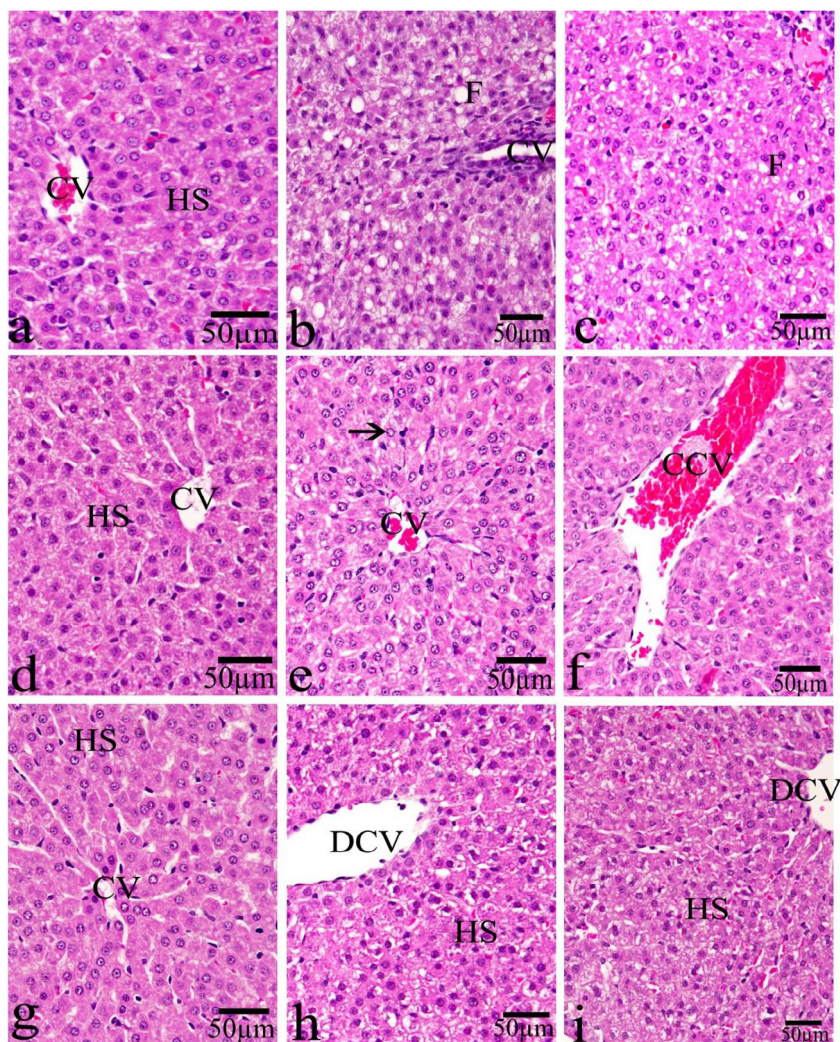
**Figure 6** Sagittal sections in the medulla oblongata show the medullary neurons (MN), neurocyte chromatolysis (NCH) and pyknosis (PKC). (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50 μm. (H&E).



**Figure 7** Transverse sections in the brachial spinal region show motorneurons (MeN), and pyknosis (PKC). (a) group C at D7, (b) group F at D7, (c) group T at D7. Scale bar = 50 μm. (H&E).

directly from the tap (group T) or after filtration using a drinking water filter (group F). The normal group (C) rats drank ideal drinking water (bottled water) which includes ideal element and salt concentration according to WHO (2011). The investigation was done postnatally to the second generation

(their mother's drank the specified type of water throughout the lives). The results in respect to the unfiltered and filtered water showed the concentration of some hazardous elements which pass readily through the placenta due to their solubility in water, becoming distributed in many foetus tissues during



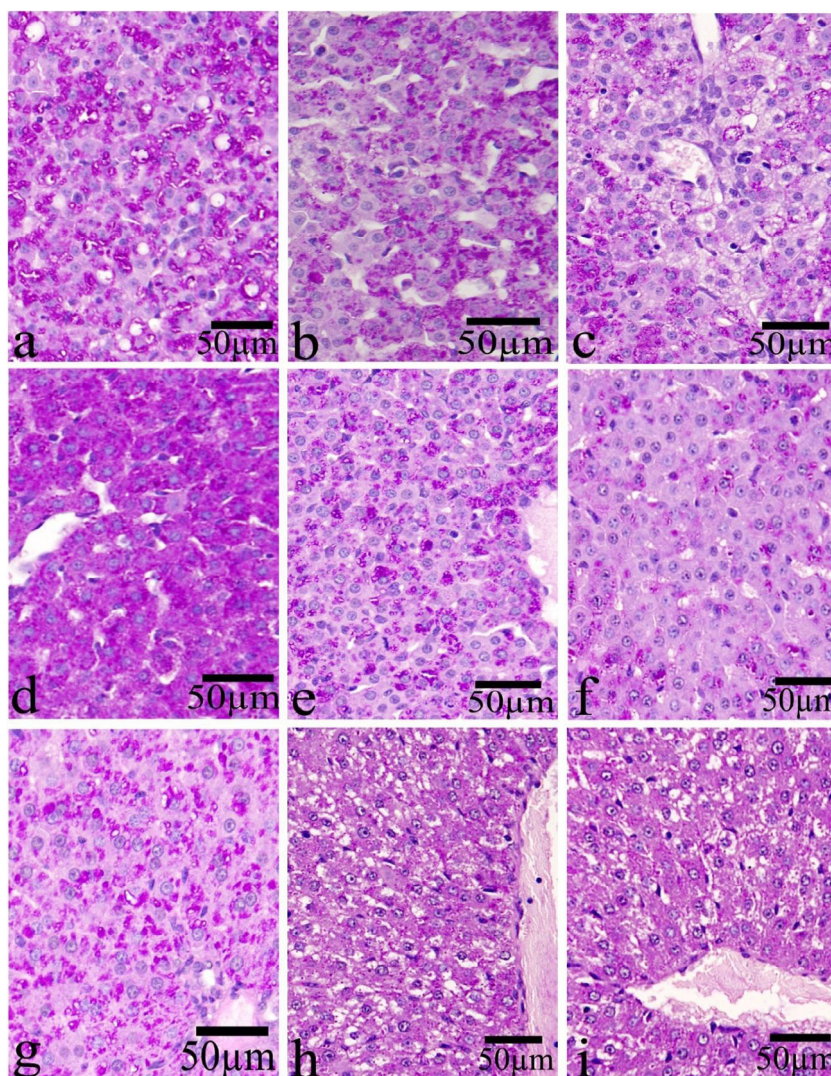
**Figure 8** Sections of liver show central vein (CV), congested central vein (CCV), dilated central vein (DCV), fatty changes (F), hepatic sinusoid (HS) and chromatolysis (arrow). (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50  $\mu$ m. (H&E).

gestation. Also, these elements pass through mother's milk to their pups during lactation (Kawashiro et al., 2008). The pups from groups T and F, therefore, suffered from exposure to excess elements perinatally. The mother's exposure to the excess elements during their life may have induced behavioural changes due, which may have an effect on the pups' lactation index, meaning that the pups from groups F and T may have been exposed to malnutrition (Allam et al., 2016).

Such malnutrition may explain the retardation in the pups' body weight development and the delay in the appearance of some external features such as fur and eye opening (Gold et al., 2000). Malnutrition causes protein and growth deficiencies which lead to developmental retardation according to Garey et al. (2005) who recorded a similar reduction in the weight of new-born rats after their mothers were exposed to toxicity during pregnancy. The main reason for this prenatal weight reduction resulted from intrauterine toxin exposures that led to growth deficiency of the developing foetus (Wise et al., 1995; Tyl et al., 2000). Indeed, according to Wise et al. (1995), pup body weight is the most sensitive indicator of

developmental toxicity. The intrauterine effect of toxins in water on embryos results from the fact that foetuses do not have the enzymes essential to deal with these toxic substances once they have entered the blood supply (Adlard and Dobbing, 1971). The small litter size in groups T and F compared to group C may be due to the exposure to harmful elements perinatally. Allam et al. (2016) reported that heavy metal (such as cadmium) toxicity induces a reduction in litter size reduction due to embryo resorption.

The expression of reflexes during development reflects the state of a certain region in the animal's central nervous system (CNS) and the rate of maturation of this region (Cassidy et al., 1992). In the current pups, the retardation in the maturation of sensorimotor reflexes is a feature of neurotoxicity (Garey et al., 2005). The exposure to toxins leads to prenatal and postnatal pup malnutrition due to alteration in the mother's behaviour (Shaheed et al., 2006). Smart and Dobbing (1971) mentioned that pup malnutrition leads to a delay in sensorimotor reflex development. The current histological results showed the neuronal loss in groups F and T which induced behavioural



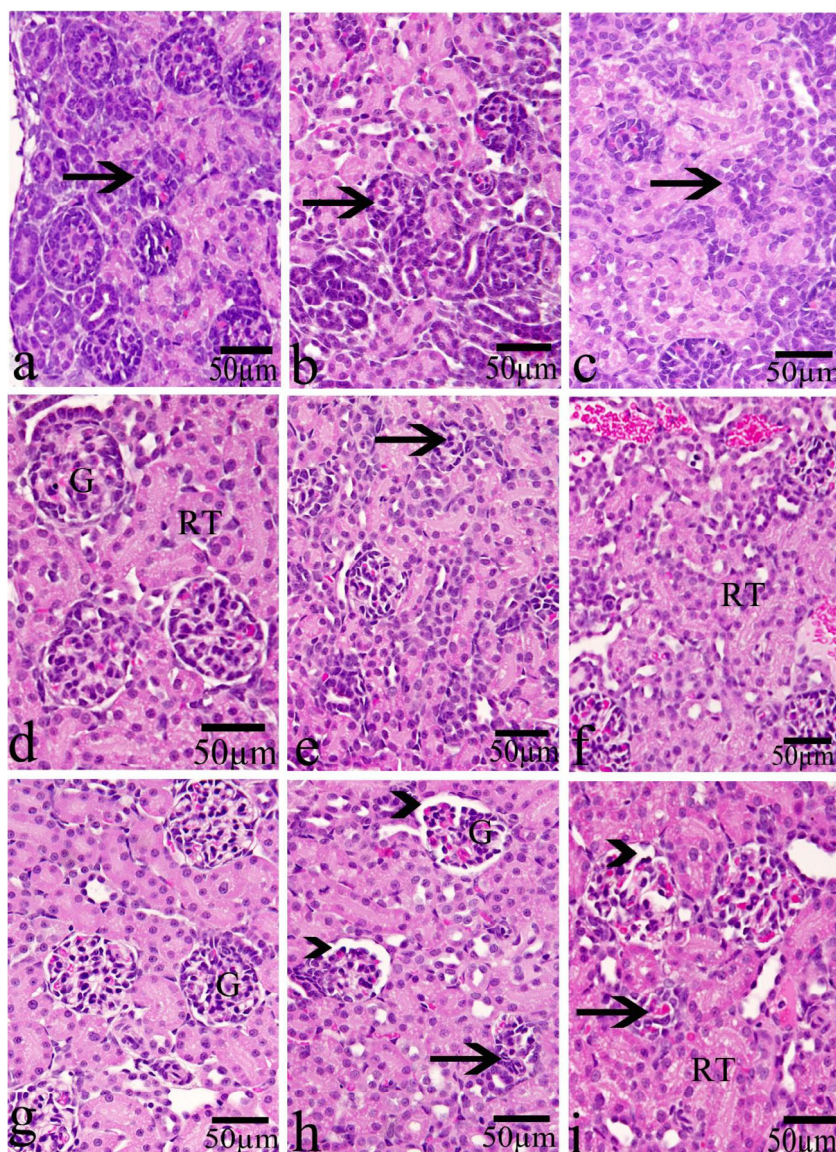
**Figure 9** Sections of liver showing the amount of carbohydrates distributed within the hepatocytes. (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50 µm. (H&E).

anomalies (Crofton et al., 1996). Cassidy et al. (1992) reported that expression of sensorimotor reflexes is mediated by the CNS neurons. The loss of these neurons will therefore affect the expression of the reflexes.

Forelimb grasping, forelimb hopping, surface body righting and chin tactile placing are spinal mediated reflexes (Cassidy et al. 1992). These appeared by postnatal day 1 because some motor neurons (reflexes mediated neurons) differentiate by this age, as seen in the brachial spinal histological sections. Body righting in the air and rota rod activity are cerebellar mediated reflexes, while grip strength, as well as vertical and horizontal pup activities are cerebral and medulla related reflexes (Ajarem and Ahmad, 1991). The regular maturation of reflexes reflects the regular rate of neuronal maturation (Cassidy et al. 1992). Conversely, a delay or abnormalities in the maturation of behavioural reflexes may be due to neuronal loss and aberration in the CNS region which mediates these reflexes (Allam et al., 2013). Excess ingestion of harmful elements leads to neuronal loss and malnutrition that delays the maturation of the

behavioural reflexes (Smart and Dobbing, 1971). Also, it impairs synapse function and neuronal connection (Agrawal and Squibb, 1981). It was obvious from the activity and grip strength results in this study that harmful elements in desalinated seawater cause weakness, ataxia and malformation in the activity of pups.

The present histological results support the above hypothesis by showing that drinking desalinated seawater, whether after or before filtration, produces neuronal loss in the CNS regions (cerebrum, cerebellum, medulla oblongata, spinal cord), abnormalities and retardation in the kidney and hepatic tissue and metabolism aberrations in pups. These deleterious changes in the tissues of pups in groups T and F may result from disturbances in the oxidative stress parameters (Allam et al., 2010, 2011). One of the cerebellar aberrations in the pups of group T is the narrow molecular layer. This confirms the high neuronal loss and tissue damage in the pups of group T because this layer depends on the numbers and size of its neurons (Bondok et al., 1991).



**Figure 10** Sagittal sections in the newborns kidney show glomerulus (G), renal tubules (RT), abnormal glomerulus (arrow head) and developed glomerulus (arrow). (a) group C at D7, (b) group F at D7, (c) group T at D7, (d) group C at D14, (e) group F at D14, (f) group T at D14, (g) group C at D21, (h) group F at D21, (i) group T at D21. Scale bar = 50  $\mu\text{m}$ . (H&E).

The pups from groups F and T, especially group T, were exposed to excess doses of harmful elements perinatally through the mother's drinking water. The investigated organs of Group T and F pups, displayed a marked elevation in oxidative stress, and a depletion of antioxidants, compared to group C. Specifically, this was shown in the significant increase in TBARS (lipid peroxidation), peroxidase and SOD, and the significant depletion of GSH in groups T and F. It has been reported that lipid peroxidation and oxidative stress enzymes increase after acute and chronic toxicity, and glutathione is one of the most important compounds for the preservation of cell integrity, due to its oxidative stress reducing properties (Conklin, 2000).

Many studies into oxidative stress have observed that a reduction in GSH induces the elevation of lipid peroxidation. GSH is, therefore, an important indicator and biomarker of oxidative stress (Recknagel et al., 1991; Allam et al., 2010).

Abu-Taweel et al. (2013) reported that metal exposure reduces the content of GSH and increases SOD and peroxidase activities. Elevations of SOD and peroxidase activities may be due to the presence of free radicals generated by the effects of certain toxins (Allam et al., 2016). Peroxidases and SOD are considered to be among the most important antioxidant enzymes that constitute a mutually supportive defence team against free radicals such as ROS, as mentioned by Allam et al. (2010). Such oxidative modifications affect several cell metabolic reactions, functions and gene expression, which in turn can cause other pathological conditions, as shown in the current study and mentioned by Young and Woodside (2001). Oxidative stress leads to neuronal damage in several brain regions and hepatic and renal tissue damage (Allam et al., 2012). For example, neuronal loss in the cerebrum impairs an animal's memory (Abu-Taweel et al., 2012), neuronal loss in the cerebellum can have an effect on balance and coordination

(Allam et al., 2011), and neuronal loss in the medulla oblongata and spinal cord can affect the physical activity of mice (Yue et al., 2014).

In conclusion, mothers drinking filtered or unfiltered desalinated seawater for a long time experienced oxidative stress causing tissue damage in the brain, liver and kidney of their pups. Disturbances in the behaviour and developmental patterns of these pups were also recorded. It may be suggested that the excess concentrations of harmful elements present in desalinated seawater produces these teratogenic effects.

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