ORIGINAL ARTICLE

Effects of the perinatal exposure of Gum Arabic on the development, behavior and biochemical parameters of mice offspring

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Abstract The effects of the perinatal oral exposure to Gum Arabic (GA) on mice offspring was examined. GA was added to the drinking water of pregnant female Swiss–Webster strain mice at doses of 1 and 4 g/kg body weight, starting from the first day of pregnancy. The treatment continued until the fifteenth day after delivery, after which mothers were switched to plain tap water. A number of tests were carried out on offspring starting one day after birth and extending up to postnatal day 30 (PD30). Pups showed a reduced gain of body weight and delayed opening of the eyes in comparison to the control group and only pups exposed to 1 g/kg body weight GA had a faster appearance of hair. Sensory motor reflex tests carried out during the weaning period (from day of birth to PD21) showed enhanced motor reflexes in pups exposed to GA. During the adolescent period (from PD22 to PD30), offspring showed dose-dependent enhanced motor activity (on PD22), reduced anxiety and fear (on PD27) and slightly enhanced memory and learning abilities (on PD30). Biochemical tests of a number of blood parameters were conducted during and after the weaning period (on PD15 and PD30, respectively). Our results indicated that GA might have a hypoglycemic and a beneficial effect on red and white blood cell counts. This study gives a first insight on the effect of GA consumption on offspring, providing a starting point for further studies.
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1. Introduction

Gum Arabic (GA) is an edible, gummy, dried exudate rich in non-viscous soluble fibers taken from the mature trees of *Acacia senegal* and *Acacia seyal* (Yebeyen et al., 2009). GA has various industrial uses. In the food industry, GA functions as a stabilizer, emulsifier and thickening agent (e.g. in marshmallows, gummy candies and soft drinks syrup). It also has

applications in the pottery, textile, lithography, cosmetic and pharmaceutical industries (Verbeken et al., 2003). Medically, GA has been used by humans, externally to cover inflamed surfaces and, internally to treat inflammatory conditions of the intestinal mucosa (Ali et al., 2009; Gamal el-din et al., 2003). In research, even though GA is widely used as a vehicle for drugs in physiological and pharmacological experiments and assumed to be an "inert" substance, recent studies have indicated that GA possesses some potential antioxidant, nephroprotectant, metabolic, digestive, antianemic and cardiovascular properties (Ali et al., 2009; Gamal el-din et al., 2003; Mohamed et al., 2015; Rehman et al., 2001).

A number of factors have significant effects on the development of offspring in the gestational period as well as in early life. For instance, the cross fostering studies of mice derived from two distinct strains suggest that the prenatal environment accounts for 61–96% of the variance in body weight gain in male and 35–92% in female offspring (Kurniato et al., 1998). Also, it has been suggested that early life nutritional exposures, combined with changes in lifestyle in adult life, can result in increased risk of chronic diseases (Christian and Stewart, 2010). Hence, the micronutrient status in fetal and early life may alter organ growth and function, metabolism and vasculature (Van Mierlo et al., 2006). The perinatal exposure to a number of different compounds has been a topic of research for many years, but the influence of GA during the gestational and postnatal periods is yet to be explored.

The aim of this study was to investigate the effects of the perinatal exposure to GA on various developmental, behavioral and biochemical factors in mice offspring. The absence of data available on the perinatal effects of this widely used component highlighted the need for this investigation. To the best of our knowledge, this is the first study to be conducted that examines the effects of oral administration of GA on offspring.

2. Materials and methods

2.1. Experimental animals

Swiss–Webster strain mice (10 males and 30 females of 8–9 weeks old) were housed in opaque plastic cages measuring $30 \times 12 \times 11$ cm, in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. Three females and one male were put in each cage. Animals were kept under reversed lighting conditions with white lights left on from 22:30 to 10:30 local time and the ambient temperature was regulated between 18 and 22 °C. Male mice were removed from the cages once pregnancy was confirmed by the appearance of a vaginal plug and this was considered to be day one of pregnancy. Food (Pilsbury's Diet) and water were available *ad libitum* unless otherwise stated (Ajarem et al., 2009). All experimental procedures were carried out in accordance with the institutional guidelines for the care and use of laboratory animals and in full compliance with the instructions of the local animal care and ethics committee.

2.2. Experimental design

A. senegal in powder form was the type of GA used in this investigation (Dar Savana Ltd., Khartoum, Sudan). Pregnant females were subjected to experimental testing starting day one of pregnancy. GA was dissolved in the drinking water to administer the required doses to the treated group of animals. The water containing the GA doses formed the only source of drinking water to the treated group of animals.

Pregnant mice were divided into 3 experimental groups of ten. The first group was given plain tap water and served as the control group. The second and third groups were administered 1 g/kg and 4 g/kg body weight of GA/day respectively, dissolved in plain tap water. According to Ajarem et al., the average consumption of water per day by pregnant or normal adult female mice is 30 mL (Ajarem et al., 2009). The two doses of GA used in the present study were dissolved in the drinking water in such a way that the desired doses were administered to the treated groups within 24 h. The water consumed by the three groups was measured daily and fresh GA doses were prepared and replaced every day. Treatment of mothers started the day of pregnancy and continued until postnatal day (PD15), after which mothers were switched to plain water. On the day of birth (PD0), the pups were culled to 8 per dam and left with their mothers until PD22.

During this weaning period, three pups from each litter were randomly selected and color marked. In total, 21 pups belonging to seven litters from each group were subjected to various behavioral and biochemical tests (described below). All observations were recorded from the same three color marked pups of each litter on PD1 and repeated every other day until PD21. For statistical purposes, the mean of all three color marked pups in each litter was considered as a single score. Hence, seven replicates from each group were considered for all the studies.

2.3. Physical assessment

Starting one day after birth (PD1), physical development in offspring was assessed through body weight, opening of the eyes and appearance of hair and continued through the weaning period (until PD21). Pups were weighed every other day and the day on which hair fuzz appeared and pups opened their eyes was recorded.

2.4. Assessment of neuromotor reflexes

The following tests were conducted on offspring during the weaning period (PD1-PD20) to evaluate the maturation of neuromotor reflexes.

2.4.1. Righting reflex
The time needed for a pup, placed on its back, to turn over and place all four paws on the surface was recorded. An upper limit of 2 min was set for this test.

2.4.2. Rotating reflex
The surface used to measure the rotating reflex was the same as that used for the righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads facing down. The time that elapsed until the pup rotated its body through 180° geonegatively and faced its head upward was recorded as the rotating time. The upper limit of this test was also set at 2 min.

2.4.3. Cliff avoidance

Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken by the pup to back away and turn from the cliff was recorded. Again, an upper limit of 2 min was chosen. A latency of 2 min was attributed when the animal fell from the "cliff".

2.5. Behavioral assessment

Offspring were weaned on PD21 and kept in groups of two or three, for 9 days. The following tests were conducted.

2.5.1. Locomotory (open-field) activity test

After the weaning period (on PD22), 10 pups from each group were subjected to locomotor activity tests in an experimental wooden arena of square shape, measuring $80 \times 80 \times 30$ cm and the floor was divided into 64 squares of equal size. Various behavioral elements like numbers of squares crossed, wall rears, rears, washes, durations of locomotion and immobility were observed. The visual observations in the arena lasted 300 s for each animal.

2.5.2. Anxiety test (Plus-Maze)

Anxiety was measured in the weaned mouse pups at PD27 using an elevated plus maze apparatus consisting of two open (30×5 cm) and two closed arms ($30 \times 5 \times 15$ cm) extending from a central square (5×5 cm), arranged in a way that the arms of the same type are opposite to each other. The floor of the entire plus maze was made of black Plexiglas and the walls of the enclosed arms were made of clear Plexiglas (Gupta et al., 2013). The apparatus was elevated to a height of 38.5 cm above the floor. Testing was conducted in a dimly illuminated (ca 8 lux) room. Each mouse was placed in the center of the plus-maze facing one of the open arms and allowed to move freely for 5 min. During the 5-min test, the number of entries into each type of arm and the time spent in each arm were recorded by an observer seated 50 cm away from the center of the maze. A mouse was taken to have entered an arm when all four legs were on the arm. At the end of each trial, the number of entries into the open arms was expressed as a percentage of the total number of arm entries. The time spent on the open arms was also expressed as a percentage of the time spent on both the open and closed arms. The maze was cleaned after each trial with a 5% Dettol solution (Reckitt and Coleman, Ltd., Hull) to minimize potential effects of odors from previous occupants.

2.5.3. Learning and memory test (T-maze)

The T-maze is an apparatus made of wood. It has three arms (main arm with a removable partition, left arm containing the food reward and right arm that remains empty without food). The apparatus is elevated to a height of 30 cm above the floor. The mice were made to fast for 24 h (received water only) before starting the T-maze test (Wenk, 2001). At PD30, each fasting mouse was placed at the start point in the main arm then the partition was removed, allowing the mouse to move freely for 120 s. After 3 h, the test was repeated for each mouse and the latency time to reach food, time durations spent in food arm and empty arm, duration of stay in main arm, and number of entries into food arm, empty arm and main arm were recorded.

2.6. Biochemical tests

2.6.1. Collection of blood samples

At PD15 and upon completing all of the above mentioned tests (at PD30), blood samples were collected from the color marked pups in each group under light anesthesia with ether, from the retro-orbital sinus plexuses of their eyes with the help of capillary tubes.

2.6.2. Analysis of blood samples

Blood samples from each group were subjected to analysis of blood parameters namely, total red blood count, total white blood count, total blood platelet count, glucose level, cholesterol level and hemoglobin content using the automated parameter hematology analyzer (T 450, USA).

2.7. Statistical analysis

Data of morphological developments, sensory motor reflexes and biochemical analyses were compared within the experimental groups by the analysis of variance (ANOVA) using the SPSS computer program, and were subsequently analyzed by Student's t-test (Yamane, 1973). Data of the locomotory testing were compared within the experimental groups by the analysis of variance (ANOVA) and subsequently were analyzed using Mann–Whitney U-tests (Sokal and Rohlfe, 1981).

3. Results

3.1. Physical assessment

At birth, no significant differences were seen in the weight of offspring between the control group and treated groups (1 g and 4 g/kg of body weight) (Fig. 1). After PD9 and extending up to PD21, a significant difference in weight was noticed between the control group (11 g) and the group treated with 4 g/kg of body weight GA (8 g). Pups that were administered GA showed reduced growth during the weaning period, with lower body weights recorded as the dose of GA increased.

With regard to other physical landmarks, pups exposed to 1 g/kg body weight GA exhibited a slightly faster appearance of body fuzz than the control group and the 4 g/kg group. However, the number of days taken for pups to open their eyes showed a significant difference between all three test groups where the two groups treated with 1 g/kg and 4 g/kg body weight GA showed delayed responses (15 and 16 days, respectively) in comparison to the control group (13 days).

3.2. Assessment of neuromotor reflexes

The results of the three tests conducted to assess the development of the neuromotor reflexes of the mice offspring are presented in Fig. 2. With regard to the testing of the righting and rotating reflexes, mice exposed to higher doses of GA (4 g/kg body weight) showed the fastest responses. Mice that were given 1 g/kg body weight GA showed slightly slower responses than the 4 g/kg test group, but faster than the
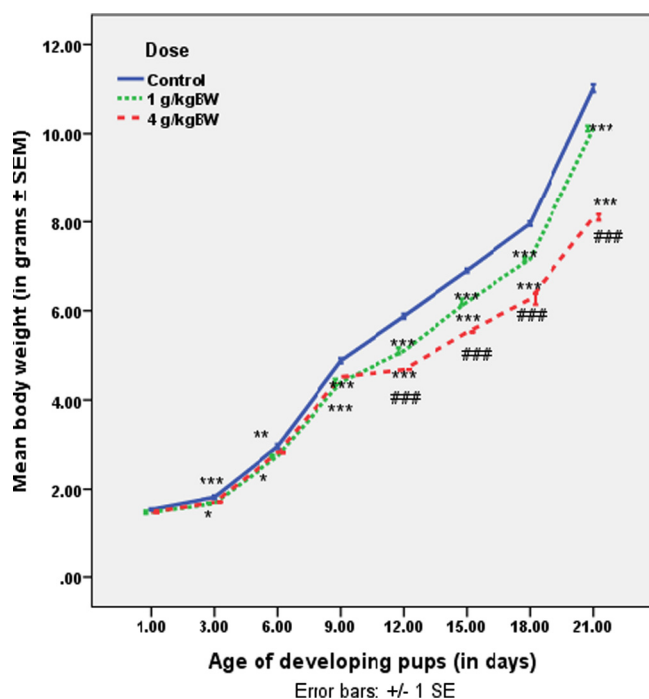
Figure 1 Effects of perinatal exposure to Gum Arabic on the body weight of mice offspring.

control group which were not exposed to GA. As for the cliff avoidance testing, pups exposed to 1 and 4 g/kg body weight GA presented faster response times than the control group, with the 1 g/kg category showing the most rapid response time.

3.3. Behavioral assessment

The Locomotory (Open-Field) Activity test (Table 1) done on PD22, showed that perinatal GA exposure had a significant inhibitory effect on the number of squares crossed in a dose-dependent manner as compared to the controls. The only other action that presented a significant difference in results was with the number of body groomings in the 4 g/kg test group, where a sharp increase in the number of times was recorded.

Fear and anxiety testing conducted on weaned mice at PD27 revealed overall lower levels of fear and anxiety in mice that were exposed to GA in comparison to the control mice. Fig. 3 shows the number of entries in the center, closed arm and open arm and the time spent in each part. The mean number of entries in the center and closed arm was significantly lower in the 1 g/kg treated group.

Next, fasting mice were subjected to learning and memory tests using the T-maze at PD30. No significant difference in the duration and number of entries into each arm was recorded between control mice and treated mice (results not shown). However, the latency period to reach the food between each trial was considerably lower in mice exposed to 1 g/kg of body weight GA and slightly lower in the 4 g/kg dose test group in comparison to the control (18 s, 21 s and 23 s respectively).

3.4. Biochemical analysis of blood samples

Blood samples taken at the intervals PD15 and PD30 showed varied results between the control group and treated groups in

all the blood parameters measured (Table 2). An elevated red and white blood cell count as well as an increased hemoglobin concentration in offspring exposed to GA was evident, taking into note the minor difference present among the two groups exposed to the different GA concentrations. At PD15, reduced platelet counts and diminished glucose levels were recorded. GA exposed mice had higher cholesterol levels.

4. Discussion

Gum Arabic is among the safest natural, versatile compounds used today, with no significant toxic or adverse effects ever being associated with its vast applications (Ali et al., 2009). Even though Collins et al. proved that GA had no teratogenic effects on rat fetuses, no recorded data are available regarding the effects of the perinatal exposure of GA on offspring (Collins et al., 1987). The results from the current study provide a preliminary basis concerning the potential results associated with the exposure of pregnant dams to GA. It was evident that this perinatal exposure influenced the rate of morphological maturation and sensory motor reflexes during the weaning period as well as behavioral modifications in the post-weaning period. An alteration in a number of blood indicators in the pups at different developmental stages was also evident. This may possibly be due to the fact that GA was available to the developing fetus in utero as well as in the milk of the lactating mothers during the weaning period as it is well established that significant quantities of compounds given to mothers during pregnancy may be transmitted to offspring in utero and/or during lactation (Martinez et al., 1994; Peters and Tang, 1982). It is therefore possible that the above discussed factors may singly or collectively affect the growing offspring during the weaning period and ultimately produce lasting behavioral effects.

Body weight, appearance of body hair and opening of the eyes serve as reliable indicators of morphological developments in animals. The postnatal depletion in body weight and the delay in the opening of the eyes in the GA exposed pups may be indicative of lasting GA-induced postnatal effects in the pups. The early appearance of body fuzz in the low dose GA treated pups suggests that GA could possibly increase the blood flow to hair follicles. However, more in-depth studies must be conducted to validate this hypothesis.

Prenatal GA administration was also found to affect the neuromotor reflexes in the weaning pups. Faster responses in the righting reflex, rotating reflex and cliff avoidance tests suggest that GA may play a positive role in the autonomic nervous system. It is well-known that GA is rich in Ca^{2+}, K^+ and Mg^{2+} salts that may have enhanced the activation and release of neurotransmitters (Ali et al., 2009). Studies that further explore the neurological effects of GA could prove to be beneficial in the treatment of some neurodegenerative disorders.

Behavioral assessment during the post-weaning period evaluated the prolonged effects of GA in mice offspring in terms of locomotor activity, anxiety and fear levels in addition to cognitive function. A dose-dependent enhanced motor activity without any significant modification in motor coordination was observed which could imply an induction of hyperactivity (Mabunga et al., 2015). In a plus-maze, rodents generally spend more time in the closed arms which was
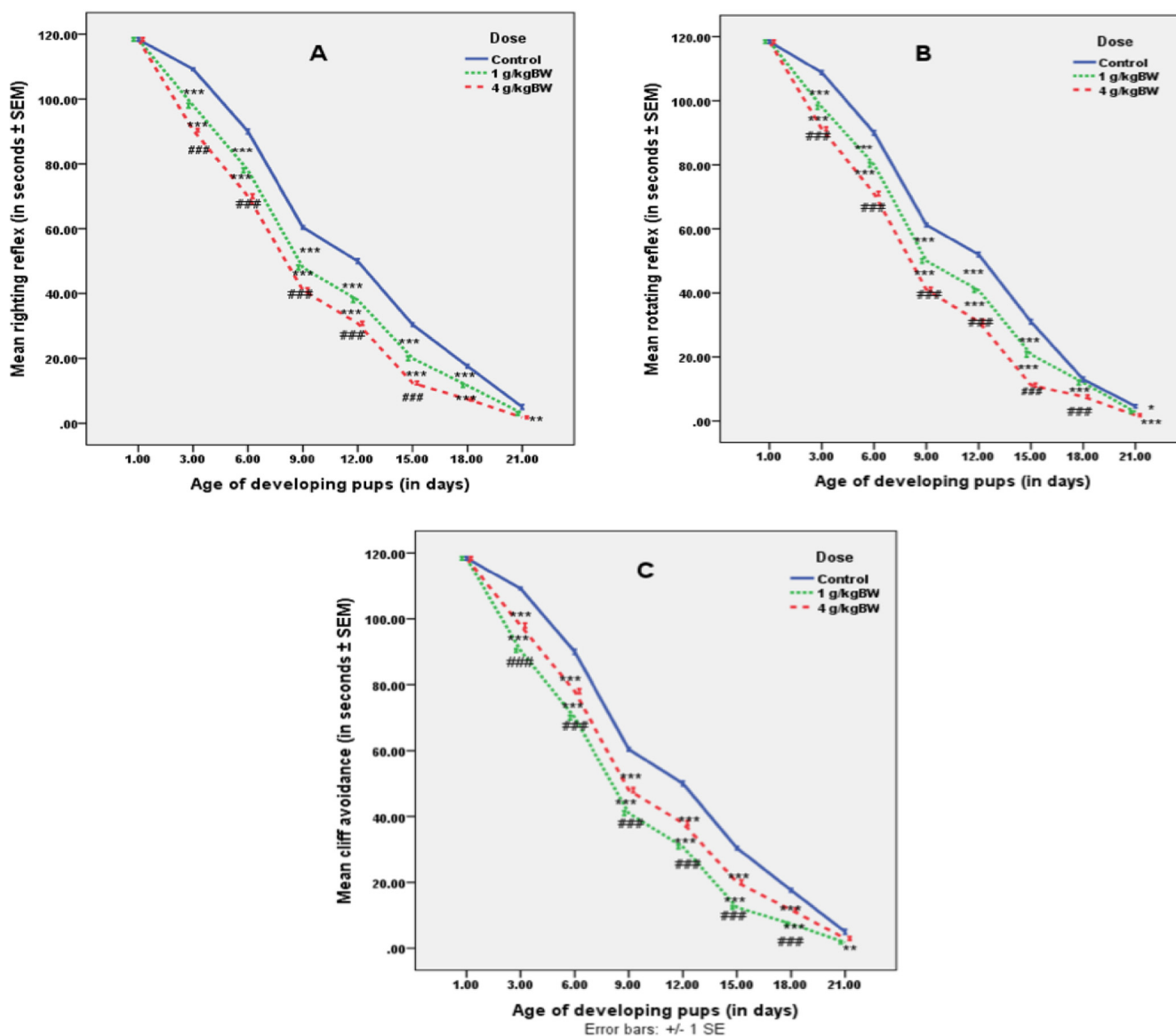
Figure 2 Effect of perinatal Gum Arabic exposure on the mean righting reflex (A), mean rotating reflex (B) and mean cliff avoidance activity (C) on mice offspring. [*** show statistically significant difference at $P < 0.001$ respectively from the control group by students t-test; ### show statistically significant difference at $P < 0.001$ from the 1 and 4 g/kg treated group by student's t-test].

Table 1 Effect of perinatal Gum Arabic exposure on the locomotor activity of mice offspring after weaning (PD22).

Treatment group	Median number (with ranges) of acts and postures					
	Squares crossed	Wall rears	Rears	Body grooming	Locomotion duration (s)	Immobility duration (s)
Control	200 (139–246)	16 (3–30)	0 (0–3)	4 (1–8)	277.5 (209–293)	22.5 (7–91)
1 g/kg	320* (170–410)	20 (10–30)	2.5** (0–5)	2.5 (1–5)	280 (250–290)	20 (7–50)
4 g/kg	350** (250–460)	19 (10–32)	0 (0–3)	10.5** (5–17)	277 (250–293)	23 (7–50)

*,**Show statistically significant difference at $P < 0.05$ and $P < 0.01$ respectively from the control group by U-test.
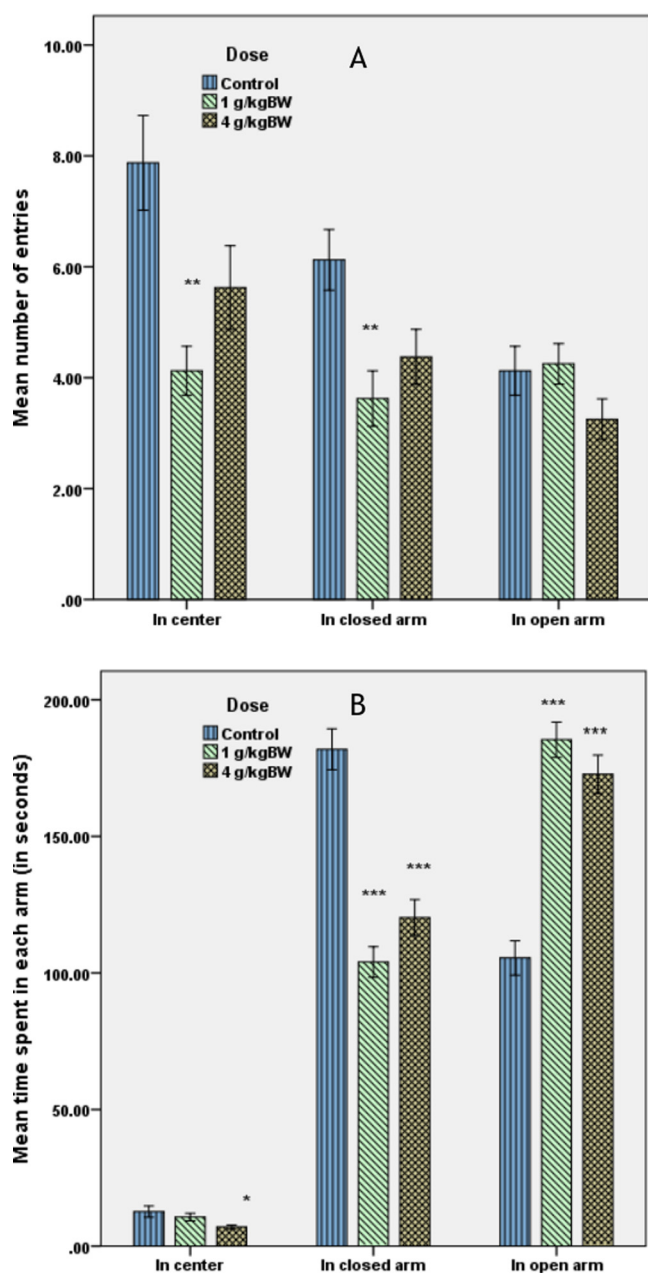
Figure 3 Effects of perinatal exposure to Gum Arabic on the mean number of entries (A) and mean time spent (B) in the center, open and closed arms during the fear and anxiety test in the Plus-Maze. [** show statistically significant difference at $P < 0.01$ from the control group by students t-test; * and *** show statistically significant difference at $P < 0.05$ and $P < 0.001$ respectively from the control group by students t-test].

Table 2 Biochemical analysis of blood samples taken from mice offspring at PD15 and PD30.

Blood parameter measured	Treatment group					
	Control		1 g/kg		4 g/kg	
	PD15	PD30	PD15	PD30	PD15	PD30
Red blood cell count (RBC) ($\times 10^6$ mL)	4.1	6	5.5	8	5.3	7
White blood cell count (WBC) ($\times 10^3$ mL)	3.1	3	4.5	4.4	4.7	4.8
Platelet count ($\times 10^3$ mL)	820	410	580	400	560	380
Hemoglobin (Hb) (g/dL)	8	11	11.2	13	10	12.3
Glucose (mg/dl)	50.3	53	14	45	16	50
Cholesterol (mg/dl)	210	50	260	160	560	380

exposed to GA in comparison to the control mice. The ability of an animal to remember the location of food within the maze is referred to as the reference memory whereas the working or episodic memory refers to the ability of the animal to use the information acquired between the test trials (Rodriguiz and Wetsel, 2006). Thus, GA appears to have improved the working or episodic memory in pups.

In the present study, the variation in a number of blood indicators between treated mice and the control group suggests that GA is not an "inert" substance and may indeed influence a number of biochemical parameters. An increase in the red blood cell count and hemoglobin levels during the weaning and post-weaning periods in both doses of GA administered reveals the important role GA has on erythropoiesis and the possible application in the treatment of anemia. Kaddam et al., in their study on the effect of GA on fetal hemoglobin production in sickle cell anemia patients, came to the conclusion that the oral administration of GA increased the percentage of fetal hemoglobin in sickle cell anemia patients and younger patients were more responsive (Kaddam et al., 2015). In their study, GA was also found to have no effect on hemoglobin concentration and white blood cell counts in contradiction to the present study.

GA has been shown to produce anti-diabetic effects by increasing the release of insulin and to possess significant hypoglycemic effects in healthy rabbits (Hou et al., 2003; Philips and Philips, 2011). Also, the oral administration of GA in diabetic rats led to a significant reduction in blood sugar (Wadood et al., 1989). The current study, showed a sharp decrease in blood glucose during the weaning period (at PD15) where GA was available to pups through the milk of the mother. This hypoglycemic effect diminished through the post-weaning period but was still evident at PD30 where treated pups had lower blood glucose levels than the control group. GA also had a clear effect in increasing blood cholesterol levels. The exact role of GA in modifying cholesterol levels is yet to be confirmed. A recent study conducted on hyperlipidemic patients showed that GA significantly reduced total cholesterol levels in the test group (Mohamed et al., 2015). In contrast, Haskell et al. showed that a GA produced no effect on the lipid profile of healthy patients compared to placebo and the study of Davidson et al. also did not support the hypocholesterolemic effect of the GA/pectin combination on hypercholesterolemic individuals (Davidson et al., 1998; Haskell et al., 1992). The perinatal effect of GA on cholesterol

verified in this study with the control group (Engel et al., 2009). Both treated groups, on the other hand, exhibited longer durations in the open arms than the control group. It has been proved that anxiolytics induce greater open arm exploration measured by the number of entries and/or time spent in the open arm indicating that GA may possess some anxiolytic properties (Walf and Frye, 2007).

Learning and memory tests conducted showed a reduced latency period in reaching the food between each trial in mice

levels was shown to be contradictory to all of the mentioned studies validating the need for further investigations.

In summary, the perinatal exposure of GA had a positive effect on sensory motor reflexes, anxiety and fear levels as well as memory. However, GA hindered growth and development in pups. Blood cells counts and cholesterol levels were increased with GA exposure and glucose levels were dramatically lowered.

Acknowledgements

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