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Allometry of Postnatal Rat Brain Development Prenatally Exposed to Aspirin

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Abstract: A total of 200 rat pups starting from the first week after birth until the fifth week were used to investigate the effects of prenatal exposure to aspirin at doses of 12.5, 25 and 37.5 mg kg^{-1} on the postnatal development of brain parts. Cerebrum and cerebellum length and width were measured in addition to brain and body weights and allometric relationships between these parameters were calculated. The results indicated that all relationships were allometric with noticeable differences between all aspirin treated groups and the control group. These differences indicate that aspirin has an effect on the development of the examined brain parts mainly on the cerebellum length and width.

Key words: Prenatal exposure, NSAID, postnatal development, allometri analysis

INTRODUCTION

Aspirin, or acetylsalicylic acid (ASA), is one of the oldest and most commonly used non-steroidal anti-inflammatory drugs (NSAID) (LaBuda and Fuchs, 2000). These drugs are useful for the treatment of pain, fever and inflammation, as well as for the reduction of platelet aggregation (Craig and Stitzel, 1997). The clinical benefits of NSAIDs are due to their ability to inhibit the production of cyclooxygenase (COX), which is required to produce prostaglandins (Vane and Botting, 1998). Aspirin can be prescribed to pregnant women that have a risk for intra uterine growth retardation (IUGR) and pregnancy induced hypertension (PIH) (Fenster *et al*., 1999). ASA has been proven to cross the placenta and circulate in fetal blood (Wilson *et al*., 1977).

ASA has been demonstrated to have teratogenic effects on animals. A review of literature suggested that exposure to NSAIDs produces a low incidence of cardiovascular defects such as ventricular septal defect (VSD) and midline defects (MDs) such as gastroschisis and omphalocele in rats (Gupta *et al*., 2003). Also, aspirin shows teratogenic effects on the developing nervous system including a decrease in crown-rump length and a significant increase in overall dysmorphology, including eye and branchial arch. The neural tube was especially vulnerable and had frequently failed to close when rat embryos were cultured in 100-300 μg mL^{-1} of salicylic acid at days 9.5-11.5 of gestation (Joschko *et al*., 1993). Furthermore, postnatal treatment of rat pups with aspirin has lead to a significant reduction in cerebellar weight, but had no effect on brainstem or total brain weight (Bonthius and West, 1989). Recently, Amateau and McCarthy (2004) found that male rats exposed prenatally to aspirin at low doses had a mild and reversible impairment in copulatory parameters and this finding indicated the sensitivity of the developing brain to perturbations in prostaglandin synthesis.

A developmental neurotoxicity study, which is used to assess neurologic development in laboratory animals following toxicant exposure, needs a careful qualitative and quantitative morphologic study of numerous brain parts at several developmental stages. Quantitative evaluation may include such basic methods as determination of brain weight and dimensions as well as the progressively more complex approaches of linear, area, or sterologic measurement of brain section (Garman *et al*., 2001). Therefore, since allometry is used to study and measure the relative growth of a part of an organism in comparison with the whole by using quantitative measurements (Reddy *et al*., 1998; Trombulak, 1991) this study aimed at examining postnatal relative growth of brain parts prenatally exposed to aspirin using allometric relationships.

MATERIALS AND METHODS

Animals: This study was conducted during the months of April to November of 2005 at the biology laboratories of the Hashemite University, Jordan.

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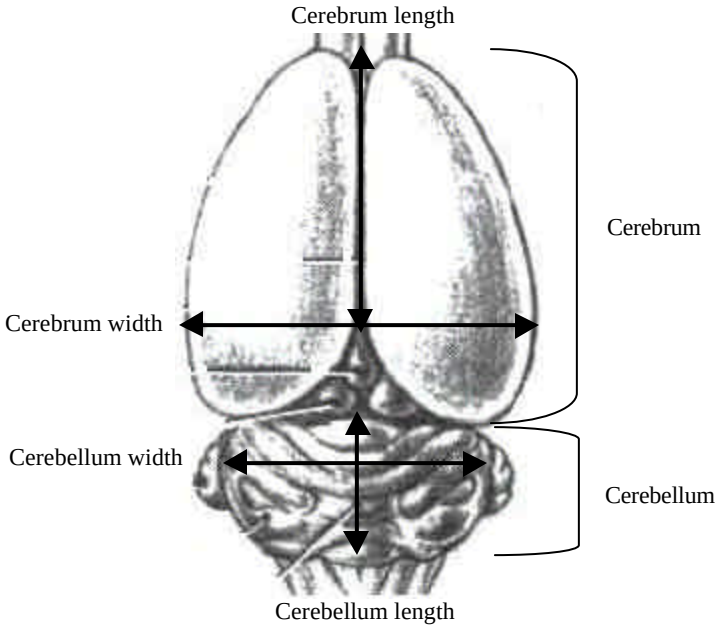


Fig. 1: Dorsal view of the cerebrum and cerebellum of the rat brain showing the measured parameters

Mature male and female Sprague Dawley rats weighing between 350-400 g for males and 150-200 mg for females were used in this study. One male and two females were housed per cage. The day which comes one day after mating was designated day 0 of gestation using copulatory plugs examination as an indicator of pregnancy. Animals were housed under standard environment conditions with free access to a standard diet and water during the entire experiment.

Aspirin: Aspirin (acetylsalicylic acid) powder row material was purchased from Scharlau Chemie S. A. (Spain). Aspirin was dissolved in distilled water and was given at doses of 12.5, 25 and 37.5 mg kg^{-1}.

Treatment: 60 Pregnant rats were divided into four groups of 15 rats each; three experimental groups and a control group. Groups 1, 2 and 3 received aspirin at a dose of 12.5, 25 and 37.5 mg kg^{-1}, respectively. The control group received distilled water. All administrations were orally and were given daily from the fifth day of gestation until birth. At day 20 of gestation, the rats were housed singly in cages and were checked regularly for the presence of pups to determine the day of birth. The rats that were delivered in the 23rd day of gestation (±2 days) were used in this study.

Brain removal: 200 pups were used starting from the first week after birth until the fifth week. At each week, 10 pups from each group were deeply anesthetized with ether and weighed using analytical balance (Precisa, 3 digits balance). For brain fixation, pups were perfused intracardially through the left ventricle with 0.1 M phosphate buffer (pH 7.4). 20, 30, 40, 50 and 60 mL of phosphate buffer were used for 1, 2, 3, 4 and 5 week aged pups, respectively. This was followed by perfusing an equal volume of fixative containing 20% buffered formalin fixative (Kiernan, 1999). Each brain was carefully removed and separated from the spinal cord and rapidly immersed for 3 days in 20% buffered formalin for further fixation.

Brain measurements: After three days in fixative, brains were removed and left for 15 min to dry. Olfactory bulbs and paraflocculus were cut and the brains were weighed using analytical balance. The cerebellum was then separated from the cerebrum. Cerebrum length and width, cerebellum length and width were measured using digital calipers (E-BASE, MC 02050282-1, China) (Fig. 1).

Data analysis: Allometric analyses were carried out using nonlinear regression analysis. In such analysis, the variables of interest (brain weight, body weight, cerebrum length and width and cerebellum length and width) were analyzed using the nonlinear equation $y = ax^b$. The relationship was allometric if the allometric coefficient (b) ≠ 1 and (a) present the mean value of the ratio y/x. The calculations were carried out using STATISTICA software for windows (StaSoft, USA).

RESULTS

The results show strong nonlinear relationships as determined by the values of the correlation coefficients (r). The values of (b) in the relationships indicate an allometric relationship ($b \neq 1$) (Table 1 and 2).

When comparing the values of the allometric coefficient (b) in the nonlinear equation $y = aX^b$, the results show that there are noticeable differences between the control group and all aspirin treated groups in the relationship between brain weight with cerebellum length (Table 1 and 2). This means that in the control group, the

Table 1: Parameters of nonlinear equations for brains in control group (n = 50)

Relationships	a	b	r
Cerebrum length X Cerebrum width	0.105730	1.724106	0.84189
Cerebrum length X Cerebellum width	1.502308	0.819220	0.94027
Brain weight X Cerebrum length	28.41357	1.602664	0.92419
Brain weight X Cerebellum length	213.3612	0.926090	0.91550
Body weight X Cerebrum length	0.871521	4.455327	0.71323

Table 2: Parameters of nonlinear equations for brains in group 1, (12.5 mg kg^{-1} n = 50), group 2 (dose 25 mg kg^{-1} n = 50) and group 3 (dose 37.5 mg kg^{-1} n = 50)

Relationship	a	b	r
Group 1			
Cerebrum length X Cerebrum width	0.355719	1.271210	0.90860
Cerebrum length X Cerebellum width	2.409163	0.626861	0.90659
Brain weight X Cerebrum length	7.065849	2.186022	0.94809
Brain weight X Cerebellum length	123.7483	1.204976	0.90149
Body weight X Cerebrum length	0.001733	7.063504	0.75084
Group 2			
Cerebrum length X Cerebrum width	0.080953	1.813101	0.85437
Cerebrum length X Cerebellum width	1.006598	0.985915	0.94836
Brain weight X Cerebrum length	30.92963	1.576175	0.93895
Brain weight X Cerebellum length	177.0651	1.021987	0.87762
Body weight X Cerebrum length	0.016138	6.150235	0.90516
Group 3			
Cerebrum length X Cerebrum width	0.022792	2.297694	0.91602
Cerebrum length X Cerebellum width	0.808291	1.083318	0.96050
Brain weight X Cerebrum length	36.64131	1.481185	0.96575
Brain weight X Cerebellum length	167.2998	1.039723	0.93786
Body weight X Cerebrum length	0.088434	5.422826	0.86513

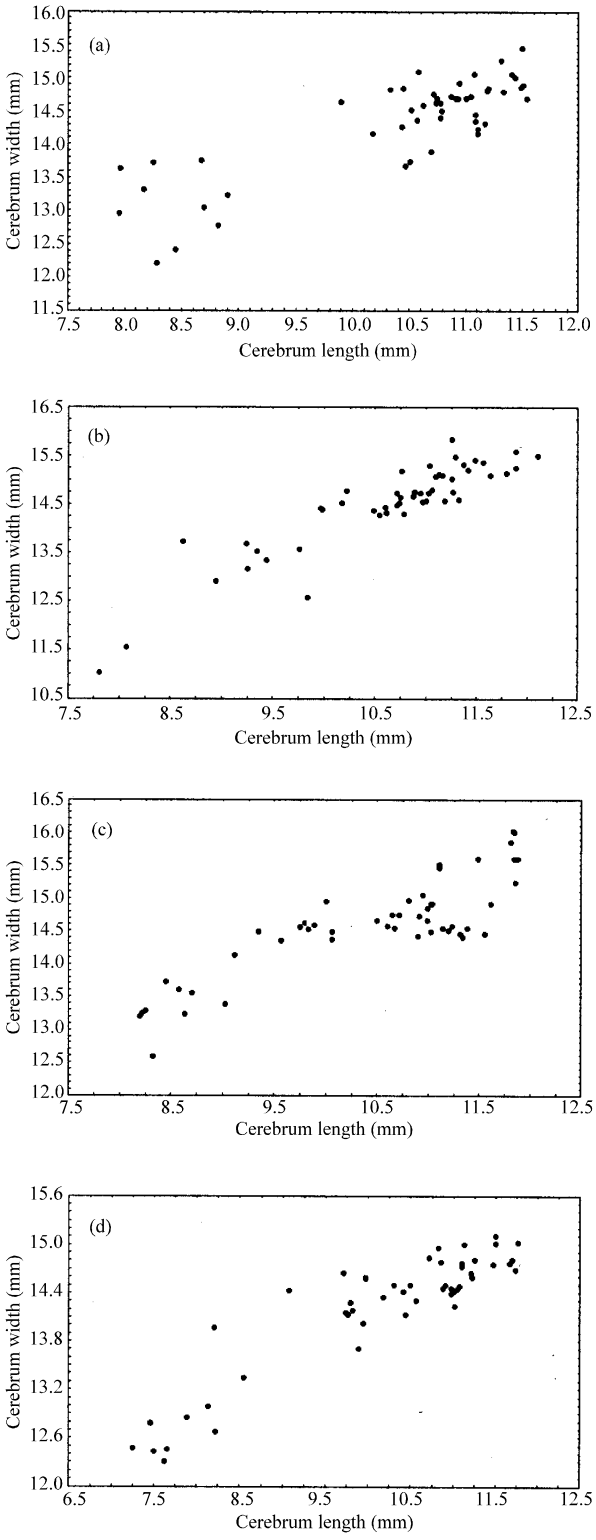
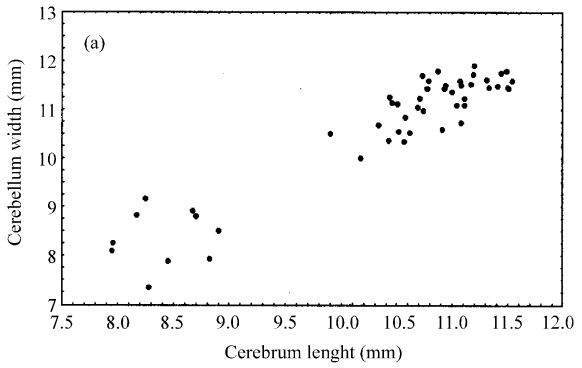
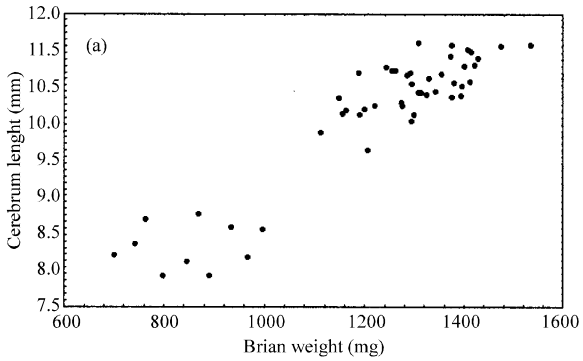
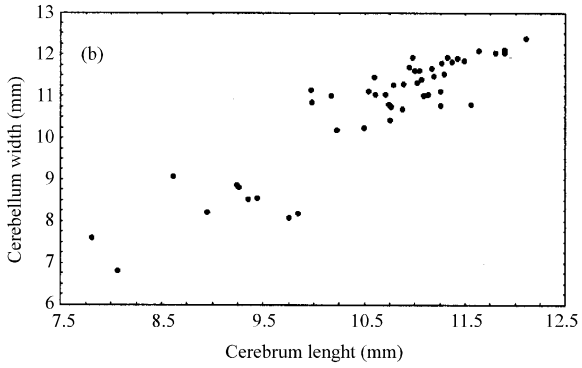
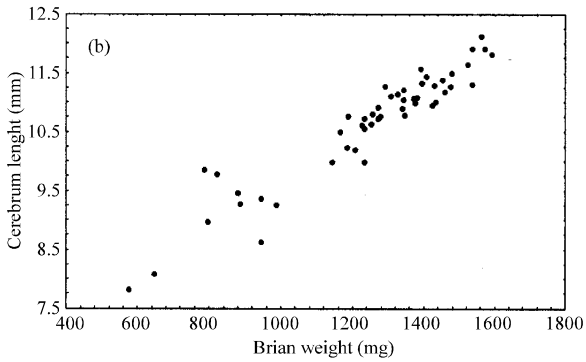


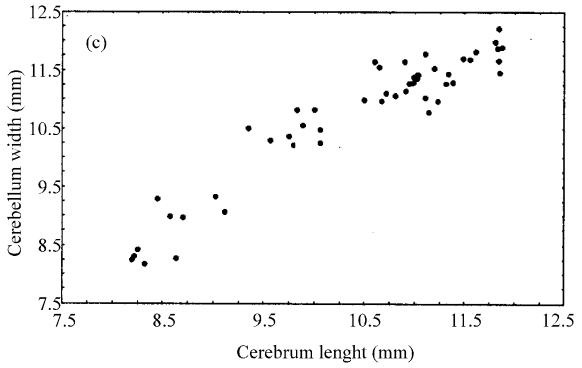
Fig. 2: Actual measurements values of cerebrum length against cerebrum width for control group (a). group1 (b). group2 (c). and group3 (d)

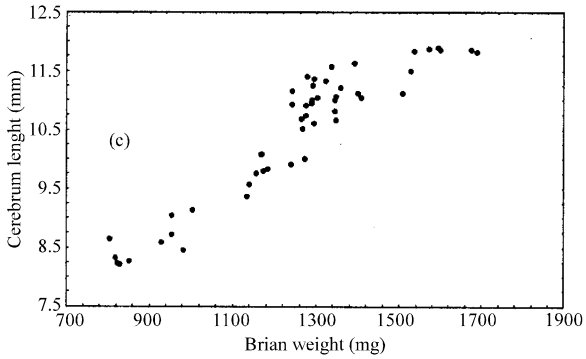


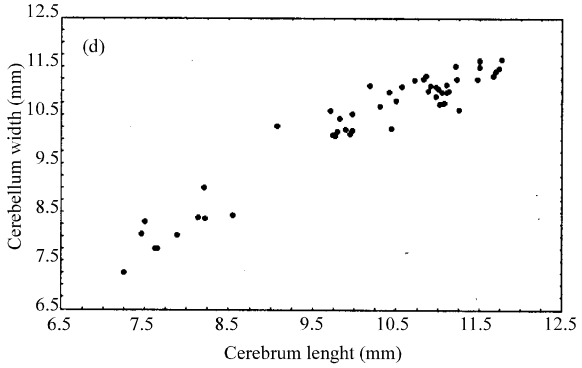












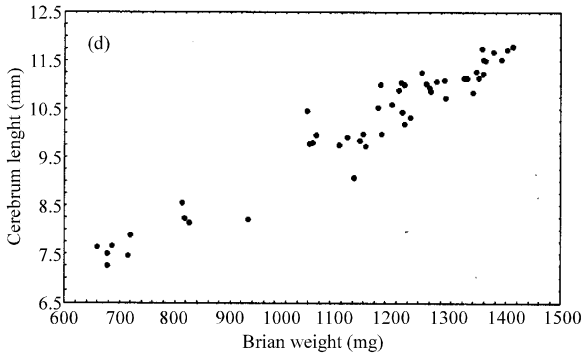


Fig. 3: Actual measurements values of cerebrum length against cerebellum width for control group (a). group1 (b). group2 (c). and group3 (d)

Fig. 4: Actual measurements values of brain weight against cerebrum length for control group (a). group1 (b). group2 (c). and group3 (d)

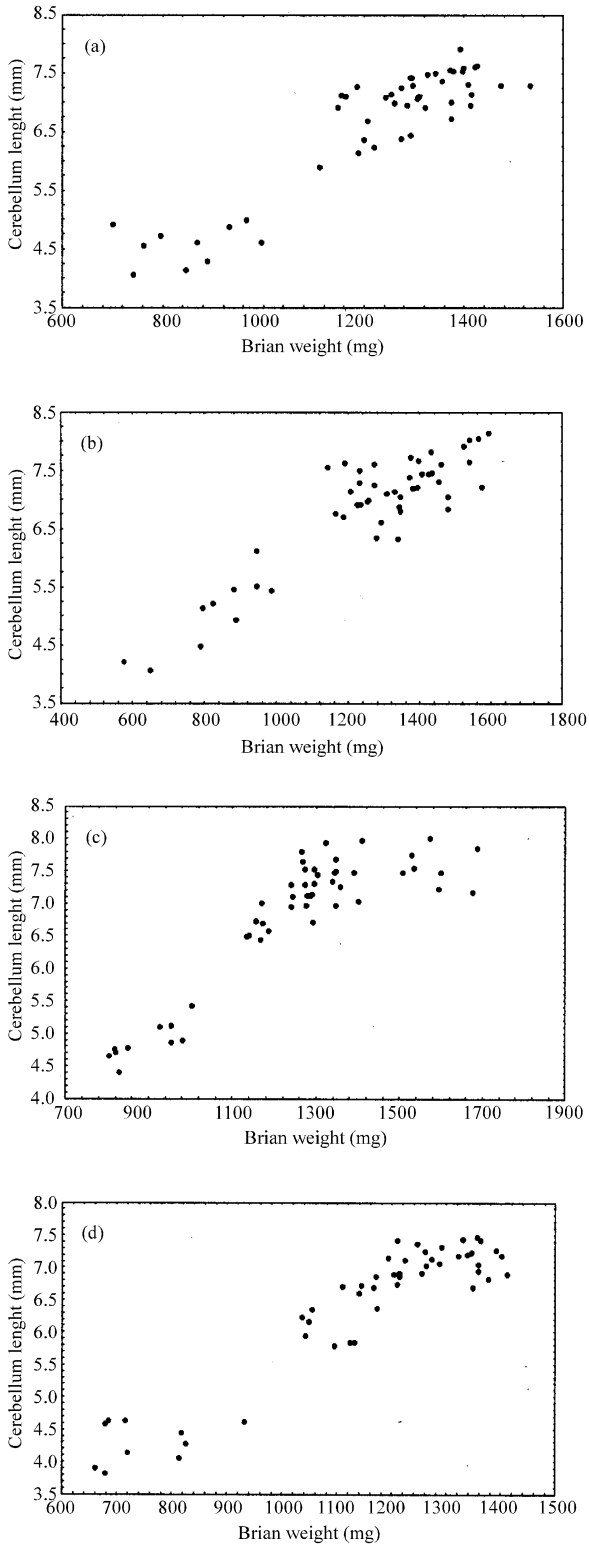


Fig. 5: Actual measurements values of brain weight against cerebellum length for control group (a). group1 (b). group2 (c). and group3 (d)

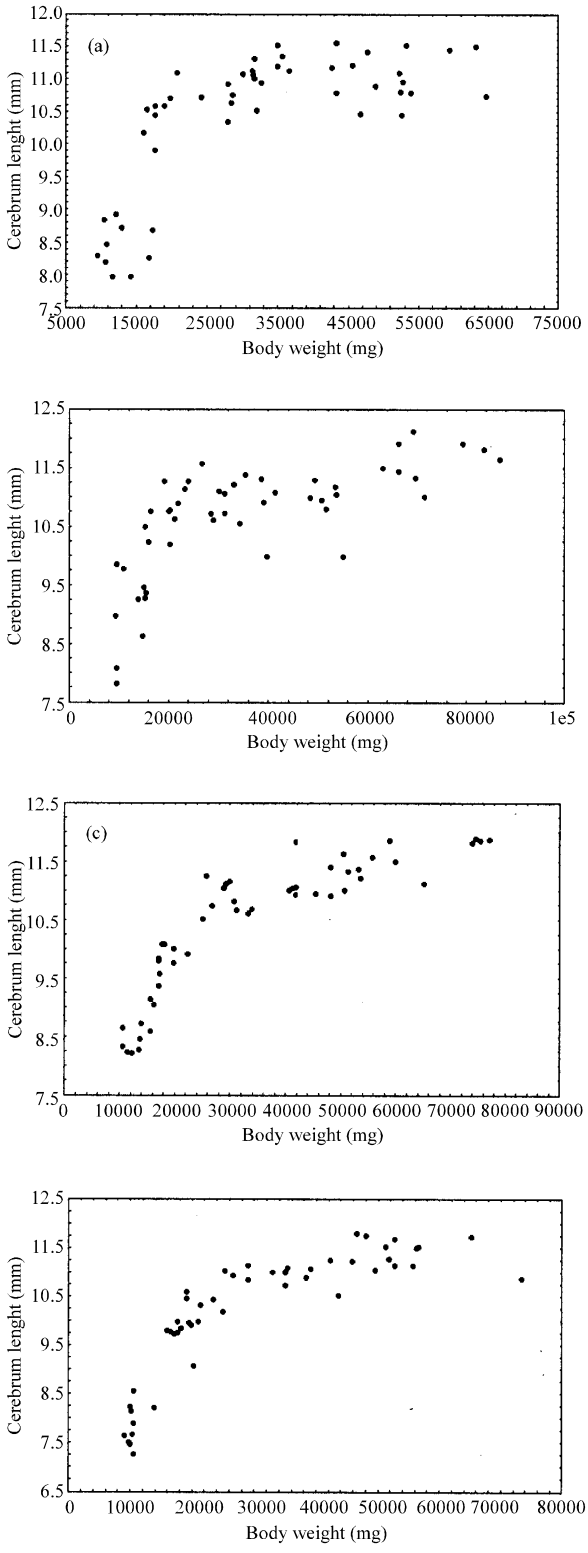


Fig. 6: Actual measurements values of body weight against cerebrum length for control group (a). group1 (b). group2 (c). and group3 (d)

brain weight increases less than the relative increase in cerebellum length while in all other experimental groups the increase in brain weight is more than the increase in cerebellum length. These relationships are shown in Fig. (2-6) and are represented in the following equations.

Brain weight = 213.3612 * cerebellum length $^{0.926090}$ (control group).
Brain weight = 123.7483 * cerebellum length $^{1.204976}$ (group 1).
Brain weight = 177.0651 * cerebellum length $^{1.021987}$ (group 2).
Brain weight = 167.2998 * cerebellum length $^{1.039723}$ (group 3).

The same pattern of relationships is also present between cerebrum length with cerebellum width. This means that the relative increase in cerebrum length is more than the cerebellum width in group 3, while in other aspirin treated groups and the control group the relative growth in cerebrum length is less than cerebellum width.

Other relationships such as cerebellum length with cerebellum width show the same behavior of relative growth between control group and aspirin treated groups in which the growth in cerebellum length is more than cerebellum width. Furthermore, the increase in brain weight is more when compared to the increase in cerebrum length. This increase was the highest in group 1 than other groups. It is worth mentioning that the increase in body weight when compared to the increase in cerebrum length in all experimental groups was higher than the control group.

DISCUSSION

The results show that there are differences in many of the allometric relationships of the brain and body after birth in the prenatally aspirin-treated groups. It seems that the main effects were on the cerebellum length and width, which did not increase at a normal ratio when compared to the increase in the whole brain or to the cerebrum length. Furthermore, the relative growth of the cerebrum length was less than the relative growth of the whole rat body. The concentration of the effects on the growth of the cerebellum is difficult to interpret due to the lack of studies in this area. Although there are a number of reports on the teratogenic effects of aspirin, few of these studies examined the effect of aspirin on brain growth. Joschko *et al.* (1993) studied the teratogenic effects of salicylic acid on the developing nervous system in rats *in vitro*. Rat embryos were subjected for 48 h to 100-300 μg mL^{-1} of salicylic acid at days 9.5-11.5 of gestation. They reported that there was a decrease in crown-rump lengths, somite numbers and also the neural tube was especially vulnerable and had frequently failed to close. These results indicate that neural tissue is sensitive to the teratogenic effects of aspirin and thus supports our results. Furthermore, Bonthius and West (1989) indicated that the highest dose of aspirin (50 mg/kg/day) treatment between gestational days 26-32 (form 4th day to 10th day postnatal) caused a reduction in cerebellum weight. In this study, aspirin shows an effect on relative growth of the cerebellum and thus is in agreement with Bonthius and West study. Vorhees *et al.* (1982), Klebanoff and Berendes (1988) and Streissguth *et al.* (1987) suggested that treatment of pregnant rats cause differences in postnatal growth and reduces behavioral performance in the progeny, some of these behavioral changes were related to reduction in body weight in aspirin-treated group and to lower IQ scores and to attention deficits. Although it is difficult to compare the results of this research with other reports due to the scarcity of research on the effects of aspirin on the development of the nervous system, it is reasonable to say that there is an agreement with the few studies that investigated this effect. Further research is required to investigate the exact mechanism of aspirin that results in these effects on the brain during development.

In conclusion, it is reasonable to say that the findings of this research clearly indicate that there were differences in the allometric relationships between groups treated with aspirin and the control group. This could indicate that there is an effect on the relative growth of mainly the cerebellum in rats prenatally exposed to aspirin.

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