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Research Article

Study of *Cinnamomum zeylanicum* and Potato during pregnancy and gestrationin swiss albino mice

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ABSTRACT

Pregnancy is the stressful period of a living life. Proper nutrition during this period leads to the development of healthy world. Nutrition demand increases in the period of gestation; hence this research article deals with importance of diet during pregnancy. In this study role of high carbohydrate diet with antioxidant *Cinnamomum zeylanicum* commonly known as cinnamon or daalchini in our Indian houses is studied during gestation in Swiss albino mice. Swiss mice were treated with three different diets during their period if gestation, diets are Control Diet group-1 [(3.85gm Wheat + 3.85gm Maize + 1.55gm Gram + 0.75gm Groundnut = 10 gm) for 1 mice], Control Diet + High carbohydrate (potato) group-2 [6 gm + 4 gm], Control Diet +cinnamon (Bark) group; this group is divided into 2 subgroups- 3 [9.5 gram + 0.5 gram; 9.75 gm + 0.25 gm]. After parturition, the body weight (BW), crown to rump length (CRL) and Body Mass Index (BMI) of pups were recorded on PND (Post Natal Day) 1, PND 7, PND 14, PND 21, PND 35 and PND 49.

Key words: *Development; Fetus; Post-natal; Diet; Carbohydrate*

INTRODUCTION

Nutrition is called as a science of food values, which was evolve from chemistry and physiology. This science consists of action, interaction and balance of nutrients in relation to health and disease. A poor diet may import an injurious impact on health, like-: It cause deficiency diseases like scurvy and kwashiorkor, Health-threatening conditions like obesity, metabolic syndrome and a common chronic systemic diseases i.e. cardiovascular disease, diabetes and osteoporosis.

Nutritional requirement changes during the different stages of a life span. Pregnancy is the stage when nutritional needs are higher and full feeling those needs impose positive effects on the health of both mother and her unborn baby. Therefore, proper nutrition is important, because nutritional status of mother plays a critical role in the outcome of pregnancy and also in fetal growth and development.[1,2]This study is done on one of the most important nutrient of human diet during pregnancy i.e. carbohydrate in addition with cinnamon (daalchini) which is a common spice in Indian kitchens.

The importance of a balance diet is emphasized especially in terms of quality of carbohydrates taken in pregnancy and lactation and the risk of developing insulin resistance and obesity is reduce by replacing low fiber grain food, such as cornflakes or white bread with whole grain higher fiber or higher amylose content products.[3] Carbohydrates are the major constituent of human diet, carbohydrate rich food is digested easily and provide large amount of energy and is also consider good for sick and old age peoples. Potato is taken as a high carbohydrate ingredient in this study by keeping its cheap availability to every strata of society.

Following wheat, maize and rice, potato is one of the world's most important crops.[4] Both US Department of Agriculture (USDA) Food Guide Pyramid and 2005 revised, include potatoes in a vegetable category as a food to be encouraged.[5] Many professionals and medical organizations, including American Dietetic Association and The American Heart Association state potato as a healthful food.[6,7] Potatoes have greater dry matter and protein per unit growing area compared with the cereal.[8] Skin of potatoes is a good dietary source of potassium instead of wheat starch, rats fed a diet high in sodium (2% sodium chloride) were better able to retain calcium and

magnesium and increase urinary pH when fed cooked potato, because potatoes have high potassium citrate content.[9] In potato cultivars phytochemicals which are the secondary products of plant metabolism and many of which acts as antioxidants, vary in amount and composition.[10-12]

Plants product improves glucose metabolism and diabetes not only by hypoglycemic effect but also by improving lipid metabolism and antioxidant status.[13] *Cinnamomumzylenicum* family of Lauraceae is a bark which has antioxidant activity.[14] *Cinnamomumzylenicum* bark is the outer skin of an evergreen tall tree which belongs to Lauraceae family.[15] Cinnamon possesses antioxidant activity in rat fed with high fat diet.[16] Cinnamon oil is an important endogenous antioxidant and also used as a protective agent against tissue damage.[17] Cinnamon is a natural product which is safe in all respect, is rich in polyphenolic compound that shows to improve the action of insulin in-vitro[18-20] in animals, and also have antioxidant activity in-vitro.[21]

MATERIALS AND METHODS

The proposed experiments were conducted in the Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, MohanlalSukhadia University, Udaipur, Rajasthan, India to observe the effect of high carbohydrate diet(potato), cinnamon and there combination during pregnancy in Swiss mice.

Animal

Healthy adult female Swiss mice 8-10 weeks old and 28- 30 g average body weight were used for this study. Animals were obtained from the animal house of our department. Male and female mice in the ratio (1:4) kept in the cage for mating. Female mice were examined every day in the morning and female showing vaginal plug were isolated and their gestation period were recorded and were given an experimental diet. Presence of spermatozoa in the vagina the following morning was considered day one of gestation. Confirmed pregnant females were housed in polyvinylchloride cages (290×320×390 mm) wrapped with rice husk bedding, and maintained under standard laboratory conditions. The laboratory animals were kept in well ventilated animal room with relative humidity of 70-80%. The room lighting consisted of alternate 12 hours' light and dark periods. The maintenance and handling of the animals were done as per the guidelines of Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University (No. CS/Res/07/759).

Experimental Design

A female which shows vaginal plugs was isolated and duration of their gestation period was be recorded. The selected pregnant females were divided in the following groups, 6 animals in each group and those pregnant females were given the following diet.

1. **Group 1-** Control diet {1 mice is given total of 10 g food in a day which have (3.85gWheat+3.85gm Maize+1.55gmGram+0.75 gm Groundnut)}.
2. **Group 2-**Control + high carbohydrate diet {Potato} (6g control+ 4g potato).
3. **Group 3-**Control+ *Cinnamomumzeylanicum* (cinnamon). **On the basis of cinnamon dose group 3 is divided into 2 sub-groups: -**
   a) Control+ *Cinnamomumzeylanicum* {higer dose} (9.5g control+ 0.50gcinnamon).
   b) Control+ *Cinnamomumzeylanicum* {lower dose} (9.75gcontrol+ 0.25 cinnamon).
4. **Group 4-**Control + high carbohydrate diet + *Cinnamomumzeylanicum* (5.5g control+ 4g potato+ 0.50g cinnamon).

Pups of the above-mentioned group were observed at the different developmental stages:

- At the time of birth (PND 1)
- At the end of first week (PND 7)
- At the end of second week (PND 14)
- At the end of lactation (PND 21)
- At the time of puberty (PND 35)
- At the end of puberty (PND 49)

Litter size, fur quality, body weight and body length are the morphological parameters which are observed during the above mention PND's.

Estimation of body weight, crown to rump length and body mass index

After treating mice with different diets, they were kept separately for 42 days (*i.e.* 21 days of gestation and 21 days of lactation). BW and CRL of pups of control and experimental animals were taken on 1st, 7th, 14th, 21st, 35th and 49th PND days after parturition. BW was estimated by WJ series electronic balance model No. WJ302A. CRL was estimated by measuring scale. From the data of BW and CRL body mass index (BMI) was calculated. The BMI was calculated of control and experimental mice at postnatal days (PND) 1st, 7st, 14st, 21st, 35th, 49th by using BW and CRL. The body mass index (BMI) was calculated by the formula as described by Novelli, 2007.

$$\text{Body mass index (BMI)} = \text{body weight (g)}/\text{length}^2\ (\text{cm}^2)$$

The data was analyzed statistically using SPSS v. 17 (SPSS, Chicago, USA). The significance differences among means were carried out using Duncan's multiple range tests at $p< 0.05$. The results are expressed as mean ± SD of four experiments.

RESULTS

1. Effect of different diets on body weight of neonates on various postnatal days 1, 7, 14, 21, 35 and 49:

Details of Body Weight (BW) in various experimental groups in comparison to control are shown in **table1 and graph 1**.

Statistically non-significant or insignificant change is observed in PND 1 (just after parturition), *i.e.* there exist a change in BW of pups but this change is not due to experimental diets ($P>0.5$; $P= 0.134$).

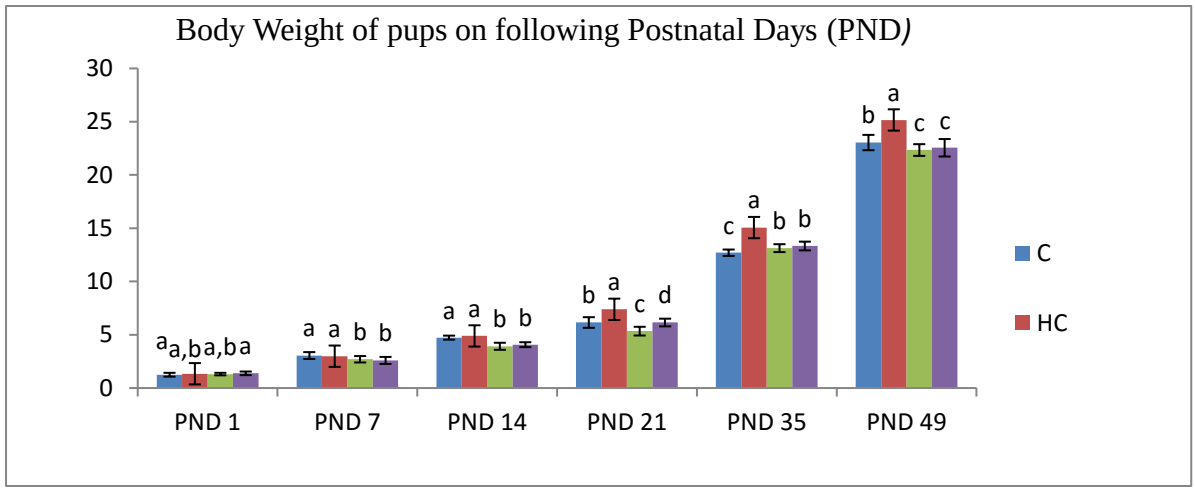
On PND 7 (at 2nd week) there is significant change (at 5% level) is observed in pups (P= 0.1-0.5; P= 0.02). Here pups of control and HC does not show much variation in BW, but BW of both the groups is high when compare with cinnamon and HC+ cinnamon group.

Highly significant result was observed in BW at PND 14, 21, 35 and 49 (P<0.1; P= 0.000). In the respective PND's HC group shows highly increase in BW of pups as compare to other 3 groups. Less decrease in BW of pups was observed in cinnamon group, but this decrease in BW was compensated by potato in 4th group i.e. HC+ Cinnamon group. Order of Change in body weight is: HC > Control > HC+ Cinnamon > Cinnamon

Table 1: Variations in Body Weight (BW) of neonates in different experimental groups on PND 1, PND 7, PND 14, PND 21, PND 35 and PND 49 days. Values are expressed as mean ± S.D. for six females per group.

Group	Body Weight of pups on following Postnatal Days (PND)					
	PND 1	PND 7	PND 14	PND 21	PND 35	PND 49
Control	1.25±0.18^{a}	3.05±0.32^{a}	4.73±0.19^{a}	6.15±0.50^{b}	12.70±0.30^{c}	23.04±0.72^{b}
High Carb	1.34±0.12^{a,b}	2.99±0.29^{a}	4.89±0.27^{a}	7.39±0.38^{a}	15.06±0.31^{a}	25.16±0.60^{a}
Cinnamon (0.25)	1.31±0.12^{a,b}	2.70±0.30^{b}	3.92±0.33^{b}	5.34±0.41^{c}	13.13±0.37^{b}	22.34±0.55^{c}
H.C. + Cinnamon (0.50)	1.38±0.17^{a}	2.59±0.33^{b}	4.07±0.23^{b}	6.15±0.36^{b}	13.33±0.41^{b}	22.55±0.82^{c}
F Value	1.94^{ns}	5.62**	38.46**	49.49**	86.56**	34.11**

P value >0.05 = non-significant (ns), <0.05 = significant (*) and <0.01 = highly significant (**). Mean followed by the same alphabet within columns are not significantly different (P < 0.05) using Duncan's multiple rang test.



Graph 1: Variations in Body Weight (BW) of neonates in different experimental groups on PND 1, PND 7, PND 14, PND 21, PND 35 and PND 49 days

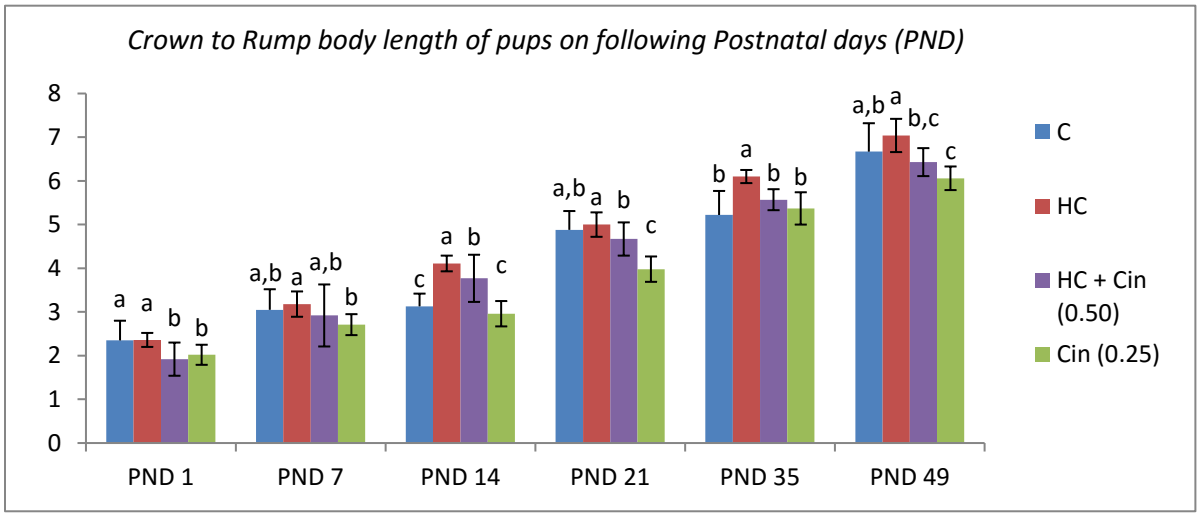
Table 2: Variations in Body Length (BL) of neonates in different experimental groups on PND 1, PND 7, PND 14, PND 21, PND 35 and PND 49 days. Values are expressed as mean ± S.D. for six females per group

Groups	Crown to Rump body length of pups on following Postnatal days (PND)					
	PND 1	PND 7	PND 14	PND 21	PND 35	PND 49
Control	2.35±0.45^{a}	3.05±0.47^{a,b}	3.13±0.29^{c}	4.88±0.43^{a,b}	5.22±0.55^{b}	6.67±0.65^{a,b}
High Carb	2.36±0.16^{a}	3.18±0.29^{a}	4.11±0.18^{a}	5±0.28^{a}	6.1±0.15^{a}	7.04±0.38^{a}
Cinnamon (0.25)	2.02±0.23^{b}	2.71±0.24^{b}	2.96±0.29^{c}	3.98±0.29^{c}	5.37±0.37^{b}	6.06±0.27^{c}
H.C. + Cinnamon (0.50)	1.92±0.38^{b}	2.92±0.71^{a,b}	3.77±0.54^{b}	4.67±0.38^{b}	5.57±0.24^{b}	6.43±0.32^{b,c}
F Value	7.08	2.35	29.77	17.53	10.85	7.978

2. Effect of different diets on body length of neonates on various postnatal days 1, 7, 14, 21, 35 and 49:

Details of Body Length (BL) in various experimental groups in comparison to control are shown in **table 2 and graph 2**. Statistically highly significant(P<0.01) changes are observed at PND 1 at the time of birth, PND 14 at the end of second week, PND 21 at the end of lactation, PND 35 and PND 49 during puberty. At all these PND's CRL reduction occurred highest in cinnamon treated group while control and HC group shows similarity in their results.

Statistically non-significant (P>0.05) changes occur at PND 7 at the end of 1st week. The decrease in body length which occurred in cinnamon treated group in comparison to other group was balanced by potato in cinnamon+ HC.



Graph 2: Variations in Body Length (BL) of neonates in different experimental groups on PND 1, PND 7, PND 14, PND 21, PND 35 and PND 49 days

2. Effect of different diets on body mass index of neonates on various postnatal days 1, 7, 14, 21, 35 and 49:

In first experiment we observed that two females died in full term and not able to delivered pups. When it was dissected both the horns of uterus shows total 8 fetous along with resorption sites as you can see 1(a) and 1(b). Next two females delivered immature dead pup where posterior region is not developed, with abdominal deformity and resorption sites were noticed in the uterine horn. Pups which are highly immature are not delivered and when the uterus having highly immature pup is dissected we observed that limbs, head region abdominal region is not fully develop 2(a), 2 (b) and 2 (c). Another female able to delivered the pups but they died after 24 hours and the last female again delivered died pups with depression and abnormality in abdominal region 3 (a), 3 (b), 3 (c) and 4.

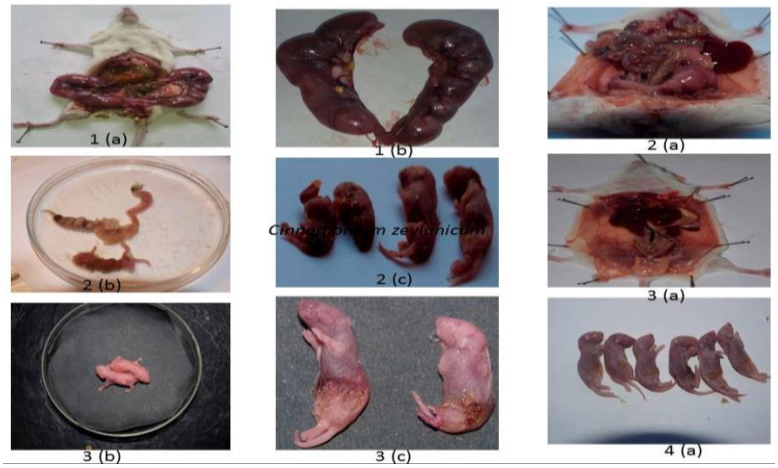


Figure. 1: 1(a) female died in pregnancy; 1(b) uterus of a female showing uterine horns; 2(b) uterus of a female having immature pup; 2(b)dissected immature pup of female 2(a); 2(c) other immature pups of female 2(a); 3(a) female delivered 2 healthy pups and 2 immature pups; 3(b) healthy pups of female 3(a); 3(c) immature pups of female 3(a); 4(a) female delivered stillborn pups.

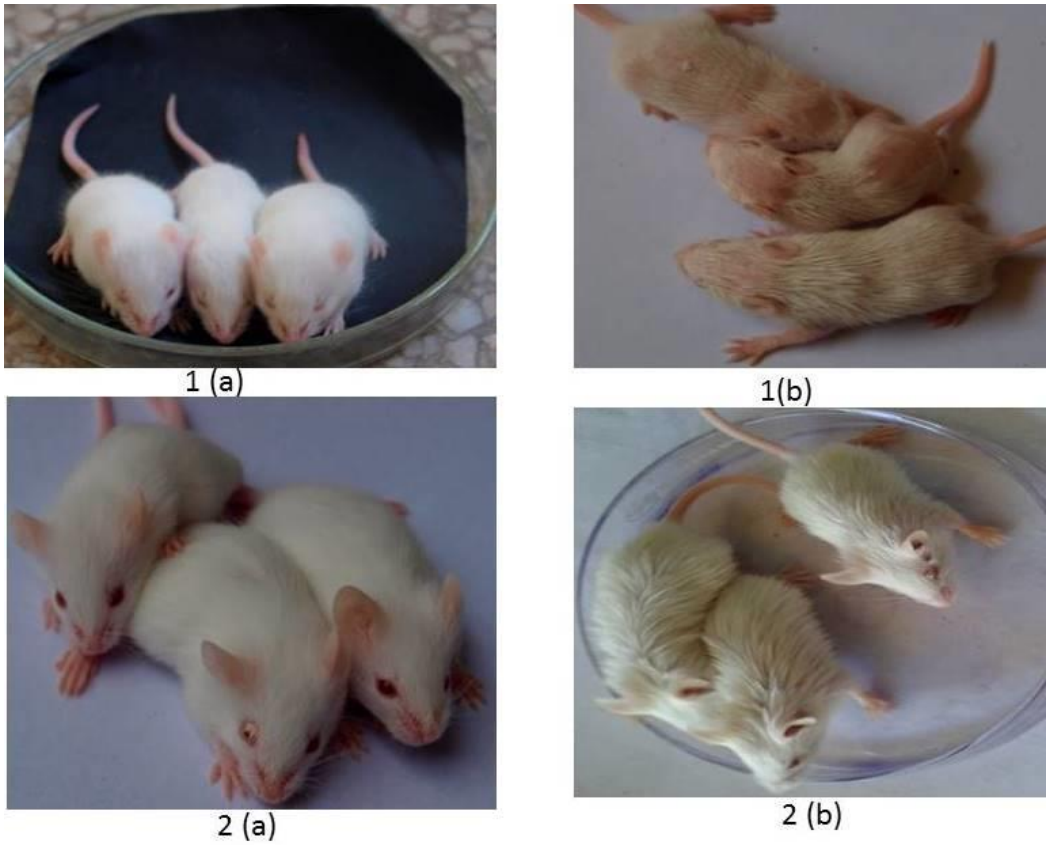


Figure 2: 1(a) shiny and voluminous fur in control at 14 day; 1(b) Non-shiny and scanty fur in cinnamon (0.25) at 14 day; 2(a) Shiny and voluminous fur of control at 21 day; 2(b) Shiny but scanty fur in cinnamon (0.25) at 21 day.

RESULT AND DISCUSSION

Cinnamomum zeylanicum is a bark of an evergreen tall tree belongs to lauracea family.[22] Cinnamon oil is important endogenous antioxidant also used as a protective agent against tissue damage.[17] Cinnamon possesses antioxidant activity in rat's fad with high fat diet.[16] According to FDA (Centre of food safety and applied nutrition) cinnamon is safe when consume in amount commonly found in food during pregnancy, but is not safe when taken orally in greater amount.[23] In the present study when cinnamon is given in an amount of 0.5 g during pregnancy, the initial gestation period and development is observed to be normalbut at the end of gestation period half of the females died [Fig. 1(a); 1 (b)]. Mantovani et. al. observed that when pregnant women consume large amount of cinnamon than its oil cause miscarriages; similarly in our study 0.5 g of its dose during pregnancy leads to the resorption of embryo; resorption sites are observed when uterus from the died pregnant female is dissected out; and if the female parturate, it delivered under developed pups and pups which are highly under developed were not parturated[24] [Fig.1: 2(a); 2 (b)].

When amount of cinnamon is reduced to half i.e. 0.25g then gestation period and parturition is normal but litter size is small and if some females have bigger size then more than half of the pups are stillborn [Fig. 1: 4(a)]. Skeletal anomalies in axial skeleton and skull of pups was observed after the treatment of cinnamon during pregnancy[24], similarly in our study all the parturated pups of 0.5g (higher dose) shows skeletal deformation [Fig.1: 2(c), ; 3(c); 4(a)]; but pups of 0.25g (lower dose) does not show any deformation like that of Hardin et. al., 1987 who did not observed any changes in progeny of mice treated during pregnancy with lower doses.[25]

Romson et al. (1981) observe normal growth and development in dogs fed with carbohydrate-free triglyceride, but survival of fetuses was lower than the dogs which fed with high carbohydrate diet; similarly in our study when females are given potato as an high carbohydrate diet during their gestation period then along with normal and healthy gestation period, females parturate healthy pups with large litter size and greater body weight.[26] Shiell et al., 2001 also said that women who consume low carbohydrate-rich foods have low weight gain in pregnancy and also have offspring of lower birth weight.[27] Further in our study the adverse effect of cinnamon is altered when cinnamon is given in the combination with potato, result of this combination is quite positive where instead of having lower litter size females parturate healthy pups of body weight and survival rate nearly equal to control and high carbohydrate group. The American Diabetes Association, The American Heart association and other national agencies from 1950'srecommend that large portion of energy in the form of glucose comes from carbohydrates.[28] Glucose is consider as an perfect fuel for foetal growth and development and glucose is a

substrate which is primarily affected by the amount and type of carbohydrates in maternal diet,[29] our study go straight with this statement because here control, high carb and high carb+ cinnamon group does not show any skeletal abnormalities which unlike that of both the cinnamon group (higher dose 0.50g and lower dose 0.25g) where either females died during the last days of gestation or they delivered immature or still born pups or they have small litter size.

But in lower dose (0.25 g) female parturate normal pups, which have normal survival rate but have less body weight and with advancing age body weight increases but less in comparison to control, high carb and high carb + cinnamon group, similar to the finding of Mantovani et. al., 1989 who treated groups of 14-16 pregnant Sprague Dawley rats between 7 and 17 day of pregnancy and observed lower increase in body weight, but its higher dosage leads to maternal toxicity.[24] Cinnamon when given in combination with potato then female does not shows the sing of resorptions and underdevelopment, our this finding correlates with the finding of Taylor et al., 1983 who shows the importance of carbohydrate during pregnancy by observing that feeding pregnant rats with carbohydrates free and fatty acid based diet from 1st day of gestation leads to resorption of implanted embryos; almost the same statement is also passed by Koski and Hill, 1986 regarding the importance of carbohydrate during pregnancy, according to them oleic acid based zero carbohydrate diet does not able to maintain pregnancy.[30,31]

Committee E (plant- base medicinal products) of drug institute of federal health office (BGA) observed skin reactions in the progeny when female were treated with cinnamon during its gestation period, similar results are observed in our study when female were treated with 0.25g (lower dose) of cinnamon then there pups shows scanty and scattered fur at 14 and 21 day [Fig. 2 shows the fur quality in control and cinnamon (0.25) treated pups at 14 and 21 day].

CONCLUSION

On the basis of above findings, we can conclude that diet especially high carbohydrate diet influences the body weight and body length of neonates it increases the body weight and body length of the neonates because carbohydrate especially potato is a source of starch and glucose. Cinnamon and cumin is the weight controlling agent so when given in combination with high carbohydrate diet balance the weight of the neonates because as we have discussed above that both cinnamon and cumin shows antioxidant property due to this property they maintain the body's physiology. But cinnamon when given in higher amount in pregnancy its shows side effects which are control by potato and when amount of cinnamon is reduce to half it does not show any side effect.

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