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RESEARCH ARTICLE

EFFECT OF SODIUM BENZOATE (SB)ON NORMAL DEVELOPMENT AND GROWTH OF PUP OF MUS MUSCULUS

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Abstract

This study was conducted to evaluate the effect of sodium benzoate on normal development and growth of pup of Mus musculus.For this study adult healthy and virgin female mice were taken anddivided in two groups, control and experimental group. They were allowed to mate with adult and healthy male mice. After the confirmation of mating the experimental group mice were treated with Sodium benzoate (500mg/kg of Body weight) till the parturition by the help of feeding needle. The different normal developmental parameters i.e. weight of pups, length of pups, onset of food intake, etc. were observed and compare it with control group pups. The obtained result were analysed statistically and conclusion has been made.

Introduction:-

Due to the food's rising chemicalization in recent years, more and more consumers have expressed interest in aspects of food including sensation, health, and most importantly, safety (Hartmann&Klaschka, 2017, Hartmann&Klaschka, 2018, Asioliet al., 2017, Cegiełka 2020, Cheung 2016).

Sodium benzoate is actually a salt of benzoic acid that is well soluble in water, tasteless, and odourless. Because of its antifungal and antibacterial qualities, sodium benzoate (or E211 in the European nomenclature) is used a preservative that is added to food in precisely controlled amounts. It prevents the development of mould, yeast, and bacteria (Davidson et al., 2021).The Food and Drug Administration (FDA) granted the first food preservative approval to sodium benzoate. Its ingestion is permitted up to a maximum of 0–5 mg/kg of body weight. The FDA also rates it as GRAS (generally recognised as safe) (CFR, 2021).

Several studies revealed that SB can be metabolized in the body under certain conditions such as irradiation to form benzene, a derivative capable of damaging mitochondria which has been implicated in kidney and liver injury (Nair, 2001).Decarboxylation of benzoate is thought to produce poisonous benzene, which, when combined with vitamin C, produces a molecule that is very toxic, mutagenic, and teratogenic (Piper, 2017). Additionally, there are claims that sodium benzoate has a negligible genotoxic effect. Additionally, it was demonstrated in vitro that it increased DNA damage in human cells. The substance reduced the rate of mitosis but had no effect on the rate of replication (Zenginet al., 2011). Another investigation using human lymphocytes showed mutagenic and genotoxic effects (Pongasavee, 2015). Chromosomes were broken and micronuclei were formed as a result of this substance. The study also demonstrates that sodium benzoate causes oxidative stress, which is harmful to the immune system, liver, kidneys, and fertility (Chatterjee, 2016).

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Taking the above mentioned studies in consideration, the present study was undertaken to assess the possible danger of the food antimicrobial preservative sodium benzoate on the normal development ofpup of Mus musculus.

Materials & Methods:-

For the present investigationadult, virgin and fertile mice (Mus musculus) of about 28-30 gm. were obtained from the animal house of University Department of Zoology, T.M.B.U. Bhagalpur.

The experimental females were allowed to mate with mature males (three females & one male). In the early morning of the next day, it was observed for the presence of the vaginal plug and /or sperms in the vaginal smears. The day in which the vaginal plug or sperms was present designated day one of pregnancy. Pregnant mice were used in the experimental work and classified as controlgroupand experimental group.

The experimental group were treated by Sodium Benzoate (500mg/kg of body weight) by the help of feeding needle from first day of pregnancy to parturition.

Body weight:
Body weight were measured by the electronic weighing machine.

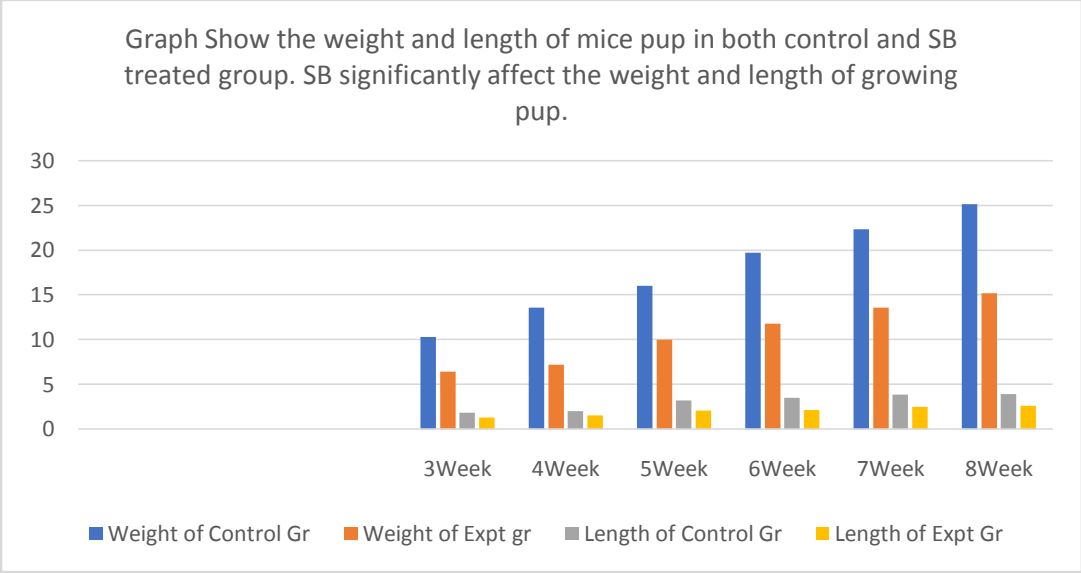
Body Length:
Body length were measured by metal scale.

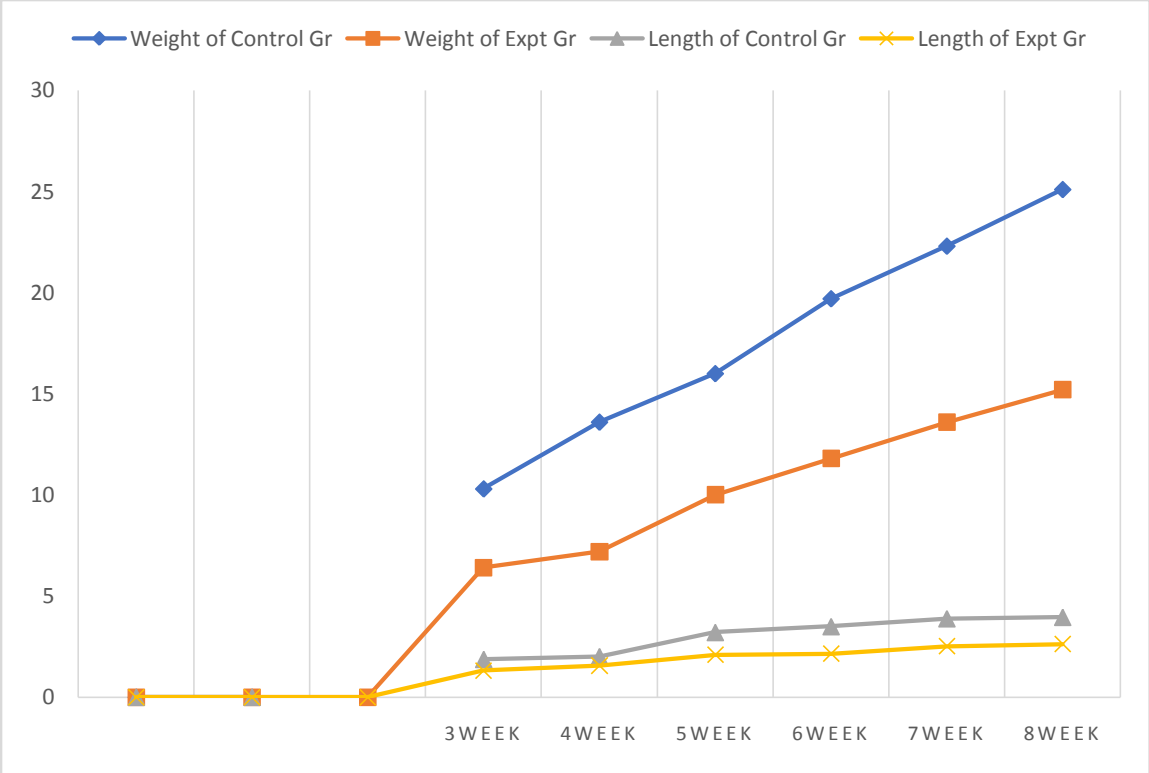
Result:-

The following result were obtained after observation.

Table 1:- Table Show the weight and length of mice pup in both control and SB treated group. SB significantly affect the weight and length of growing pup.

Age	Weight Control Group (In gm)	Weight Experimental Group (In gm)	Test of Significance	Length Control Group (In Inch)	Length Control Group (In Inch)	Test of Significance
3Week	10.3	6.4	T value is 2.663 and P value is 0.011 Result is significantat p < .05	1.87	1.32	The t-value is 2.39648. The p-value is .01877. The result is significant at p < .05
4Week	13.6	7.2		2.01	1.56	
5Week	16.0	10.0		3.20	2.09	
6Week	19.7	11.8		3.49	2.14	
7Week	22.3	13.6		3.88	2.51	
8Week	25.1	15.2		3.95	2.62	





Graph 1:- Graph Show the weight and length of mice pup in both control and SB treated group. SB significantly affect the weight and length of growing pup.

Table 2:- Table Show the Eye opening time and onset of food intake time of mice pup in both control and SB treated group. SB significantly affect the eye opening and onset of food intake time of growing pup.

No of pup	Eye Open Control Group (Days)	Eye Open Expt. Group (Days)	Test of Significance	Starting of Food Intake ControlGroup (Days)	Starting of Food Intake Expt Group (Days)	Test of Significance
1	11	15	The t-value is -11.87939. The p-value is < .00001. The result is significant at p < .05.	13	14	The t-value is -5.43215. The p-value is .000018. The result is significant at p < .05.
2	09	16		13	15	
3	12	16		13	14	
4	11	17		12	14	
5	09	15		15	15	
6	11	17		13	15	
7	12	17		12	16	
8	10	18		12	15	
9	10	15		12	15	
10	11	16		12	14	
Average	10 ± 0.34	16 ± 0.33		12 ± 0.30	14 ± 0.21	

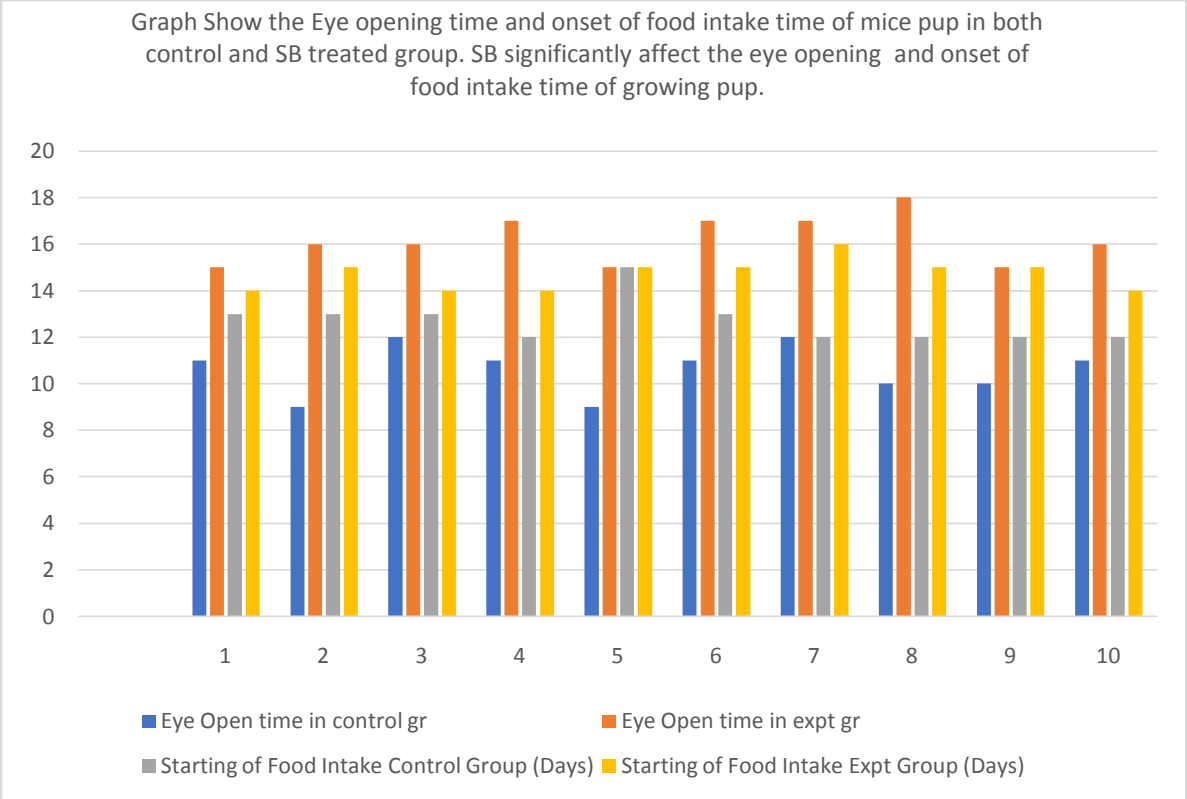


Fig A:- Mice pup Expt. Group (8 Week old). **Fig B:-** Mice pup Control Group (8 Week Old).

Discussion:-

From the obtained result it is clear that SB significantly reduces the growing process of mice pup. At the third week the average weight and length of control gr of mice were 10.3gm and 1.87inch respectively but at same time the average weight and length of SB treated gr mice was noted only 6.4gm and 1.32inch respectively. The weight and length of pup of both groups were recorded from 3rd week to 8th week. From the obtained result (Table1) it is very clear that the SB dosage significantly affect the weight and length of pup.

SB dosage also interfere with the one set of food intake of pup. In control group the average food intake time of pup was recorded 12± 0.30 days from the birth but this phenomenon was delayed in experimental group i.e. 14± 0.22 days. The eyes opening time period also affected by the SB. This process was 6days delayed (Table 2) than the control group.

Afshar et. al. in 2012, observed developmental defects in mouse fetuses where potassium benzoate was administered (280 and 560 mg/kg b.w.).Eyedevelopment defects, such as deformed lenses and also retinal folds with undevelopedlayers accompanying hemorrhages, were observed in these fetuses. The same effects were find in another study where potassium benzoate was administered (280 and 560 mg/kg b.w.) (Afsar et. al. 2019).

In contrast, in another study, sodium benzoate(doses 9.3 and 18.6 mmol/kg b.w.) decreased the fetal weight of rats and increased theirmortality (Taheri&Sohrabi, 2002). In addition, one more study found fetal deformities after prior treatment ofpregnant females with benzoate (280 and 560 mg/kg b.w.) (Ajpt, 2014). Among them, the followingwere observed: skin hemorrhages, craniofacial deformities, limb defects, spine defects,and neural tube defects.Benzoate's teratogenicity was found to have variable effects on chickens (5-200 mg/kg b.w.).This compound did not adversely affect neural tube development in these embryos (Emon et. al.).A zebrafish model was used in a teratogenic investigation on sodium benzoate (Tsayet.al., 2007). The embryos had a 100% survival rate at low dosages (1–1000 ppm), while the larvae were deformed at higher concentrations. The same model-based study also shown that time and dose affected embryo survival (Chen et. al., 2009). The larvae also exhibited lower locomotor activity, tyrosine hydroxylase, and dopamine transporter expression.It was noted in a different study that benzoate's impact on hormones may be cumulative (Sabour&Ibrahim, 2019).It's possible that other parameters have also seen this effect. Additionally, some studies were carried out on pregnant female rats given sodium benzoate (0.5, 1, and 1.5 mg/mL) (Saatciet.al., 2016) Despite having no impact on maternal weight gain, this substance demonstrated no harmful side effects. The 1% and 1.5% benzoate dosage groups significantly increased perinatal mortality.

Conclusion:-

From the above result and discussion, it can be concluded that the SB affect the development of pups, embryo, larvae as well as maternal health. It interferes with the weight, length, onset of feeding, eye opening, etc. in mice pup. These parameters and phenomenon are very important in normal growth consideration. It can be suggested after this study that its use should be limited, especially in pregnant women, due to itspotential teratogenic properties.

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