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PROTECTIVE EFFECT OF OKRA, *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON DEVELOPING BRAIN OF RATS DURING PRE- AND POST-NATAL FLUORIDE EXPOSURE

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ABSTRACT: This study reports protective effect of *Abelmoschus moschatus* seed extract against sodium fluoride (NaF) induced alterations in behavior correlated with neurochemical changes in developing brain of rats. Excessive intake of fluoride during pregnancy cross the blood brain barrier (BBB) and cause adverse effects on neonatal development. As the BBB in fetuses, neonates and infants is immature, it cannot provide protection against the entries. The pregnant wistar rats were randomly categorized into six groups of five animals each. Group I is of control rats received normal tap water. Group II is NaF exposed group with 20 ppm (or 20 mgkg^{-1} body wt.) in their drinking water. Group III rats were treated with *A. moschatus* aqueous extract (AMAE) (300 mgkg^{-1} body wt./day/rat) along with NaF water (20 ppm). Group IV rats were treated with *A. moschatus* ethanolic extract (AMEE) (300 mgkg^{-1} body wt./day/rat) along with NaF water (20 ppm).Group V and VI rats were treated with AMAE (300 mgkg^{-1} body wt./day/rat), and AMEE (300 mgkg^{-1} body wt./day/rat) respectively. On 1st, 7th, 14th, 21st and 30th day (postpartum days), the pups were sacrificed to assess oxidative stress markers (LPO, CAT), and measured body weight, brain weight, BSI, and estimated protein content of brain tissue of all experimental groups. On post-natal day 21 and day 30 pups behavioral activity (rota rod, hot plate test) was measured. Fluoride exposure significantly increased lipid peroxidation and decreased the activity of catalase, and decreased body and brain weight, BSI and also protein content of the brain of pups indicating oxidative stress and inhibited antioxidant system and protein synthesis which were reverted on administration of AMAE and AMEE against NaF intoxication. The altered behavioral responses on NaF exposure were also reversed to that of control. Hence, this study proves the vulnerability of developing brain to fluoride toxicity during development and growth and protection offered by AMAE and AMEE towards neurotoxicity of NaF.

INTRODUCTION: Prolonged ingestion of fluoride through various sources, mainly drinking water caused fluorosis [1]. Fluorosis, as a global public health problem, has been receiving wide and deserving attention in recent years.

Prolonged consumption of fluoride in excess during gestation cause adverse effects on neonatal development [2]. The mechanisms of maternal fetal transmission of fluoride are poorly understood.

Therefore, the present study focused on maternal exposure of fluoride and it's effects on developing CNS and it's amelioration with extract of okra seeds. Fluoride is known to cross the placenta [3, 4] and the blood brain barrier [5] and ultimately increase fluoride levels in fetal brain tissue. The blood brain barrier in the embryo, fetus, and newborn is "immature" that means it is poorly developed,

leaky, or even absent, rendering the developing brain more vulnerable to fluoride entering the fetal circulation from the mother [6]. Once it enter into the brain it can cause adverse effects on the brain cell ultra structure, metabolism, enzymes, the oxidant-antioxidant status and on neurotransmitters and thus overall metabolism of brain [7, 8]. The maternal exposure to fluoride and fluoride insobriety at the early periods of life is shown to cause more prominent effects on the oxidant-antioxidant status in the brain than the fluoride exposure at the later stages of life [9, 10]. Fluoride increases production of reactive oxygen species (ROS), lipid peroxidation, and decreased catalase activity which have been considered in pathogenesis and recent studies suggest that oxidative stress is a possible pathologic mediating factor in fluoride toxicity [11, 12].

Exposure to fluoride during early developmental stages of life results in long-term irreversible consequences with their structure and function and account for qualitative differences in age related susceptibility [13]. The neuronal growth-spurting in rats occurs in the first 3 weeks of postpartum [14]. During this period the brain undergoes many profound structural and functional transformations, which makes vulnerable to fluoride [15].

The high intake of plant products is associated with a reduced risk of a number of chronic diseases [16]. Earlier reports on natural products which aid in protection against NaF induced behavioral alterations are: Curcumin [17], Quercetin [18], Rutin (extracted compound of Okra) [19], Vitamin A [20], and *Spirulina platensis* [21]. These natural products reverse behavioral alterations which are induced on NaF exposure. These beneficial effects have been moderately attributed to the compounds which possess antioxidant activity.

Abelmoschus moschatus L., (Okra) is one of the most commonly known and utilized species of the family Malvaceae. The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids [22]. Nutritionally, the richest part of the okra plant is the dried seed. Okra seed is rich in high quality protein especially with regard to its essential amino acids [22] and unsaturated fatty acids such as linoleic acid [22]. Seeds are rich in phenolic compounds with derivatives, catechin oligomers and hydroxyl-cinnamic derivatives. The nutrients content of Okra seed showed that 21% protein, 14% lipids and 5% ash. Proteins play a particularly important role in human nutrition [22]. Okra is a popular health food due to its high fiber, Vitamin C, and folate content and also a good source of calcium and potassium. High fiber, "helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract" [23]. Okra seeds will make available the essential energy to the body and important antioxidants that could boost immune system and prevent diseases [24]. The neuroprotectant effects of extract of Okra against fluoride induced behavior and neurodegenerative changes in brain of adult rat were reported in our earlier studies [25]. In view of this, the efficacy of AMAE and AMEE in reducing fluoride induced neurobehavioral, and neurochemical alterations in rats of pre and post-natal fluoride exposed is reported in this paper.

MATERIAL AND METHODS: Healthy albino wistar rats (*Rattus norvegicus*) weighted between 180 to 200 gm were assigned randomly to experimental groups and housed 2 females and 1 male for cage (polypropylene cages) to allow breed. Light cycles were maintained as 12 Light: 12 Dark hours (6.00 AM to 6.00 PM), and temperature between $22 \pm 2^{o}C$. The rats were maintained on standard diet and water was supplied *ad libitum.* After their pregnancy was confirmed, from the first day pregnancy, the females made into six groups and allowed to drink on fluoridated water at the rate of 20 ppm NaF and also *Abelmoschus moshatus* seed extract was given to the rats at the rate of 300 mgkg^{-1} body weight as follows;

Experimental Groups:

- **Group I:** Received normal tap water
- **Group II:** Fed on fluoridated drinking water (20ppm)
- **Group III:** NaF (20 ppm) + *Abelmoschus moschatus* seed aqueous extract (AMAE) (300mgkg^{-1} b. wt.)
- **Group IV:** NaF (20 ppm) + *Abelmoschus moschatus* seed ethanolic extract (AMEE) (300mgkg^{-1} b. wt.)
- **Group V:** *Abelmoschus moschatus* seed aqueous extract (AMAE) (300 mgkg^{-1} b. wt.) only.

- ➢ **Group VI:** *Abelmoschus moschatus* seed ethanolic extract (AMEE) (300 mgkg^{-1} b. wt.) only.

Chemicals: Potassium dichromate, glacial acetic acid, Thiobarbituric acid (TBA), H_2O_2, sodium carbonate, sodium potassium tartrate, Folin reagent *etc.* were purchased from local laboratory chemical suppliers.

Physical Parameters:
Body Weight: The rat pups were weighted at the age of post-natal Day 1, Day 7, Day 14, Day 21 and Day 30 and noted down the readings.

Brain Weight: The same pups were killed by decapitation and their brains were dissected out. These brains weighted and noted down the values.

Brain Somatic Index (BSI): For calculating the brain - somatic index, the weight of the individual rat was noted and the brain was removed carefully and weighed on an electronic weigh balance after removing the blood with tissue paper. BSI was calculated by using the value of brain weight of control rat divided by the same rat body weight value and multiplied with 100 (brain weight / body weight $\times$ 100). This formula was applied to all experimental groups to calculate BSI. The Brain - Somatic Index of the rat pups were calculated by the use of equation cited by Ashwini [26].

$$\text{Brain Somatic Index} = \frac{\text{Brain weight}}{\text{Body weight}} \times 100$$

Behavioral Assessments:
Rota Rod: The rota rod test, in which rat pups must balance on a rotating rod, is widely used to assess motor deficit in neurodegenerative disease models (in rodents). Performance is measured by the duration that an animal stays on the rod as a function of rotating rod speed. In the present study, the instrument (Dolphin^{TM} instruments) had incremental fixed-speed (about 30 rpm) rotating rod. Before experimental testing, rat pups were trained to run on the rotating rod in 3 training trials per day for three consecutive days with a constant speed of 30 rpm. For this purpose, animals were kept on the rotating rod for 2 min, and time of their first falling off and the frequency of falling off the rod are recorded for each rat. After the training period, on the day of testing, the performance of the rats was measured as maximal time spent on the rod at 30 rpm before falling off and endurance time noted in min [27].

Hot Plate Test: The hot plate test is used for evaluating thermal pain sensitivity. The hot plate test evaluates thermal pain reflexes due to footpad contact with a heated surface. An apparatus consisting of an aluminium plate that is heated and cooled by Peltier elements in contact with its lower surface. During the experiment, the rat was introduced into Remi hot plate kept at 52 ± 0.5 °C. Hot-plate latencies are determined as described by placing each rat pup on a hot plate and observing the occurrence of a nociceptive response (licking of a hind paw, jumping). The time of exhibiting these behaviors was noted as response time and recorded in seconds. To observe the rat behavior, observation area covered with a colourless plastic cylinder was placed on the hot-plate. In order to avoid possible tissue injury, a cut-off time of 12 seconds was used to avoid skin damage. One hot plate test was carried out for each rat [28].

Oxidative Stress Markers: Lipid per-oxidation and catalase were assessed.

Lipid per-oxidation (LPO): The LPO was assessed by the method of Bhuyan [29]. This assay is based on the reactivity of an end product of lipid peroxidation, malondialdehyde (MDA) with TBA to produce a pink adduct, which is measured at 533 nm on spectrophotometer. The Levels of lipid peroxidation products expressed as μmol of MDA/gr. weight of tissue.

Catalase: Catalase activity was estimated by measuring the rate of decomposition of H_2O_2. Disappearance of peroxide in the presence of enzyme source is the basis for assay which is followed spectrophotometrically at 505 nm. One unit decomposes one micromole of H_2O_2 per minute [30].

Protein Estimation of Brain: Brain tissue total protein content was estimated by the method of Lowry [31]. The principle of Lowry method is the peptide nitrogen(s) with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin - Ciocalteau phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper catalyzed oxidation of aromatic

acids. The color was read at 540 nm. Protein content in the brain tissue expressed as mg of protein / gm weight of tissue.

Per cent of change between groups was calculated by the formula of;

$$\% \text{ change} = \frac{\text{Experimental} - \text{Control}}{\text{Control}} \times 100$$

Data Analysis: Statistical significance was determined by one way analysis of variance. Differences between means were determined by t-test.

RESULTS:

Body Weight: The body weight was progressively decreased (**Table 1**) in NaF treated rats from post-natal day 1 to post-natal day 30 rats as compared to control. Among these experimental days the maximum per cent (-14.34%) of decreased body weight was recorded in post-natal day 30 NaF treated one.

Furthermore, the reverted body weight was noticed in F+AMAE and F+AMEE compared to fluoride alone treated group of respective age and it was similar to control group.

TABLE 1: EFFECT OF *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON BODY WEIGHT, BRAIN WEIGHT, BRAIN – SOMATIC INDEX (BSI) OF RAT PUPS (FROM POST-NATAL DAY 1 TO POST - NATAL DAY 30) EXPOSED TO NaF

Day interval	Organ (brain, body)	Control	Fluoride	% of change from control	F+AMAE	% of change from control	F+AMEE	% of change from control	AMAE	% of change from control	AMEE	% of change from control
Day 1	Brain weight	0.23±0.005	0.20±0.003***	-13.04%	0.21±0.005**	-8.69%	0.22±0.003*	-4.34%	0.23±0.006	0%	0.22±0.008	-4.34%
	Body weight	5.18±0.162	4.83±0.085*	-8.86%	5.12±0.105*	-1.88%	5.14±0.121na	-3.01%	5.33±0.021	1.50%	5.11±0.070	-3.58%
	BSI	0.68±0.161	4.10±0.021**	-7.02%	4.19±0.156na	-4.98%	4.25±0.107na	-3.62%	4.39±0.136	-0.45%	4.31±0.187	-2.26%
Day 7	Brain weight	0.68±0.017	0.58±0.022*	-6.06%	0.65±0.016**	-1.51%	0.65±0.015na	-3.03%	0.66±0.010	0%	0.66±0.015	0%
	Body weight	10.67±0.244	9.44±0.077*	-13.06%	10.57±0.182*	-0.27%	10.37±0.197***	-3.89%	10.46±0.328	-3.05%	10.53±0.270	-0.55%
	BSI	6.41±0.255	6.19±0.276na	-3.43%	6.23±0.203na	-2.80%	6.31±0.255na	-1.56%	6.34±0.207	-1.09%	6.30±0.190	-1.71%
Day 14	Brain weight	1.25±0.005	1.11±0.015*	-4.95%	1.23±0.008*	2.47%	1.21±0.005*	0%	1.25±0.008	3.30%	1.24±0.013	2.47%
	Body weight	20.25±0.301	18.71±0.768*	-8.69%	19.78±0.250**	-1.44%	20.07±0.426na	-1.54%	20.15±0.265	-0.77%	20.60±0.404	-2.02%
	BSI	6.20±0.092	5.93±0.258na	-4.35%	6.20±0.119na	0%	6.06±0.133na	-2.25%	6.23±0.076	0.48%	6.04±0.143	-2.58%
Day 21	Brain weight	2.03±0.014	1.79±0.044*	-5.5%	1.92±0.010*	-1.00%	1.89±0.024*	-1.5%	2.02±0.007	1.00%	1.96±0.008	-1.5%
	Body weight	26.98±0.375	24.36±0.424*	-7.59%	25.73±0.374*	1.27%	25.50±0.326**	-1.95%	26.87±0.472	3.34%	26.04±0.099	-2.03%
	BSI	7.52±0.140	7.36±0.279na	-2.12%	7.48±0.07na	-0.53%	7.42±0.147na	-1.32%	7.53±0.146	0.13%	7.52±0.032	0%
Day 30	Brain weight	2.87±0.160	2.23±0.031*	-22.29%	2.57±0.141na	-10.45%	2.55±0.048***	-11.14%	2.85±0.069	5.92%	2.85±0.026	-1.39%
	Body weight	40.37±0.716	34.58±0.689na	-14.34%	38.52±0.653na	-4.58%	36.70±0.647na	-9.09%	40.24±0.622	4.13%	39.83±0.364	-9.11%
	BSI	7.12±0.433	6.47±0.130na	-9.12%	6.69±0.397na	-6.03%	6.96±0.231*	-2.24%	7.08±0.164	-0.56%	7.17±0.082	0.70%

Percentage of decreased body weight, brain weight and BSI of all experimental groups compared with control was presented. There is no significant change in case of mean value of control group and experimental group. Data presented as mean ± S.E.M. (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. t-test was conducted in all possible combinations to compare significance of data. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of $P < 0.01$, ** for the p-value of $P < 0.05$, *** for the p-value of $P < 0.001$, and na represent non-significant difference between groups.

Brain Weight: The brain weight was decreased in NaF intoxicated rats when compared to control as shown in the **Table 1**. But there is no uniform decrease in the brain weight of experimentally treated rats. The reverted brain weight was reported in F+AMAE and F+AMEE compared to fluoride alone treated group of respective age. AMAE and AMEE received rats brain weight is similar to control.

Brain Somatic Index (BSI): Decreased BSI (**Table 1**) was observed in Day 1 (-7.02%), Day 7 (-3.43%), Day 14 (-4.35%), Day 21 (-2.12%) and Day 30 (-9.12%) aged pups of NaF intoxicated

when compared to control group of respective aged pups. Among these day 30 post-natal rats showed the highest per cent of decreased BSI. AMAE and AMEE treated groups against NaF exposed pups showed the reversed BSI as compared to NaF group.

Furthermore, AMAE and AMEE alone treated groups BSI is same as that of control.

Rota Rod Test: Decreased latencies and repeated fall offs (**Fig. 1**) from the rotating rod of rota rod apparatus were noted down in NaF intoxicated pups than control. In day 21 experimental groups the per cent of decreased retention time is -21.69% in NaF and similarly, in day 30 it is decreased by -25.52% when compared to respective control group.

It was increased in AMAE and AMEE treated protective groups against NaF as compared to NaF alone treated. AMEE treated group exhibited superior efficacy than AMAE treated group.
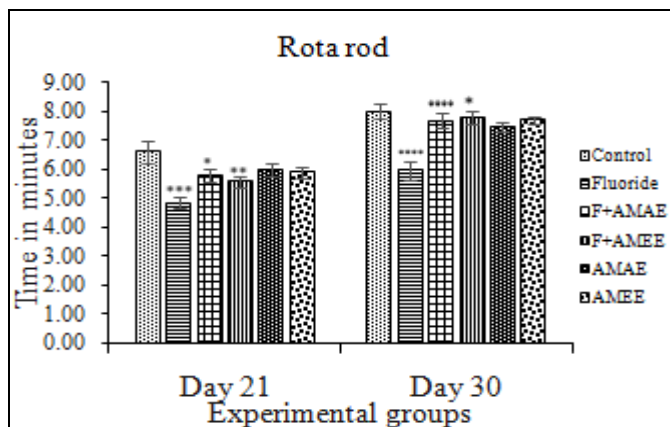
FIG. 1: EFFECT OF ABELMOSCHUS MOSCHATUS SEED EXTRACT ON MOTOR CO-ORDINATION OF RAT PUPS (OF POST – NATAL DAY 21 AND POST-NATAL DAY 30) EXPOSED TO NaF) Each bar denotes the mean value, and error bars represent standard error of mean (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of P < 0.01, ** for the p-value of P < 0.05, *** for the p-value of P < 0.001, and **** for the p-value of P < 0.005.

Hot Plate Test: The threshold intensity of stimulus for thermal sensitivity was conspicuously increased (P < 0.001) in NaF administered group (**Fig. 2**) by 44.38% in Day 21 pups with compared to control pups of respective age group and it was 48.45% on Day 30 rats. The response to thermo nociceptive

pain in AMAE and AMEE treated groups towards NaF toxicity was found to be reversed. AMEE treated group showed better efficacy than AMAE treated group.
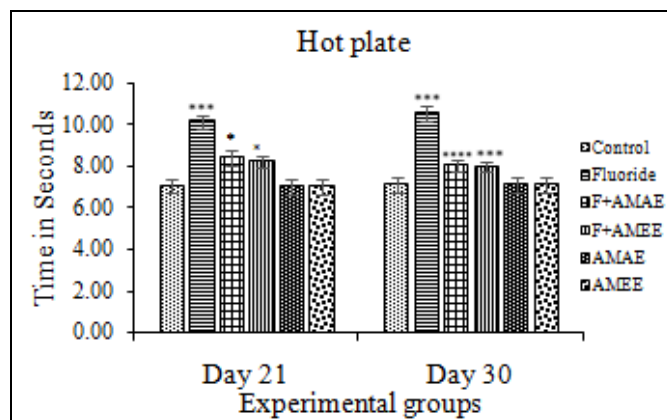
FIG. 2: EFFECT OF *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON THERMOCEPTION OF RAT PUPS (OF POST-NATAL DAY 21 AND POST-NATAL DAY 30) EXPOSED TO NaF
Each bar denotes the mean value, and error bars represent standard error of mean (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of P < 0.01, *** for the p-value of P < 0.001 and **** for the p-value of P < 0.005.

Oxidative Stress Markers: Lipid per-oxidation and catalase were assessed.

Lipid Per-oxidation (LPO): Significant increased LPO levels were found in brain tissues of NaF exposed pups of all age groups when compared to control group of respective age. Noticeably diminished levels of LPO were found in AMAE and AMEE towards NaF fed rats. AMAE exhibited better protective effect than AMEE. (**Table 2**)

Catalase: Reduced catalase activity was noticed in the NaF received rats with compared to control. Simultaneous treatment of AMAE and AMEE restored the catalase activity. AMAE presented better result than AMEE. (**Table 3**)

Protein Estimation of Brain: Decreased protein content (**Fig. 3**) in the brain tissue of NaF intoxicated pups was observed when compared to control group of rats. Protein content was reverted in AMAE and AMEE treated towards control with compared to NaF alone treated group.

TABLE 2: EFFECT OF *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON LPO LEVELS IN BRAIN TISSUE OF RAT PUPS (FROM POST-NATAL DAY 1 TO POST-NATAL DAY 30 WITH ONE WEEK INTERVAL) EXPOSED TO NaF

	Control	Fluoride	% of change from control	F+AMAE	% of change from control	F+AMEE	% of change from control	AMAE	% of change from control	AMEE	% of change from control
Day 1	0.84± 0.023	1.19± 0.022***	41.66%	0.95± 0.036***	13.09%	1.07± 0.01***	27.38%	0.85± 0.026	1.19%	0.85± 0.03	1.19%
Day 7	0.86± 0.022	1.14± 0.045****	33.73%	1.07± 0.051***	24.41%	1.08± 0.059*	25.58%	0.84± 0.016	1.20%	0.85± 0.029	2.40%
Day 14	1.05± 0.057	1.32± 0.038*	25.71%	1.20± 0.054*	14.28%	1.20± 0.041*	14.28%	1.00± 0.03	-4.76%	1.01± 0.02	-3.80%
Day 21	1.21± 0.065	1.57± 0.062***	29.75%	1.34± 0.024*	10.74%	1.32± 0.024*	9.09%	1.21± 0.05	0%	1.19± 0.048	-1.65%
Day 30	1.26± 0.03	1.60± 0.062*	26.98%	1.38± 0.031*	9.52%	1.40± 0.014***	11.11%	1.31± 0.034	3.96%	1.32± 0.042	4.76%

Data presented as mean ± S.E.M. (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of $P < 0.01$, *** for the p-value of $P < 0.001$, **** for the p-value of $P < 0.005$. LPO levels expressed as Nano-mole MDA/gm weight of tissue.

TABLE 3: EFFECTS OF *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON CATALASE ACTIVITY IN BRAIN TISSUE OF RAT PUPS (FROM POST-NATAL DAY 1 TO POST-NATAL DAY 30 WITH ONE WEEK INTERVAL) EXPOSED TO NaF

	Control	Fluoride	% of change from control	F+AMAE	% of change from control	F+AMEE	% of change from control	AMAE	% of change from control	AMEE	% of change from control
Day 1	0.37± 0.019	0.27± 0.023*	-27.02%	0.34± 0.019***	-8.10%	0.30± 0.017*	-18.91%	0.36± 0.01	-2.70%	0.35± 0.007	-5.40%
Day 7	0.45± 0.023	0.35± 0.012***	-22.22%	0.39± 0.026**	-13.33%	0.41± 0.025*	-8.88%	0.43± 0.023	-4.44%	0.42± 0.025	-6.66%
Day 14	0.48± 0.026	0.36± 0.028***	-25.00%	0.43± 0.026*	-10.41%	0.41± 0.03*	-14.58%	0.47± 0.029	-2.08%	0.46± 0.029	-4.16%
Day 21	0.57± 0.027	0.44± 0.022***	-22.80%	0.49± 0.025*	-14.03%	0.49± 0.026*	-14.03%	0.57± 0.037	0%	0.55± 0.027	-3.50%
Day 30	0.61± 0.029	0.48± 0.04***	-21.31%	0.57± 0.026*	-6.55%	0.55± 0.028*	-9.83%	0.59± 0.03	-3.27%	0.59± 0.029	-3.27%

Data presented as mean ± S.E.M. (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of $P < 0.01$, ** for the p-value of $P < 0.05$, *** for the p-value of $P < 0.001$. CAT activity expressed as µ mole/min/mg of tissue.
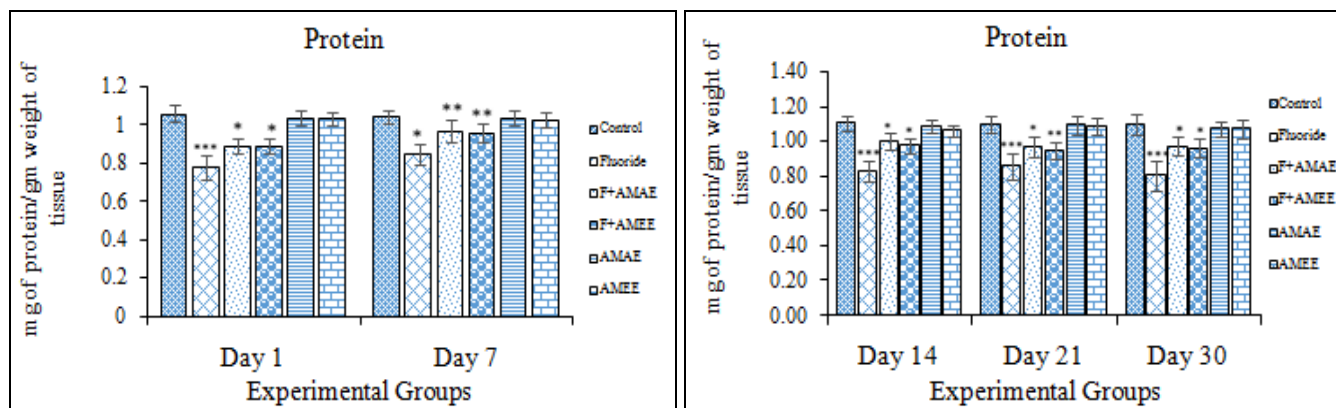
FIG. 3: EFFECT OF *ABELMOSCHUS MOSCHATUS* SEED EXTRACT ON PROTEIN CONTENT OF BRAIN TISSUES OF RAT PUPS (FROM POST-NATAL DAY 1 TO POST-NATAL DAY 30 WITH ONE WEEK INTERVAL) EXPOSED TO NaF
Each bar denotes the mean value, and error bars represent standard error of mean (n = 5). Data exposed to one-way ANOVA and t-test to determine the statistical differences between groups. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-value of $P < 0.01$, ** for the p-value of $P < 0.05$, *** for the p-value of $P < 0.001$. Units: protein content in the brain tissue expressed as mg of protein/gm weight of tissue.

DISCUSSION: The present study demonstrated that the protective effects of *Abelmoschus moschatus* seed extract against pre- and post-natal NaF fluoride induced behavioral and neurochemical milieu in albino wistar rat. Fetus is not well protected against fluoride that circulates in the maternal blood. This is due to placenta does not block the passage of fluoride from maternal circulation to foetal circulation [32] and poorly developed blood brain barrier (BBB). The poorly formed BBB vasculature fails to control the entry of fluoride into the brain [6].

Further, the research reports also demonstrated that the fluoride also transferred to the infant through human breast milk [32]. That means during foetal life and early infancy, the blood brain barrier provides only partial protection against the entry of chemicals into the central nervous system [33, 34]. Thus the fetus and new born pups are much vulnerable to fluoride. In the present research, the results have shown that exposure to 20 ppm of NaF during pre- and post-natal and lactation period affect the body weight, brain weight, and brain somatic index, and decreased behavioral response measured on rota rod and hot plate apparatus. And also found that the increased levels of LPO levels and decreased activity of catalase and protein content of brain tissues of fluoride intoxicated rat pups. These all were reversed on treatment of AMAE and AMEE along with NaF.

Excessive fluoride ingestion significantly decreased animal growth evidenced by the depressed appetite, decrease in the rate of feed and water consumption that eventually lead to the poor growth rate in pups, witnessed by decrease in the body weight, brain weight and BSI [8]. The ingested fluoride not only disturbed nutrient digestibility (absorption) but also attributed to weight loss, degeneration of structure of organs, and decreased protein and lipid levels, which may be the prime factor in causing fluoride neuro-toxicity. Decreased organ and body weight was noticed on NaF treatment in group of animals [35]. And also decreased organo-somatic index (OSI) with loss of body weight and decreased protein content was found in brain tissue.

Shashi [36] found significant decreased in acidic, basic, and total proteins in rabbits treated with NaF for 100 days. A decline in protein content occurred in various soft tissues of rodents treated with different doses of NaF after 30 to 70 days [37]. The results showed that the administration of AMAE and AMEE along with NaF showed the increased body weight, brain weight and BSI. Treatment with the 60 mg/kg of quercetin, rutin or okra showed a significant reversal in their body weights when compared with the dexamethasone treated group [38]. The phyto-chemical studies showed that the okra pods contains high ash content which would provide essential valuable and useful minerals needed for body growth [39]. *A. esculentus* peel and seed powder increases body weight [40].

Behavioral assays are important in toxicology, teratology, phenotype screening, and in other applications that require animals' functional status to be measured. When assessment involves infant animals, it can be especially challenging because an immature animal is often more fragile, prone to fatigue, has limited sensory and motor capabilities, and may be differentially responsive to standard challenges such as water or food deprivation. Fluoride exposed rat pups showed the decreased latencies and frequent falls from the rod with compared to control group pups. On the contrary, F+AMAE, and F+AMEE treated group of pups were showed increased latencies and infrequent falls from the rod. And AMAE and AMEE alone treated pups were displayed similar latency and fall off times of controlled pups.

These results demonstrating that the higher centers of brain were protected from fluoride-induced damage. The significant decline in endurance time of fluoride-exposed animals compared with the control. This is presumably due to its negative impact of fluoride on the cerebellum [21]. An inhibition of spontaneous motor activity and rota-rod performance in adult rats [21, 41] and mice [42] treated with NaF for 60 days indicates that motivation of these animals has been impaired by fluoride.

A deficiency in these behaviors may lead to suppression of eating process. Thus a behavioral impairment has been accounted by these investigators for diminished food ingestion in rats and mice treated chronically with NaF. Thus NaF exposed pups showed decreased body weight, brain weight and also BSI. F-induced inhibition of motivated behavior has been accompanying with the locomotor behavioral deficit that occurred in workers who have chronic exposure to high level of industrial F [43]. Moreover, increased time to respond thermoceptive pain on hot plate was noted in NaF intoxicated pups which was reverted in AMAE and AMEE treated rats. Fluoride exposure during gestational period significantly exacerbated the levels of LPO along with the decreased activities of antioxidant enzyme like catalase.

The results observed were in agreement with the previous findings [11, 44]. The elevated lipid peroxidation in fluorosis may result from the

decreased activities of antioxidant enzymes [45]. The brain is susceptible to oxidative stress. This is because of the presence of high levels of polyunsaturated fatty acids, relatively low antioxidant capacity, the presence of redox metal ions and high oxygen utilization [2, 46]. Further, the oxidative stress not only increases free radical damage but also boosts excito-toxicity, since ROS, reactive nitrogen species and lipid peroxidation products can trigger the excito-toxic process [47, 48].

Administration of natural plant extracts to fluoride intoxicated pups alleviated the altered cascade of free radical damage and / or oxidative stress [2]. AMAE and AMEE along with NaF treated pups showed the reversal levels of LPO and catalase activity. Free radical scavenging activity is the most important mechanism by which antioxidants inhibit lipid peroxidation [49]. The present results stand evident for fluoride activity was decreased with increase in the age. This is because of either increasing complexity of BBB with respect to the age of rats or simultaneous administration of AMAE and AMEE, nullifying production of free radicals from fluoride. Inhibition in the docking phenomenon of free radical and excitotoxicity of NaF by antioxidant properties of AMAE and AMEE produces a decline in NaF toxicity.

The antioxidant activity of phenolics (AMAE and AMEE) is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [50]. Natural polyphenols exert their advantageous health properties by their antioxidant activity, these compounds are capable of eliminating free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α- tocopherol radicals and inhibit oxidases [51]. Okra is abundant with several vitamins, minerals and nutrients that handles the health advantages the plant provides [52]. Occurrence of hyperoside, quercetin, coumarin scopoletin, uridine, and phenylalanine is reported by several authors [53 - 54]. Shui and Peng [55] have reported that quercetin derivatives and (−)-epigallocatechin as chief antioxidant compounds in okra. 70% of the total antioxidant activity comes due to the quercetin derivatives (quercetin 3- O- xylosyl (1″→2″) glucoside, quercetin 3- O- glucosyl (1″→6″) glucoside, quercetin 3-O-glucoside and quercetin 3-O- (6″-O-malonyl) - glucoside). Liao [52] reported a new flavonol glycoside named 5, 7, 3', 4'-tetrahydroxy- 4″- O- methyl flavonol- 3- O- β- D-glucopyranoside. The dietary supplementation of above mentioned quercetin derivatives have an important role in alleviating fluoride induced toxic effects in brain of developing rat pups.

Decreased protein content in brain of fluoride intoxicated rats is due to either increased breakdown of proteins or decreased synthesis of it. Moreover, inhibition of oxidative decarboxylation of branched chain amino acids is supposed to be involved in protein breakdown in fluorosis [56]. Additionally, decreased brain protein contents in fluoride exposed animals may result from decreased ability of brain tissue to synthesize amino acids as a result of suppressed activity of certain metabolic enzymes like glutamine synthetase and methionine activating enzymes [57]. Ge *et al.,* [58] observed down-regulation of the G protein in the brains of rats exposed to high F concentration (100 mg/LF) in attempt to avoid overstimulation by this ion. The fluoride ions act as enzymatic poisons, interrupting protein synthesis, increasing glycolysis, activating anti-oxidative pathways, these changes may decrease brain weight and body weight.

Emblica officinalis fruit as a food supplement is beneficial in reducing fluoride induced alterations in body and brain weight and recovery of health of the animal [59]. *E. officinalis* is a good antioxidant, prevent the production of F ions thus the normal role of enzymes involved in protein synthesis helps in normal body and organs' mass. Natural antioxidants play an important protective role against the fluoride, indicating that the dietary intake of antioxidant rich foods protect from neurodegeneration of fluoride induced [60].

Thus the neuroprotective effect of seed extract may be because of afore said mediatory mechanisms.

CONCLUSION: The present study concludes that the developing brain is more prone to NaF exposure due to poorly formed BBB and less established antioxidant system. Free radicals generated by NaF interfere with protein synthesis and disturb antioxidant system. These alterations led to decrease in the rate of feed and water consumption that eventually lead to the poor

growth rate in pups, witnessed by decrease in the body weight, brain weight and BSI. Ultimately, these changes led to neurobehavioral alterations. AMAE and AMEE possess quercetin derivatives which reduce the production of free radicals and provide rich nutritional value to the diet, thus reversing the neurotoxic effect of NaF.

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