Availableonlineatwww.jcsonline.in

Journal of Current Science &Humanities 11(4),2023,1-10.

ImpactFactor-2.05

PROTECTIVEEFFECTOFOKRA,*ABELMOSCHUSMOSCHATUS*SEEDEXTRACTONDEVELOPINGBRAIN OFRATSDURING PRE-AND POST-NATALFLUORIDE EXPOSURE

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ABSTRACT: This study reports protective effect of *Abelmoschusmoschatus*seedextractagainstsodiumfluoride(NaF)inducedalterationsinbehaviorcorrelated with neurochemical changes in developing brain of rats. Excessiveintake of fluoride during pregnancy cross the blood brain barrier (BBB) andcause adverse effects on neonatal development. As the BBB in fetuses, neonatesand infants is immature, it cannot provide protection against the entries. Thepregnant wistar rats were randomly categorized into six groups of five animalseach. Group I is of control rats received normal tap water. Group II is NaFexposed 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 weretreatedwith*A.moschatus*ethanolicextract(AMEE)(300$mgkg^{-1}$bodywt./day/rat) along with NaF water (20 ppm).Group V and VI rats were treatedwithAMAE(300$mgkg^{-1}$bodywt./day/rat),andAMEE(300$mgkg^{-1}$bodywt./day/rat) respectively. On 1^{st}, 7^{th}, 14^{th}, 21^{st} and 30^{th} day (postpartum days), thepupsweresacrificedtoassessoxidativestressmarkers(LPO,CAT),andmeasured body weight, brain weight, BSI, and estimated protein content of braintissueofallexperimentalgroups.Onpost-natalday21andday30pupsbehavioral activity (rota rod, hot plate test) was measured. Fluoride exposuresignificantly increased lipid peroxidation and decreased the activity of catalase,anddecreasedbodyandbrainweight,BSIandalsoproteincontentofthebrainof pups indicating oxidative stress and inhibited antioxidant system and proteinsynthesis which were reverted on administration of AMAE and AMEE againstNaF intoxication. The altered behavioral responses on NaF exposure were alsoreversedtothatofcontrol.Hence,thisstudyprovesthevulnerabilityofdevelopingbraintofluoridetoxicityduringdevelopmentandgrowthandprotectionoffered byAMAEandAMEE towards neurotoxicityofNaF.

Keywords: Sodium fluoride,Oxidativestress,Neurotoxicant,Abelmoschus,Behavior

INTRODUCTION: Prolongedingestionoffluoride through various sources, mainly drinkingwatercausedfluorosis[1].Fluorosis,asaglobalpublic health problem, has been receiving wide anddeservingattention in recentyears.Prolonged consumption of fluoride in excess duringgestationcauseadverseeffectsonneonataldevelopment [2]. The mechanisms of maternal fetaltransmission offluoridearepoorlyunderstood.

Therefore, the present study focused on maternalexposure of fluoride and it's effects on developingCNSandit'sameliorationwithextractofokraseeds. Fluoride is known to cross the placenta [3,4]and the blood brain barrier [5] and ultimately increasefluoride levels in fetal brain tissue. The blood brainbarrierintheembryo,fetus,andnewbornis"immature"thatmeansitispoorlydeveloped,leaky, orevenabsent,renderingthedevelopingbrain more vulnerable to fluoride entering the fetalcirculation from the mother [6]. Once it enter into thebrain it can cause adverse effects on the brain cellultra structure, metabolism, enzymes, the oxidant-antioxidantstatusandonneurotransmittersandthus overall metabolism of brain [7,8]. The maternalexposure to fluoride and fluoride insobriety at theearlyperiodsoflifeisshowntocausemoreprominent effects on the oxidant-antioxidant statusin the brain than the fluoride exposure at the laterstages of life [9,10]. Fluoride increases production ofreactive oxygen species (ROS), lipid peroxidation,anddecreasedcatalaseactivitywhichhavebeenconsideredinpathogenesisandrecentstudiessuggest that oxidative stress is a possible pathologicmediatingfactor in fluoride

toxicity[11,12].

Exposuretofluorideduringearlydevelopmentalstagesofliferesultsinlong-termirreversibleconsequences with their structure and function andaccountfor qualitativedifferencesinage relatedsusceptibility [13]. The neuronal growth-spurting inrats occurs in the first 3 weeks of postpartum [14].Duringthisperiodthebrainundergoesmanyprofound structural and functional transformations,whichmakesvulnerableto fluoride [15].

The high intake of plant products is associated witha reduced risk of a number of chronic diseases [16].Earlierreportsonnaturalproductswhichaidinprotectionagainst NaFinducedbehavioralalterationsare :Curcumin[17],Quercetin[18],Rutin(extracted compound of Okra) [19], Vitamin A [20], and*Spirulinaplatensis*[21].Thesenaturalproductsreverse behavioral alterations which are induced onNaF exposure. These beneficial effects have beenmoderatelyattributedtothecompoundswhichpossessantioxidant activity.

*Abelmoschusmoschatus*L., (Okra) is one of themost commonly known and utilized species of thefamilyMalvaceae.Themajorantioxidantsofvegetables are vitamins C and E, carotenoids, andphenoliccompounds,especiallyflavonoids[22].Nutritionally,therichestpartoftheokraplantisthe dried seed. Okra seed is rich in high qualityprotein especially with regard to its essential aminoacids [22] and unsaturated fatty acids such as linoleicacid [22]. Seeds are rich in phenolic compounds withderivatives,catechinoligomersandhydroxyl-cinnamic derivatives. The nutrients content of Okraseed showed that 21% protein, 14% lipids and 5%ash. Proteins play a particularly important role inhuman nutrition [22]. Okra is a popular health fooddue to its high fiber, Vitamin C, and folate contentand also a good source of calcium and potassium.Highfiber,"helpstostabilizebloodsugar byregulating the rate at which sugar is absorbed fromtheintestinaltract"[23].Okraseedswillmakeavailabletheessentialenergytothebodyandimportantantioxidantsthatcouldboostimmunesystem and prevent diseases [24]. The neuroprotectanteffects of extract of Okra against fluoride inducedbehavior and neurodegenerative changes in brain ofadult rat were reported in our earlier studies [25]. Inview of this, the efficacy of AMAE and AMEE inreducingfluorideinducedneurobehavioral,andneurochemical alterations in rats of pre and post-natalfluoride exposedisreported inthis paper.

MATERIALANDMETHODS: Healthyalbinowistarrats(*Rattusnorvegicus*)weightedbetween180to200gmwereassignedrandomlytoexperimental groups and housed 2 females and 1maleforcage(polypropylenecages)toallowbreed.Lightcycleswereremaintainedas12Light:12Darkhours(6.00AMto6.00PM),andtemperaturebetween22±2^{o}C.Theratsweremaintained on standard diet and water was supplied*ad libitum.* After their pregnancy was confirmed,from the first day pregnancy, the females made intosixgroupsandallowedtodrinkonfluoridated waterattherateof20ppmNaFandalso*Abelmoschusmoshatus*seed extract was given tothe rats at the rate of 300 mgkg^{-1} body weight asfollows;

ExperimentalGroups:

- **GroupI:**Receivednormaltapwater
- **Group II:** Fed on fluoridated drinking water(20ppm)
- **GroupIII:**NaF(20ppm)+*Abelmoschusmoschatus*seedaqueousextract(AMAE)(300mgkg^{-1} b. wt.)
- **GroupIV:**NaF(20ppm)+*Abelmoschusmoschatus*seedethanolicextract(AMEE)(300mgkg^{-1} b. wt.)
- **GroupV:***Abelmoschusmoschatus*seedaqueous extract (AMAE) (300 mgkg^{-1} b. wt.)only.

- **GroupVI:***Abelmoschusmoschatus*seedethanolic extract (AMEE) (300 $mgkg^{-1}$ b. wt.)only.

Chemicals: Potassiumdichromate,glacialaceticacid,Thiobarbituricacid(TBA),H_2O_2,sodiumcarbonate, sodium potassium tartrate, Folin reagent*etc.* were purchased from local laboratory chemicalsuppliers.

PhysicalParameters:

Body Weight: The rat pups were weighted at theage of post-natal Day 1, Day 7, Day 14, Day 21 andDay30 and noted down thereadings.

BrainWeight: Thesamepupswerekilledbydecapitationandtheirbrainsweredissectedout.Thesebrainsweightedand noteddownthevalues.

Brain Somatic Index (BSI): For calculating thebrain - somatic index, the weight of the individualrat was noted and the brain was removed carefullyand weighed on an electronic weigh balance afterremovingthebloodwithtissuepaper.BSIwascalculated by using the value of brain weight ofcontrol rat divided by the same rat body weightvalue and multiplied with 100 (brain weight / bodyweight×100).Thisformulawasappliedtoallexperimental groups to calculate BSI. The Brain - SomaticIndexof the ratpupswere calculatedbythe useof equation citedbyAshwini[26].the rats was measured as maximal time spent on therod at 30 rpm before falling off and endurance timenoted in min [27].

HotPlateTest: Thehotplatetestisusedforevaluating thermal pain sensitivity. The hot platetest evaluates thermal pain reflexes due to footpadcontactwithaheatedsurface.Anapparatusconsisting of an aluminium plate that is heated andcooled by Peltier elements in contact with its lowersurface.Duringtheexperiment,theratwasintroduced into Remi hot plate kept at 52 ± 0.5 °C.Hot-plate latencies are determined as described byplacing each rat pup on a hot plate and observingthe occurrence of a nociceptive response (licking ofa hind paw, jumping). The time of exhibiting thesebehaviors was noted as response time and recordedinseconds.Toobservetheratbehavior,observation area covered with a colourless plasticcylinder was placed on the hot-plate. In order toavoid possible tissue injury, a cut-off time of 12seconds was used to avoid skin damage. One hotplatetest was carried outforeach rat[28].

OxidativeStressMarkers: Lipidper-oxidationandcatalasewere assessed.

Lipidper-oxidation(LPO): TheLPOwasassessed by the method of Bhuyan[29]. This assay isbased on the reactivity of an end product of lipidperoxidation,malondialdehyde(MDA)with TBA

BrainSomaticIndex=Brainweight

Bodyweight×100

BehavioralAssessments:to produce a pink adduct, which is measured at 533nmonspectrophotometer.TheLevelsoflipidperoxidationproductsexpressedasμmolofMDA/gr. weight of tissue.

RotaRod: The rota rod test, in which rat pupsmust balance on a rotating rod, is widely used toassessmotordeficit in neurodegenerativedisease models (in rodents). Performance is measuredby the duration that an animal stays on the rod as afunction of rotating rod speed. In the present study,theinstrument(Dolphin™instruments)hadincrementalfixed-speed (about30rpm)rotatingrod.Beforeexperimentaltesting,ratpupsweretrained to run on the rotating rod in 3 training trialsper day for three consecutive days with a constantspeed of 30 rpm. For this purpose, animals werekept on the rotating rod for 2 min, and time of theirfirst falling off and the frequency of falling off therodarerecordedforeachrat.Afterthetrainingperiod,onthedayoftesting,theperformanceof

Catalase: CatalaseactivitywasestimatedbymeasuringtherateofdecompositionofH_2O_2.Disappearanceofperoxideinthepresenceofenzymesourceisthebasisforassaywhichisfollowedspectrophotometricallyat505nm.Oneunitdecomposesone micromoleofH_2O_2perminute [30].

ProteinEstimationofBrain: Braintissuetotalproteincontentwasestimatedbythemethodof Lowry [31]. The principle of Lowry method

isthepeptide nitrogen(s) with the copper [II] ions underalkaline conditions and the subsequent reduction oftheFolin-Ciocalteauphosphomolybdicphosphotungstica cidtoheteropolymolybdenumbluebythecopperc atalyzedoxidationofaromatic

acids.Thecolorwasreadat540nm.Proteincontentint hebraintissueexpressedasmgofprotein/ gm weight of tissue.

Per cent of change between groups was calculatedbythe formulaof;

RESULTS:

Body Weight: The body weight was progressivelydecreased (**Table 1)** in NaF treated rats from post-natal day 1 to post-natal day 30 rats as compared tocontrol.Amongtheseexperimentaldaysthema ximumpercent(-14.34%)ofdecreasedbody

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

weightwasrecordedinpost-natalday30NaFtreatedone.

DataAnalysis: Statisticalsignificancewasdetermin edbyonewayanalysisofvariance.Differences between means were determined by t-test.

Furthermore, the reverted body weight was noticedin F+AMAE and F+AMEE compared to fluoridealone treated group of respective age and it wassimilarto 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)EXPOSEDTONaF

Dayinterval	Organ (brain, body)	Control	Fluoride	% ofchangefromcontrol	F+AMAE	% of changefromcontrol	F+AMEE	% of changefromcontrol	AMAE	% of changefromcontrol	AMEE	% of changefromcontrol
Day1	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%
Day7	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%
Day14	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%
Day21	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%
Day30	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 nosignificant change in case of mean value of control group and experimental group. Data presented as mean ± S.E.M. (n = 5). Data exposed toone-way ANOVA and t-test to determine the statistical differences between groups. t-test was conducted in all possible combinations tocompare significance of data. Superscript symbols indicated significant differences observed from experimental groups; * denotes the p-valueofP<0.01,**for thep-valueofP < 0.05,***forthep-valueofP<0.001,andnarepresentnon-significantdifferencebetweengroups.

Brain Weight: The brain weight was decreased inNaF intoxicated rats when compared to control asshowninthe**Table1**.Butthereisnouniformdecreaseinthebrainweightofexperimentallytreated rats. The reverted brain weight was reportedin F+AMAE and F+AMEE compared to fluoridealonetreatedgroupofrespectiveage.AMAEand

AMEEreceivedratsbrainweightissimilartocontrol.

Brain SomaticIndex(BSI): DecreasedBSI(**Table1)**wasobservedinDay1(-7.02%),Day7 (-3.43%),Day14(-4.35%),Day21(-2.12%)and Day30(-9.12%)agedpupsofNaFintoxicated

when compared to control group of respective agedpups. Among these day 30 post-natal rats showedthe highest per cent of decreased BSI. AMAE andAMEE treated groups against NaF exposed pupsshowedthereversedBSIascomparedtoNaFgroup.

Furthermore,AMAEandAMEEalonetreatedgroups BSIis same as thatof control.

Rota Rod Test: Decreased latencies and repeatedfall offs (**Fig. 1)** from the rotating rod of rota rodapparatuswerenoteddowninNaFintoxicatedpups than control. In day 21 experimental groupsthe per cent of decreased retention time is -21.69%in NaF and similarly, in day 30 it is decreased by -25.52%whencomparedtorespectivecontrolgroup.

ItwasincreasedinAMAEandAMEEtreatedprotective groups against NaF as compared to NaFalonetreated.AMEEtreatedgroupexhibitedsuperiorefficacythan AMAE treated group.

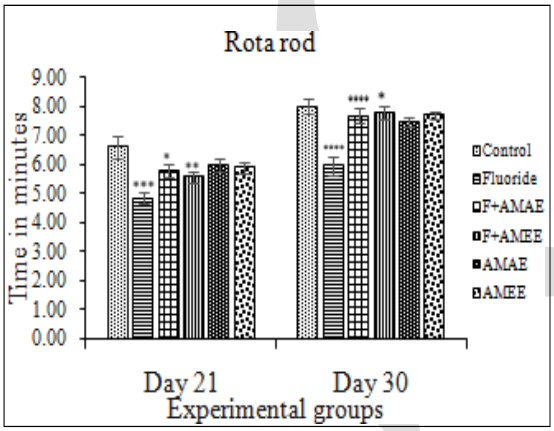

FIG.1:EFFECTOFABELMOSCHUSMOSCHATUSSEED EXTRACT ON MOTOR CO-ORDINATION OFRATPUPS(OFPOST–NATALDAY21ANDPOST-NATAL DAY 30) EXPOSED TO NaF) Each bar denotesthe mean value, and error bars represent standard error ofmean (n = 5). Data exposed to one-way ANOVA and t-test todeterminethestatisticaldifferencesbetweengroups.Superscriptsymbolsindicatedsignificantdifferencesobserved from experimental groups; * denotes the p-valueofP < 0.01, ** for the p-value of P < 0.05, *** for the p-value ofP<0.001, and ****forthe p-valueofP< 0.005.

Hot Plate Test: The threshold intensity of stimulusfor thermal sensitivity was

conspicuously increased($P < 0.001$) in NaF administered group (**Fig. 2)** by44.38% in Day 21 pups with compared to controlpups of respective age group and it was 48.45% onDay30rats.Theresponsetothermonociceptivepain in AMAE and AMEE treated groups towardsNaFtoxicitywasfoundtobereversed.AMEEtreated group showed better efficacy than AMAEtreated group.

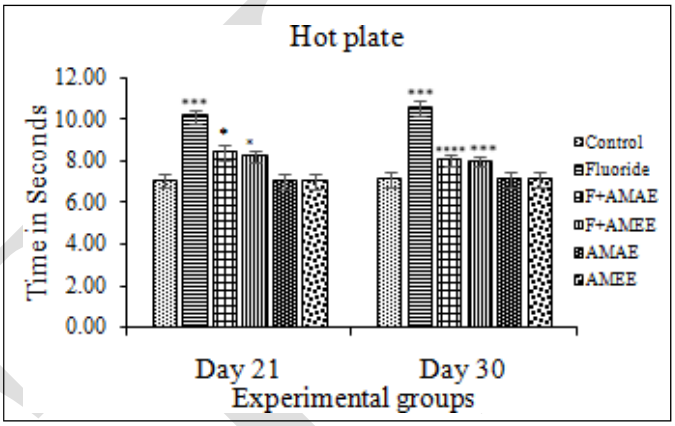

FIG.2:EFFECTOF*ABELMOSCHUSMOSCHATUS*SEEDEXTRACTONTHERMOCEPTIONOFRATPUPS (OF POST-NATAL DAY 21 AND POST-NATALDAY30) EXPOSED TONaF

Each bar denotes the mean value, and error bars representstandard error of mean (n =5). Data exposed to one-wayANOVAandt-testtodeterminethestatisticaldifferencesbetweengroups.Superscriptsymbolsindicatedsignificantdifferences observed from experimental groups; * denotes thep-valueofP<0.01,***forthep-valueofP<0.001and ****forthep-valueofP<0.005.

OxidativeStressMarkers: Lipidper-oxidationandcatalasewere assessed.

Lipid Per-oxidation (LPO): Significant increasedLPOlevelswerefoundinbraintissuesof NaFexposed pups of all age groups when compared tocontrolgroupofrespectiveage.Noticeablydiminished levelsofLPO were found inAMAEand AMEE towards NaF fed rats. AMAE exhibitedbetterprotectiveeffect thanAMEE. (**Table 2)**

Catalase: Reduced catalase activity was noticed inthe NaF receivedratswithcomparedtocontrol.SimultaneoustreatmentofAMAEandAMEErestoredthecatalaseactivity.AMAEpresentedbetterresult than AMEE.(**Table3)**

Protein Estimation of Brain: Decreased proteincontent(**Fig.3**)inthebraintissueofNaFintoxicated pups was observed when compared tocontrol group of rats. Protein content was revertedin AMAE and AMEE treated towards control withcomparedto NaFalonetreatedgroup.

TABLE2:EFFECTOF*ABELMOSCHUSMOSCHATUS*SEEDEXTRACTONLPOLEVELSINBRAINTISSUEOFRATPUPS(FROMPOST-NATAL DAY 1TOPOST-NATAL DAY30WITHONEWEEKINTERVAL) EXPOSEDTO NaF

	Control	Fluoride	% of changefrom control	F+AMAE	% of changefrom control	F+AMEE	% ofchange fromcontrol	AMAE	% ofchange fromcontrol	AMEE	% of changefrom control
Day1	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%
Day7	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%
Day14	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%
Day21	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%
Day30	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-valueofP<0.001,****forthep-valueofP<0.005.LPOlevels expressedasNano-mole MDA/gmweightoftissue.

TABLE3:EFFECTSOF*ABELMOSCHUSMOSCHATUS*SEEDEXTRACTONCATALASEACTIVITYINBRAINTISSUEOFRATPUPS (FROMPOST-NATALDAY1TOPOST-NATALDAY 30 WITHONEWEEK INTERVAL) EXPOSEDTONaF

	Control	Fluoride	% ofchange fromcontrol	F+AMAE	% of changefrom control	F+AMEE	% ofchange fromcontrol	AMAE	% of changefrom control	AMEE	% ofchange fromcontrol
Day1	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%
Day7	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%
Day14	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%
Day21	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%
Day30	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-valueofP<0.05, ***for thep-valueofP<0.001. CATactivityexpressedas µ mole/min/mgoftissue.

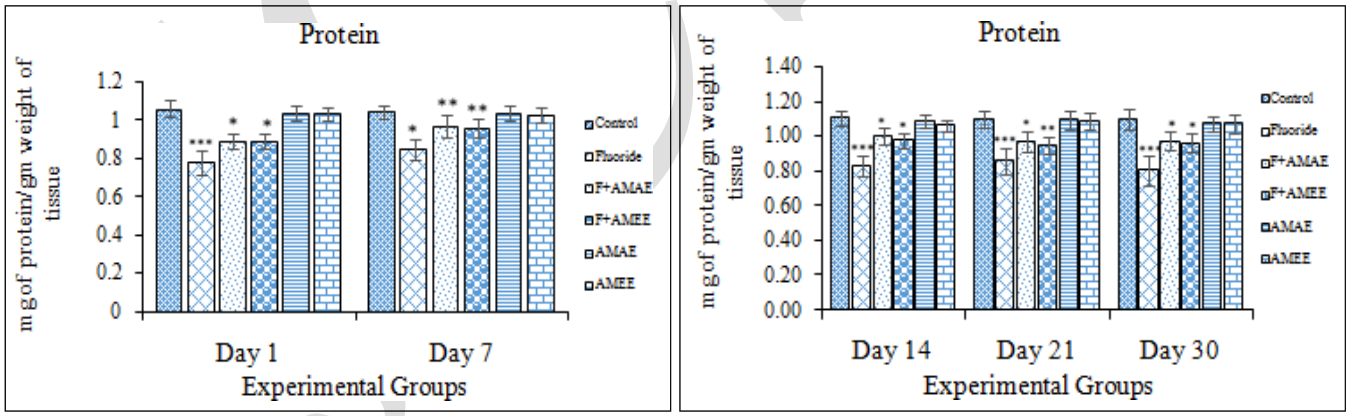

FIG.3:EFFECTOF*ABELMOSCHUSMOSCHATUS*SEEDEXTRACTONPROTEINCONTENTOFBRAINTISSUESOFRATPUPS (FROMPOST-NATALDAY 1 TOPOST-NATALDAY 30WITHONE WEEK INTERVAL) EXPOSEDTONaF

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 todeterminethestatisticaldifferencesbetweengroups.Superscriptsymbolsindicatedsignificantdifferencesobserved fromexperimentalgroups;
* 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 tissueexpressed asmgofprotein/gmweight oftissue.

DISCUSSION: Thepresentstudydemonstratedthat the protective effects of *Abelmoschusmoschatus*seedextractagainstpre-andpost-natalNaFfluorideinducedbehavioralandneurochemicalmilieuinalbinowistarrat.Fetusisnotwellprotectedagainstfluoridethatcirculates inthematernal blood. This is due to placenta does notblockthepassageoffluoridefrommaternalcirculation tofoetalcirculation[32]andpoorlydeveloped blood brain barrier (BBB). The poorlyformedBBBvasculaturefailstocontroltheentryof

fluoride into thebrain[6].

Further, the research reports also demonstrated thatthe fluoride also transferred to the infant throughhuman breast milk [32]. That means during foetal lifeand early infancy, the blood brain barrier providesonlypartialprotectionagainsttheentryofchemicalsintothecentralnervoussystem[33,34].Thusthefetusandnewbornpupsaremuchvulnerable to fluoride. In the present research, theresults have shown that exposure to 20 ppm of NaFduringpre-andpost-natalandlactationperiodaffectthebodyweight,brainweight,andbrainsomatic index, and decreased behavioral responsemeasured on rota rod and hot plate apparatus. Andalso found that the increased levels of LPO levelsanddecreasedactivityofcatalaseandproteincontent of brain tissues of fluoride intoxicated ratpups.TheseallwerereversedontreatmentofAMAEand AMEEalong withNaF.

Excessive fluoride ingestion significantly decreasedanimal growth evidenced by the depressed appetite,decrease in the rate of feed and water consumptionthat eventually lead to the poor growth rate in pups,witnessed by decrease in the body weight,brainweight and BSI [8]. The ingested fluoride not onlydisturbed nutrient digestibility (absorption) but alsoattributed to weight loss, degeneration of structureof organs, and decreased protein and lipid levels,which may be the prime factor in causing fluorideneuro-toxicity. Decreased organ and body weightwasnoticedonNaFtreatmentingroupofanimals [35]. And also decreased organo-somatic index (OSI)withlossofbodyweightanddecreasedproteincontentwas found in brain tissue.

Shashi[36]foundsignificantdecreasedinacidic,basic, and total proteins in rabbits treated with NaFfor 100 days. A decline in protein content occurredinvarioussofttissuesofrodentstreatedwith different doses of NaF after 30 to 70 days [37]. Theresults showed that the administration of AMAEand AMEE along with NaF showed the increasedbody weight, brain weight and BSI. Treatment withthe 60 mg/kg of quercetin, rutin or okra showed asignificantreversalintheirbodyweightswhencompared with the dexamethasone treated group [38].The phyto-chemical studies showed that the okrapodscontainshighashcontentwhichwouldprovideessentialvaluableandusefulmineralsneeded for body growth [39]. *A. esculentus*peel andseedpowder increases bodyweight[40].

Behavioralassaysareimportantintoxicology,teratology,phenotypescreening,andinotherapplications that require animals' functional statusto be measured. When assessment involves infantanimals, it can be especially challenging because animmatureanimalisoftenmorefragile,pronetofatigue, has limited sensory and motor capabilities,andmay bedifferentially responsivetostandardchallengessuchaswaterorfooddeprivation.Fluorideexposed ratpupsshowedthedecreasedlatenciesandfrequentfallsfromtherodwithcompared to control group pups. On the contrary,F+AMAE,andF+AMEEtreatedgroupofpupswereshowedincreasedlatenciesandinfrequentfalls from the rod. And AMAE and AMEE alonetreated pups were displayed similar latency and falloffitimes of controlled pups.

These results demonstrating that the higher centersofbrainwereprotectedfromfluoride-induceddamage. The significant decline in endurance timeoffluoride-exposedanimalscomparedwiththecontrol.Thisispresumablyduetoitsnegativeimpactoffluoride onthecerebellum[21].Aninhibition of spontaneous motor activity and rota-rodperformanceinadultrats[21,41]andmice[42]treatedwithNaFfor60daysindicatesthatmotivation of these animals has been impaired byfluoride.

CONCLUSION: The results of this research show that the developing brain has a less developed antioxidant system and a poorly constructed BBB, making it more vulnerable to NaF exposure. The production of free radicals by NaF disrupts the antioxidant system and messes with protein synthesis. Because of these changes, feed and water consumption rates dropped, which exacerbated the poor Puppies' development rate is shown by the decline in their body weight, brain weight, and BSI. Neurobehavioral modifications were the end result of these changes. Both AMAE and AMEE include

quercetin derivatives, which counteract NaF's neurotoxic effects by lowering free radical generation and increasing dietary nutritional value.

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