RESEARCH ARTICLE

Insights of *In-silico* Neurotoxicity Studies of Glucuronolactone, Taurine and Gluconolactone Correlating the Induced Neuronal Alteration in Rat Pups

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ABSTRACT

Background: Significant concentrations of food additives found in energy drinks have the potential to be neurotoxic and promote oxidative stress, among other negative consequences. Pregnant rats were split up into six groups for the current study. Group 1 received vehicle, CAF standard (25 mg/kg p.o.), groups 3-6 received GLUR (5 mg/kg p.o.), TAU (8 mg/kg p.o.), GLU (84 mg/kg p.o.), and combinations of the three chosen food additives (CF), respectively. From prenatal day 3 through postnatal day 15, certain food additives were administered to pregnant rats at significant doses. After parturition on PND 21, behavioral changes were assessed using the Rotarod, active avoidance, and elevated plus maze tests. On PND 30, 45, and 60, rat brain tissue had its acetylcholine and epinephrine levels evaluated. Further, on days 30 and 60, brain tissue was assessed for the presence of oxidative stress markers such as lipid peroxidation, catalase, superoxide dismutase, and glutathione peroxidase. Finally, histopathological studies were carried out in brain hippocampal region. Further *in-silico* studies were carried out on selected receptors.

Results: Rat pups fed with food additives showed a significant ($p < 0.001$) change in behavior, including memory, cognition, and motor activity. Increased lipid peroxidation and decreased anti-oxidant enzymes were significant in TAU and CF groups. Further *in-silico* studies were carried out, where GLUR showed high binding affinity to specific receptor targets, GABA A and NMDA1 receptors, and specific enzyme targets MAO A and MAO B neurotransmitter metabolic enzymes compared to caffeine hinted the decrease in neurotransmitters as *in-vivo* studies.

Conclusion: The current findings support the hypothesis that the chosen dose and mix of food additives altered rat pups' neurobehavioral and neurotransmitter profiles.

Keywords: Energy drinks, Food additives, Neurobehavioural alterations, Neurotransmitters, Oxidative stress markers, Rat pups.

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BACKGROUND

Oxidative stress has been well-defined as destructive because oxygen-free radicals attack biotic particles such as lipids, proteins, and DNA. Oxidative stress is due to disturbance in the regulation of free radical and antioxidant enzyme (glutathione, superoxide dismutase, and catalase) balance. Free radicals consist of unpaired electrons which have responsible for cellular functioning. In our body, the brain is the ultimate utiliser of energy. An increase in the utilization of oxygen in the brain to form ROS results in increased oxidative stress leads to various CNS disorders.[1]

Reduced and oxidized glutathione shows a dynamic part in the determination of oxidative stress. The decrease in the antioxidant enzymes results in an increase in the superoxide radicals, leading to the degeneration of neurons which finally causes alteration in behavior.[2]

An increase in energy drinks consumption above the acceptable level result in toxic effects, was stated in earlier studies.

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But, the exact individual food additive responsible for oxidative consequences is not documented. Previous studies stated that oxidative stress may be due to the synergistic interaction of ingredients in energy drinks.[3] In the present study, selected food additives used in energy drinks were evaluated for individual and combination effects on oxidative stress markers and learning behaviors in animals after pre and postnatal treatment at high doses.

In-silico studies made possible the understanding of structural mechanisms involved by graphical correlation of biological activity. Over the last decade, several computational methods were used to interpret and assess the pharmacological hypothesis of drugs, chemicals, and food additives. These studies were used to characterize the binding sites structurally and to better understand the mechanism involved in ligand-receptor binding/ligand-enzyme binding. In recent reports, the prevalence of behavior disorders was high and risk assessment has increased.

Using computational methods, the hypothesis of neurobiology and drug affinity to bind with the various CNS receptors and enzymes made it possible to understand the molecular mechanisms. Based on the functional activities of CNS receptors, they are selected by researchers to interpret the drugs used in the treatment of CNS disorder or to interpret the toxic effects of drugs, chemicals and food additives.[4]

METHODS

Animals

The animal house of the MLR Institute of Pharmacy in Hyderabad provided pregnant Sprague Dawley (SD) albino rats that were between 160 and 180 days old—the MLR Institute of Pharmacy in Hyderabad. Institutional Animals Ethics Committee (IAEC) gave its approval to the experimental methods (CPCSEA/IAEC/PR3/2019).

Experimental Protocol

Each of the six groups, made up of four animals each, was created from all the pregnant rats between the ages of 160 and 180 days.[5] Each animal was given a newly made dosage that was dissolved in water and given through oral gauge.

Treatment

Treatment began on gestational day 3 (GD 3), and it lasted until postpartum day 15 (PN 15). Group 1 was kept on the vehicle, whereas CAF received caffeine (CAF) 25 mg/kg p.o., TAU with taurine (TAU) 8 mg/kg p.o. GLUR with glucuronolactone (GLUR) 5 mg/kg p.o, and GLU with gluconolactone 84 mg/kg p.o (GLU) and CF with all three additives.

Neurobehavioural Studies

Rota Rod Test

Rota rod test was used to evaluate the motor co-ordination and behavioral response on PND 32. Rats were allowed to make aware of the balance and co-ordination for 3 times, followed for three consecutive days with speed of rotation 4 rpm. Animals were positioned on the rod at 4 rpm on day 4, and the speed was constantly increased until the animal fell off, or up to 40 rpm in 300 seconds. The rat pups' latency time to fall was measured.[6,7]

Active Avoidance Test

On PND, 25 animal pups were placed in the wooden box to measure the active avoidance response. A long-length wooden box was partitioned into compartments with a door which can be opened and close. The shuttle box was set up with an automatic reflex conditioner. Animal pups were allowed to acquaint themselves for the environment for 2 minutes, with no conditioned stimulus. Following that, a buzzer at 670 Hz and 70 dB and a light at 21 W were turned on as conditioned stimuli (CS). Unconditional stimulus (US) 5 s was used to get this going. By using an electric scrambler, the US shocked the grid floor (1 mA for 4 s). The shuttle box's microcontroller recorded an avoidance reaction once the CS started, and this was thought to be a conditioned avoidance response to avoid the electrical shock. The animal avoided the US by rushing into the other compartment within 5 s. Each individual received 50 trials with a predetermined 15 second intertrial period. The number of avoidances was determined during the 50 trial session for the particular animal. To prevent shock therapy, the entire time it took each animal to reach the opposite compartment was calculated (latency of avoidance response in seconds).[8]

Elevated Plus Maze

Anxiety-like behaviors were measured using this test. It has a center chamber (10x10 cm) that is elevated to a height of 50 cm above the ground and is surrounded by two open arms and two closed arms (50x10 cm) with walls that are 40 cm high. Apparatus was arranged in plus shape. The animals were allowed to get habituated to the maze. On PND 30 animals were placed inside the maze in the middle chamber facing a closed arm. Each animal was tested for 5 mins. Entries with open arms are counted. It was timed how long the arm was outstretched.[6]

Measurement of Neurotransmitters

Adrenaline

To determine the amount of adrenaline present, 0.2 mL of the tissue extract's aqueous layer was obtained, and the methodology described by N. Mesram *et al.* (2017) was

Table 1: PDB IDs of targets used in molecular docking

S. No	*Name of target*	*PDB ID*
1	GABA(A) receptor	4COF
2	NMDA 1 (Glutamate receptor)	5I2N
3	Nicotinic receptor (α4,β2 nicotinic receptor)	5KXI
4	5-Hydroxytryptomine ($5HT_4$)	5EM9
5	MAO A	Z5X
6	Tyrosine hydroxylase	4J6S
7	Dopamine beta-hydroxylase	4ZEL
8	Phenylethanolamine N-methyl transferase	4MIK
9	MAO-B	2BK3
10	Glutamate dehydrogenase 2	6G2U

followed. The spectrofluorometer read the excitation and emission spectra between 410 and 500 nm.[9]

Acetylcholine

After homogenizing 10 mg of brain tissue, 1-mL of 3% $HClO^4$ was added. The homogenized tissue was then centrifuged for 10 minutes (1200 rpm). The supernatant was collected and utilized in the spectrophotometer for quantification at 540 nm.[9]

Estimation of Oxidative Stress Markers

The brains of 30 and 60 killed rats were separated, weighed, and kept at ice-cold temperatures.

Lipid Peroxidation[10]

Cerebral and hippocampal brain tissues were homogenated, centrifuged, and the supernatant was taken to measure the quantity of MDA, a secondary product of peroxidation of lipids. MDA was estimated by reaction with thiobutaric acid (TBA). The absorbance of pink color generated by the reaction was recorded at 533 nm in a spectrophotometer. Calculated as nanomoles MDA/gm of tissue weight.

Superoxide Dismutase[11]

It was assessed by removing the supernatant from the homogenate of the rat brain tissue's cerebral cortex. In this test, pyrogallol underwent an autooxidation process while being exposed to EDTA. At 420 nm, the spectrophotometer read the absorbance. Results are expressed as units per mg of protein**.**

Catalase

The rate of decomposition of H_2O_2 measures catalase action. The supernatant of homogenate of the cerebral cortex was used, and the changes were measured at an absorbance of 240 nm using a spectrophotometer. The results were expressed in milli moles H_2O_2/min per mg protein.

Glutathione Peroxidase (GSH Px)[11]

An antioxidant enzyme is GSH Px. GSH Px reduces hydrogen peroxide in the presence of the cofactor NADPH to GSH. The GSH Px activity, which decreased NADPH resembles, was detected at 340 nm and estimated as micromoles of GSH consumed per minute per milligram of protein.

Histopathological studies

Haematoxylin and Eosin Staining

Rats were killed on PND 90, and their brains were removed. After being fixed in 10% neutral buffered formalin for histopathology examinations, brain tissues were dried with a graded ethanol series. A 5 m thin transverse segment of the hippocampus was cut using a microtome, and paraffin-embedded tissue blocks were utilized for fixation. The brain tissue was stained using hematoxylin and eosin staining. The 40X observations of the brain segment were performed.[10]

Methodology for *In-silico* docking studies

Ligand Preparation

SMILES (Simplified Molecular Input Line Entry System) structure is a chemical notation of ligands' structures (GLU, TAU, and GL) that enabled for computer-readable representation of chemical structures and was acquired from Pub chem. SMILES structure adheres to the fundamental tenets of chemistry, thus it has been verified and entered into the computer.

Preparation of Targets (Receptors)

All the structures were 3D-X-ray crystallization graphics, and the PDB IDs were obtained from Protein Data Bank [www.rcsb.com]. Selected targets are used based on the previous literature of *in-silico* neurotoxicity studies in Table 1.

Molecular Docking Analysis

A statistical ligand-target docking technique was used to study the neurotransmitter receptors and enzyme molecular complexes with GLU, TAU, and GL (ligands) to understand the ligands' structural basis mechanism to the binding site affinity of targets. One ligand docked in around one to two minutes. Auto Dock VINA with an Intel i3 CPU and 1GB of RAM was used to dock in Windows 7. Caffeine was the accepted dosage (ligand). Docking was carried out to generate the binding scores, 2D and 3D views of ligand-target interactions were saved. Type of hydrogen bonding and amino acids at the binding sites were distinguished.[12]

Statistical Analysis

Rat pups have shown significant variance in learning behavioral activity and oxidative stress markers. The values were all presented as mean SEM. Dunnett's multiple comparison tests, a two-way ANOVA, were used to compute each outcome statistically. Statistical computations were performed using the 2020 version of Graph Pad Prism software.

RESULTS

The pregnant rats were treated with food additives at the selected doses from GD 3 to PND 15. After parturition on PND 21, eight rat pups from each group were isolated and evaluated

Neurobehavioural Studies

Rota Rod Test

Animals given dietary additives at particular concentrations demonstrated a decrease in the fall time. When compared to controls and CAF, the time of fall was significantly reduced in CF-treated pups (animals fed with a mixture of food additives) (Figure 1). When compared to the control group, TAU's period of decline was shown to be shorter.

Active Avoidance Test

Rat pups fed with food additives demonstrated a substantial decline in avoidance behavior on PND 25 compared to the control group. Compared to the control group, the animals' overall avoidance latency—the time it took them to go to another compartment—was much longer. The number of avoidances was significantly reduced in CF pups compared to CAF (p 0.05), but only slightly in TAU and GLUR compared to controls (Figure 2).

Elevated Plus Maze

Animals avoid the open arm for fear and anxiety. Animals, after habituating to the maze were evaluated for anxiety

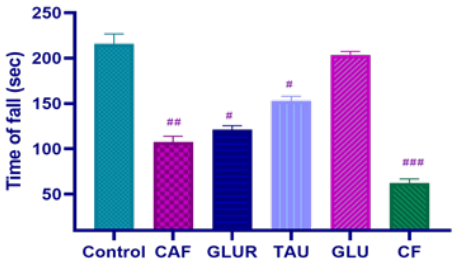

Figure 1: Effect of food additives (GLUR, TAU, GLU and CF) on Rota rod test in rat pups on PND 32. Values were expressed as mean ± SEM of 8 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using one-way ANOVA followed by Dunnett's test.

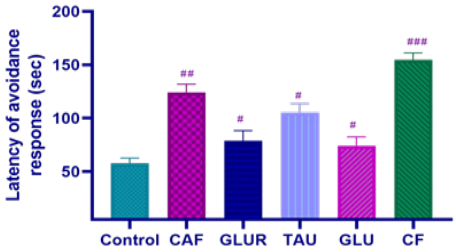

Figure 2: Effect of food additives (GLUR, TAU, GLU and CF) on Active avoidance test in rat pups on PND 25. Values were expressed as mean ± SEM of 8 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using one-way ANOVA followed by Dunnett's test.

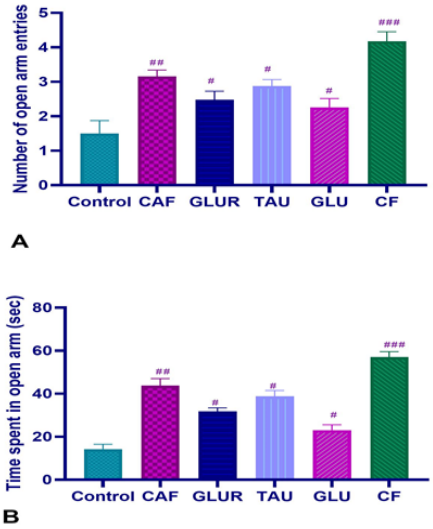

Figure 3: Effect of food additives (GLUR, TAU, GLU and CF) on Elevated plus maze test in rat pups on PND 30. A) Number of open arm entries. B) Time spent in open arm. Values were expressed as mean ± SEM of 8 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using one-way ANOVA followed by Dunnett's test.

behavior. In 5 minutes, the number of open arm entries was distinctly increased (p <0.001) in CF rat pups and GLUR. Time spent in the open arm was increased in food additive-treated animals compared with control, indicating altered behavior (Figure 1).

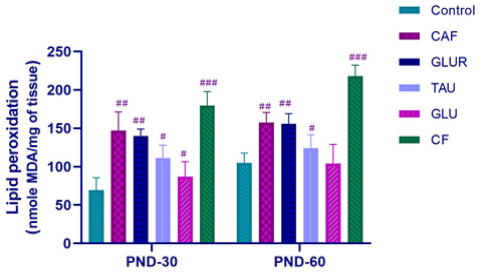

Figure 4: Effect of food additives (GLUR, TAU, GLU and CF) on lipid peroxidation in brain tissue of rat pups on PND-30 and 60. Values were expressed as mean ± SEM of 4 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using two-way ANOVA followed by Dunnett's test.

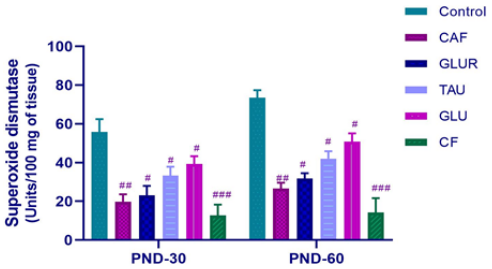

Figure 5: Effect of food additives (GLUR, TAU, GLU and CF) on superoxide dismutase in brain tissue of rat pups on PND 30 and 60. Values were expressed as mean ± SEM of 4 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using two-way ANOVA followed by Dunnett's test.

Estimation of Neurotransmitters

On days PND 30, 45, and 60, the homogenate was prepared after calculating the rat brain's wet weight.

Estimation of Adrenaline

Compared to the control group, the pups in the CAF-treated group had lower levels of adrenaline. Adrenaline levels varied from PND 30 to 60 (Figure 2). GLUR, TAU, and CF-treated group pups increased levels on PND 60. Marked increased adrenaline levels in TAU and CF-treated group pups compared with CAF group.

Estimation of Acetylcholine

CAF-treated group pups showed a decrease in the acetylcholine levels compared with the control group on PND 60. Decreased acetylcholine levels were observed in the GLUR, TAU, and CF-treated group pups compared with the control group. But, GLU-treated animals showed no significant variation in acetylcholine levels.

Among these CF-treated groups, pups showed a prominent decrease in acetylcholine levels compared to compare with CAF group (Figure 3).

Oxidative Stress Markers

After parturition, rat brain tissues were isolated and evaluated for effect on oxidative stress markers in rat pup brains on days 30 and 60. Raise in lipid peroxidation was observed in food additives treated group rat pups, indicating a significant

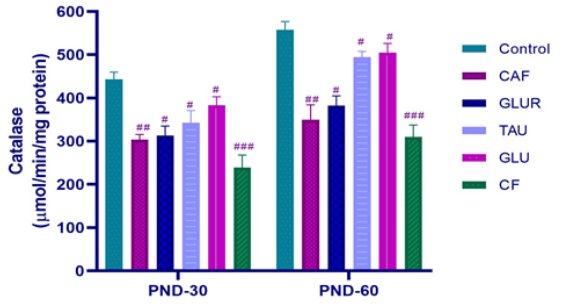

Figure 6: Effect of food additives (GLUR, TAU, GLU and CF) on catalase in brain tissue of rat pups on PND 30 and 60. Values were expressed as mean ± SEM of 4 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using two-way ANOVA followed by Dunnett's test.

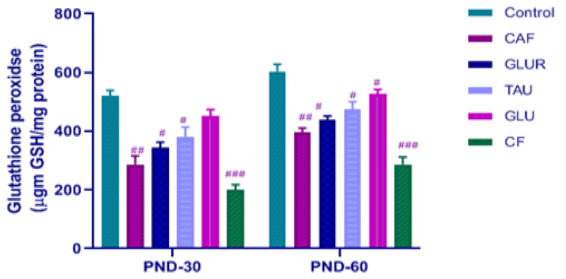

Figure 7: Effect of food additives (GLUR, TAU, GLU and CF) on glutathione peroxidase in brain tissue of rat pups on PND 30 and 60. Values were expressed as mean ± SEM of 4 observations. ###=p<0.001, ##=p<0.01, #= p<0.05 compared with control group using two-way ANOVA followed by Dunnett's test.

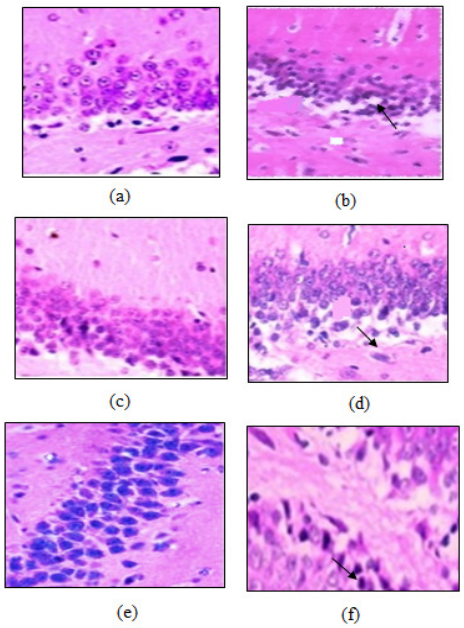

Figure 8: Effect of food additives (GLUR, TAU, GLU and CF) on histopathological changes in the hippocampus region of the brain in rat pups (40X). (Scale 150µm) a) Control, b) CAF, c) GLUR, d) TAU, e) GLU, f) CF.

increase in MDA concentration. Marked increase in lipid peroxidation in CF and GLUR rat pups (Figure 4).

A marked decrease in superoxide dismutase enzyme was observed in food additives-treated rat brain tissues as compared with the control (Figure 5). CF pups showed a significant decrease in superoxide dismutase enzyme levels compared to CAF. In CF and group 3 decreased catalase indicated an increase in free radical formation in brain tissue compared to the control group (Figure 6). A significant reduction of catalase levels was observed on day 60 of pups. GLUR and five rat pups showed a less significant effect on oxidative stress markers. A decrease in glutathione peroxidase levels, an anti-oxidant enzyme, was observed significantly ($p < 0.05$) in TAU and CF pups on day 60 (Figure 7), indicating oxidative stress in brain tissue.

Histopathological Examination

On PND 60, rat pups' cerebral cortex (Figure 8) and hippocampus areas were microscopically examined using hematoxylin and eosin stain.

The control group had typical morphology in the cortical area. In contrast to the control group, neuronal degeneration in the CAF-treated group was investigated. Whereas the TAU group indicated less degeneration of neurons, the CF-treated group pups showed more degeneration of neurons than the CAF group. No degeneration of neurons was observed in GLUR, and GLU-treated group pups.

The control group's hippocampus region had normal neuron morphology. In CAF pups, the shrinkage of neurons

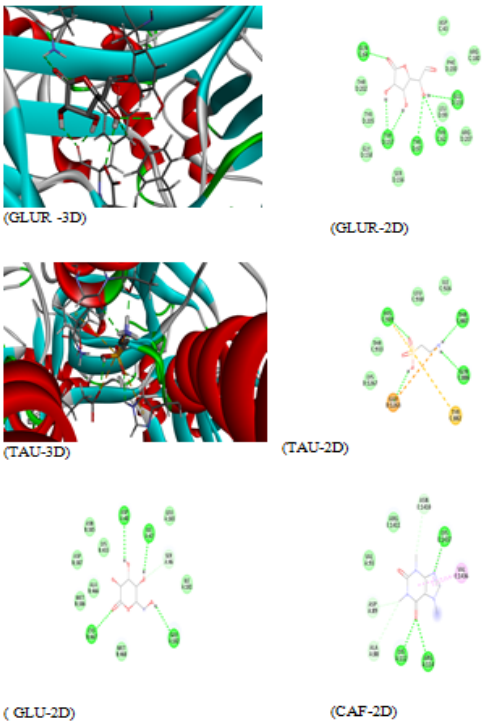

Figure 9: Molecular docking of ligands (GLUR, TAU and GLU) with target 4COF (GABA A receptor). Green lines indicate the hydrogen bonds.

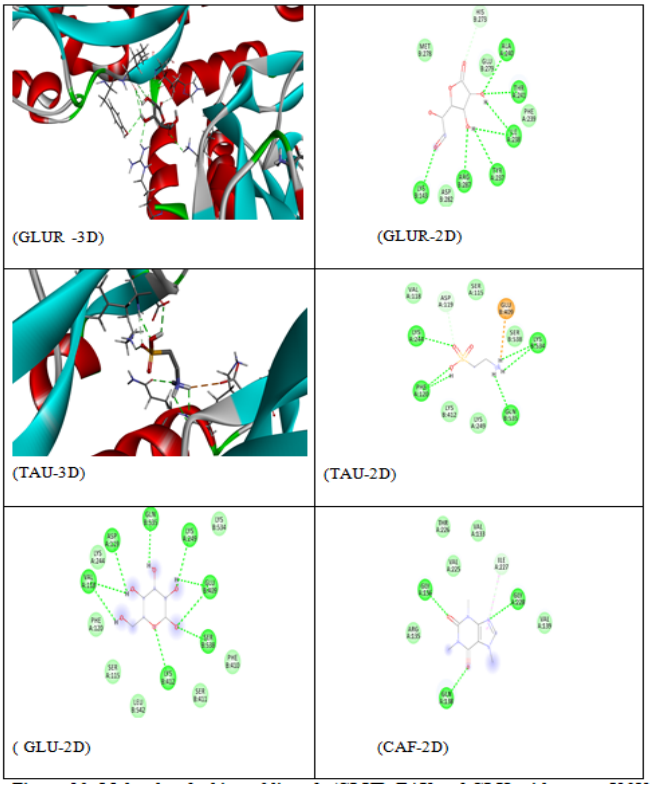

Figure 10: Molecular docking of ligands (GLUR, TAU and GLU) with target 512N (NMDA receptors). Green lines indicate hydrogen bonds.

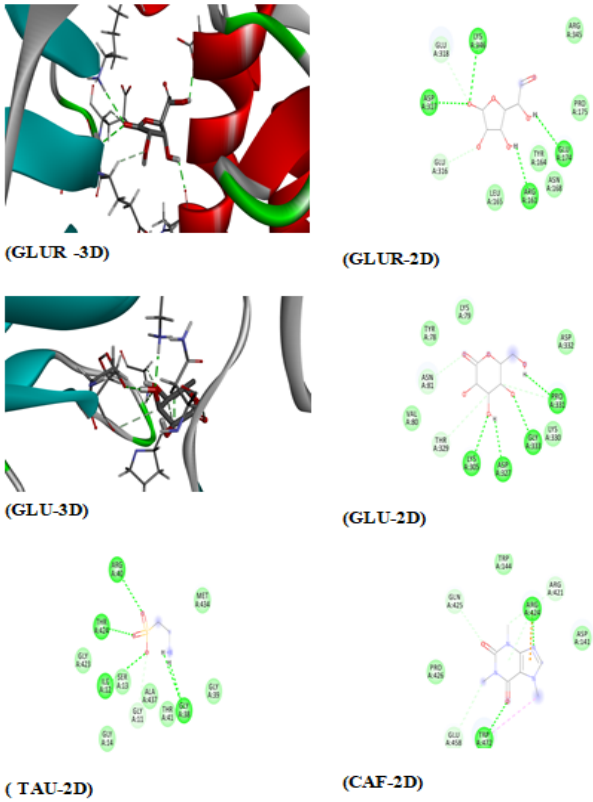

Figure 11: Molecular docking of ligands (GLUR, TAU and GLU) with target Z5X (MAO A enzyme). Green line indicates the hydrogen bonds.

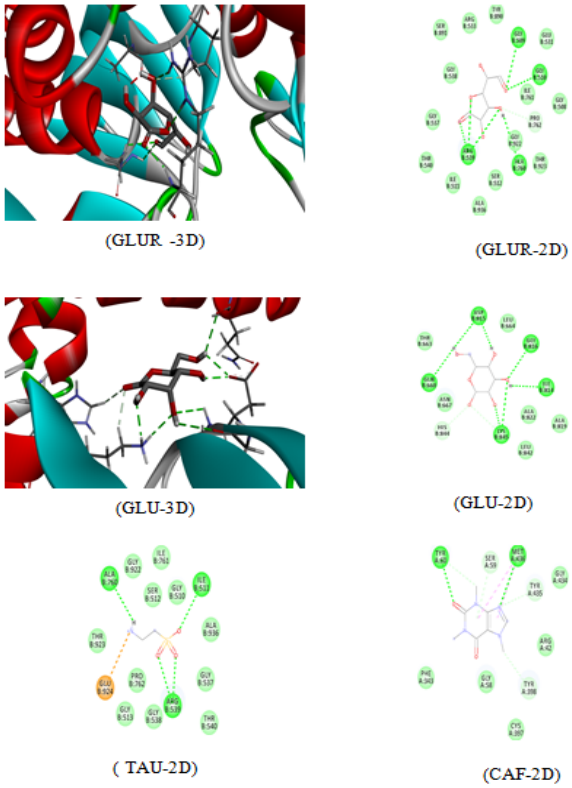

Figure 12: Molecular docking of ligands (GLUR, TAU and GLU) with target 2BK3 (MAO B).

was examined compared with the control. Similarly, TAU showed shrinkage of neurons, but CF showed prominent shrinkage compared to the CAF group. GLUR, GLU-treated pups showed no shrinkage of neurons.

Hence, the CF group showed marked degeneration in the cortex region and shrinkage of neurons in the hippocampal region, indicating the toxic effects on brain tissue.

In-silico Studies

Ligands (GLUR, TAU, and GLU) showed high interaction to targets (neurotransmitter receptors) compared to the CAF indicated by docking score and hydrogen bonds interaction with amino acids at the binding site. GLUR and TAU showed high binding affinity to 4COF and 512N targets (GABA A and NMDA 1 receptors) among the studied receptor targets, indicating the probable mechanism to produce neurobehavioral effects (Figures 9 and 10).

From the docking scores and hydrogen bonding with amino acids at the binding site, the study hypothesis was that GLUR, TAU, and GLU showed more specific binding of ligands with protein targets (neurotransmitter metabolic enzymes). Among the studied protein targets, the food additives have shown prominent binding with MAO-A (PBD ID: Z5X) and MAO B (PBD ID: 2BK3) enzymes (Figures 11 and 12). This helps us to understand the increase in the rate of metabolism of neurotransmitters, which might be the reason for the decreased neurotransmitter levels of dopamine, serotonin, and nor-adrenaline observed in biochemical estimation.

However, these studies were not able to explain the alteration in the level of acetylcholine in the studied animal models in phase II. So principally, food additives altered biogenic amines more than other neurotransmitters.

DISCUSSION

The current study provides proof of harmful neurodevelopmental effects, including behavioral modifications and oxidative stress, for several food additives that are listed within acceptable limits and further validated by *in-silico* investigations. This technique administered food additives to pregnant animals starting on day 1 and continuing through day 15 after delivery. After parturition, F1 generation studies from PND 21 were conducted to provide evidence of food additives-induced neuronal oxidative stress and behavioral changes in rat pups.[13]

Motor coordination was assessed using a rota rod.[6] It is used to rule out the motor deficit. The performance (time of fall) of pups on rota rods decreased across the individual food additive-treated groups compared with the control. As suggested in previous literature, TAU and six rat pups showed a significant decline in performance on rota rod indicated motor deficit.[6] The learning behavior in an active avoidance test is determined by the animals' time to go to a different compartment after receiving a shock (escape latency).[8,10] Food additives treated groups showed a significant variation in learning behavior. The group (CF) given with a combination of food additives had a noticeable fall in open-arm performance, indicating a decrease in learning behavior. Linked to the previous studies, the results showed the animals treated with food additives were poor learners as they took significantly high escape latency time compared to control on PND 30 and 60.

Adrenaline, a hormone and neurotransmitter produced from small neurons in the medulla oblongata, acts on all body tissues. An increase in adrenaline levels increases in behavioral activity of animals reported in previous studies.[14] In the present study, a significant increase in adrenaline levels was observed in GLUR, TAU, and CF treated group, hints an increase in neurobehavioural toxic effects.

Lipid peroxidation is one of the parameter to be considered important in oxidative stress. It is measured by MDA-treated pups showed raised MDA levels, indicates increased peroxidation in brain tissue linked to previous studies. Glutathione is a fundamental anti-oxidant enzyme, as it eliminates the oxygen species. Catalase and superoxide dismutase are the families of antioxidants. A decrease in the anti-oxidant enzyme results in the degeneration of neurons and altered behavior.[16] As suggested in the literature, a decrease in antioxidant enzymes in food additive-treated groups indicated an increase in brain oxidative damage. The current study showed a significant increase in adrenaline levels in the GLUR, TAU, and CF-treated groups, which may indicate increased neurobehavioural toxic effects.

Haemoxylin and eosin staining in the brain cortex and hippocampal region showed degeneration and shrinkage of neurons in GLUR, TAU, GLU, and CF-treated groups. Based on the previous studies, neurodegeneration and shrinkage of neurons were caused due to oxidative damage. Hence CF showed marked shrinkage of neurons linked to an increase in lipid peroxidation, leading to neurodevelopmental toxic effects in pups.[10]

Previous studies reported that neurotoxicity was evaluated by understanding the modulation and binding affinity of ligands to the neurotransmitter receptors and enzyme targets.[17] In the present *in-silico* docking studies, GLUR, TAU, GLU, and CF showed a high affinity of binding to the target proteins and the modulation of neurotransmitter receptors and enzymes compared with caffeine indicated neurotoxicity correlating the observed neurotoxic effects in neurobehavioural studies.

Previous studies indicated caffeine is well known for its GABA action with a high binding affinity towards the active site of GABA A receptor showed neurobehavioural alteration when taken in high doses.[18] Based on the previous studies, taking caffeine as a reference, the binding affinity of food additives was evaluated by docking with GABA A receptors. GLUR showed high binding affinity at the active site of GABA A receptors, hinted at the neurobehavioural alteration produced in pharmacological studies.

Previous studies stated that the modulation of NMDA receptors induced neurotoxicity, and the studies were supported by docking the drugs to NMDA receptors.[19] In the present study, molecular docking of NMDA receptors with GLUR, TAU, and GLU was carried out. GLUR showed high binding affinity at the active site, showed modulation of NMDA receptors, and hints at the neurotoxicity of food additives.

The neurotransmitters nor-adrenaline, serotonin, and dopamine are metabolized by a set of enzymes known as MAO. Neurotransmitter metabolism is reduced as a result of an increase in MAO-A enzymes, which forms the basis for a number of neurodegenerative diseases.[20,21] Thus, based on the previous literature, the high binding affinity of food additives such as GLUR and GLU at the binding sites of MAO A and MAO B indicated their role in enhancing neurotransmitter metabolism, which resulted in a decrease in neurotransmitters as shown in pharmacological studies.

From all these results, we can summarise the neurodevelopmental toxic effects in pregnant animals. Pre and post-natal exposure to food additives is also fatal in pups and caused mood disorders. The reason for the altered behaviors can be attributed to the affinity of food additives to MAO A and MAO B enzymes which lead to alteration in neurotransmitter levels.

The study also provided strong evidence for oxidative stress caused by selected food additives in animal models. The severity of the neurotoxicity increased with the combination of food additives. Further studies are mandatory to understand the molecular mechanism of neurotoxicity caused by food additives. The study clearly highlights the need for extensive research and revision of acceptable daily intake of food additives, especially in the case of energy drinks.

CONCLUSION

The current findings support the hypothesis that the chosen dose and mix of food additives altered rat pups' neurobehavioral and neurotransmitter profiles. This demonstrated the neurotoxic effects on neurodevelopment, which were further corroborated by an increase in oxidative stress and neurobiological changes in the cerebral cortex of the growing rat brain. This study provides proof that food additives used in energy drinks can be hazardous to neurodevelopment when ingested in excess of the recommended daily dosage.

The order of neurodevelopmental toxicity for all the parameters (neurobehavioural effects, biochemical studies, and histopathological studies) in rat pups was found to be CF>TAU>GLUR>GLU.

The results of *in-silico* indicated a higher binding affinity of selected food additives with target proteins (neurotransmitter receptors and metabolic enzymes) when compared to caffeine, hinting at neurotoxicity.

The chosen dietary additives combined produce neurotoxic effects on neurodevelopment. Further systematic mechanistic research is advised to elucidate the molecular mechanism of the synergistic harmful impact caused by food additives.

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