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Research Article

Ameliorative Potential of Ginger (*Zingiber officinale*) following Repeated Coexposure with Fluoride and Dimethoate in Blood and Brain of Wistar Rats

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Coexposure to exogenous neurotoxins, such as fluoride (F^-) and dimethoate (DM), is a serious public health concern. In the current study, Wistar rats were exposed to DM ($1/10^{th}$ LD_{50}) and F^- (4.5 ppm) in drinking water individually as well as in combination continuously for 28 days and the effectiveness of ginger and quercetin was assessed in combating the oxidative stress-mediated combined toxic effects on blood and brain. Significant ($p < 0.05$) reductions were observed in the levels of total antioxidant status (TAS), glutathione peroxidase (GPx), blood glutathione (GSH), activities of catalase (CAT), total thiols (TTH), superoxide dismutase (SOD), aryl esterase (AE), and acetylcholinesterase (AChE) whereas significant elevations ($p < 0.05$) in malondialdehyde (MDA) and advanced oxidation protein products (AOPP) were recorded in blood and brain of coexposed rats. Administration of ginger and quercetin significantly ($p < 0.05$) ameliorated the combined F- and DM-induced hematotoxicity as well as neurotoxicity as indicated by the levels of markers of oxidative injury in brain and blood in coexposed rats, which showed significant ($p < 0.05$) improvement. Moreover, pathological changes, such as neurodegeneration, perivascular edema, and gliosis, observed after combined F^- and DM toxicity in the brain (cerebrum and cerebellum) were markedly reduced after ginger supplementation.

1. Introduction

The organophosphorus (OP) compounds have been rampantly used as insecticides to improve productivity in agriculture, horticulture, and allied industries and have also found applicability in the field of medicine [1–3]. Nonetheless, the residues of these agrochemicals bioaccumulate in the soil causing environmental pollution and contaminating grains, vegetables, and fruits and ultimately entering the human food chain [4–6]. OP poisoning causes multisystemic dysfunctions, particularly neurotoxicity which is a global community health problem. Manifestations of chronic low-dose exposure include electroencephalographic changes and neurodevelopmental disorders such as autism, attention deficit hyperactivity disorder, dementia, and Parkinson's disease [7, 8]. However, acute neurotoxicity can result in acute cholinergic syndrome, OP-induced intermediate syndrome, and delayed neuropathy [9–12]. Dimethoate (O, O-dimethyl-S-methyl carbamoyl methyl phosphorothioate) (DM), an OP compound, is widely used for the control of agricultural and household pests [13, 14]. The WHO has enlisted DM as a class II (moderately hazardous) agent and

a putative neurotoxin due to its ability to inhibit acetylcholinesterase (AChE) activity as a result of which a buildup of ACh occurs at nerve synapses, causing overstimulation of muscles which eventually leads to seizures, paralysis, exhaustion, and death [1]. Additionally, continuous low-level exposure to DM incurs damage to antioxidant enzymes, and the ensuing oxidative stress has been incriminated as a main risk factor for neurological disorders (Alzheimer's disease, Huntington's disease, and Parkinson's disease) [15, 16]. Furthermore, DM also causes nephrotoxicity, hepatotoxicity, cardiotoxicity, genotoxicity, and hematotoxicity [4, 17–19].

Fluoride (F) is another neurotoxin that is a highly abundant, reactive, and electronegative element and often exists as a complex with other minerals in rocks and soil from where it readily leaches into groundwater due to natural weathering and erosion. In addition, volcanic emissions, industrial effluents or byproducts, and anthropogenic activities, such as coal burning and mining, pollute natural ecosystems with F. Drinking water containing geogenic F constitutes the largest source of F ingestion for humans and animals. Besides, the consumption of tea, tomatoes, spinach, grapes, fluoridated salt and the use of cosmetics, dental gels, and toothpaste also potentiates F intake [20, 21]. Endemic fluorosis is common worldwide, and Indian states (Jammu and Kashmir, Gujarat, Andhra Pradesh, Rajasthan, and West Bengal) are at higher risk of fluorosis due to heavy contamination of groundwater [22–24].

The European Food Safety Authority (EFSA) recommendation limits the F intake to 50–70 μg/kg/day which is safe and prevents the development of dental caries; however, due to its narrow safety margin, an intake of >0.1 mg/kg/day predisposes to the development of dental fluorosis [21, 25, 26]. The WHO recommended upper limit for F in drinking water is 1.5 ppm. Skeletal fluorosis can occur after an intake of 6–10 mg of F per day for at least 10 days [20]. Apart from skeletal deformities, such as arthritis and osteoporosis, fluorosis also predisposes to neurological developmental disabilities and heightens the risk of formation of certain brain tumors as it can readily permeate the blood-brain barrier [27–29]. Intellectual impediments in humans, such as impaired memory, slow learning ability, and low intelligence quotients, have been reported in areas where the groundwater carries high F content [30]. Research on the role of F as a neurotoxicant is ongoing, and despite the controversies, several past and recent *in vitro* studies have revealed its toxic nature to neuronal cells and human embryonic stem cells [21, 31, 32]. F-mediated oxidative damage to erythrocytes impedes their oxygen-carrying capability through methemoglobin formation and induces derangements in erythrocytic energy and redox metabolism [33]. Many OP compounds employed as broad-spectrum insecticides accumulate and inhibit plasmatic antioxidant machinery [34]. ROS-mediated metabolic and antioxidant enzymatic alterations can impinge perfusion of nervous tissue and glucose uptake by the brain and can be a potential risk factor for neurodegenerative disorders [35]. Therefore, a concoction of environmental toxicants may interact to exacerbate direct neuronal damage inflicted by individual toxicant exposure. Besides, existing brain damage can be further potentiated by a hindrance to oxygen supply to the brain after oxidative damage to red blood cells (RBCs) by coexposure to extraneous poisons. In the current scenario, a high likelihood of simultaneous coexposure to F and DM exists in animals and humans; however, only a few studies have evaluated the consequences of their coexposure on erythrocytes and the brain, and need scientific attention.

Ginger (*Zingiber officinale* Roscoe, *Zingiberaceae*), a perennial rhizome, is a popular spice that has been widely consumed in Chinese, Ayurvedic, and Unani herbal medicine systems for the treatment of a wide range of illnesses such as catarrh, sore throat, rheumatism, neurological disorders, asthma, gingivitis, toothache, stroke, constipation, dementia, and diabetes [36–38]. In a recent study, ginger was found to provide protection against DM-induced hormonal disturbances [39]. Bioactive components identified in ginger include 6-gingerol, 8-gingerol, 10-gingerol, zingerone, 6-shogoal, sesquiterpenes, phenols, quercetin, and curcumin, which collectively confer antioxidant, anticancer antimicrobial, antiulcerative, and anti-inflammatory properties [18]. It also inhibits vascular smooth muscle proliferation and may be useful for the treatment of vascular diseases [40]. Therefore, the present study was designed to evaluate the effect of combined exposure of F- and DM-induced oxidative stress-mediated hematotoxicity and neurotoxicity in Wistar rats and the ameliorative potential of ginger.

2. Materials and Methods

2.1. Preparation of ZO Extract. After being identified by the University of Kashmir Taxonomists (dated 03/03/2020, voucher specimen No. 2921), the required quantity of *Zingiber officinale* (ZO) rhizomes was cleaned and dried and converted to a fine powder in the laboratory using an electric grinder. The ethanol and distilled water in a ratio of 1 : 1 were used as a solvent for the extraction purpose in a Soxhlet apparatus while setting the temperature of the hot plate at 65–70°C. The extract from ZO was dried using a rotatory evaporator (15 rpm, 55–60°C) and kept in a glass jar under desiccation and refrigeration.

2.2. Drugs and Chemicals. Serine salicylate, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS), and quercetin were supplied by Sigma-Aldrich, USA, whereas ethyl alcohol, sodium fluoride, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and nicotinamide adenine dinucleotide sodium salt (NADPH) were sourced from Hi-Media company (India, Mumbai). The remaining chemicals were supplied by SD Fine Chemicals (India, Mumbai) and were of analytical grade. AgroShopy, Rallis India Limited (Mumbai, India), supplied a commercial formulation of dimethoate (Tafgor, 30% EC) for the study.

2.3. Experimental Design. The present study (a 28-day *in vivo* experiment) was performed using 54 Wistar rats (180–190 g adult and healthy animals) of either sex

purchased from the CSIR Lab (Indian Institute of Integrative Medicine, Jammu, India) which were maintained under standard managemental conditions having access to clean drinking water and pelleted ration *ad-libitum*. The pelleted feed was manufactured by CSKHPKV, Palampur, H.P., India. An experiment with nine groups with six rats in each ($n = 6$) was designed following a treatment regimen presented in Table 1. After acclimatizing for 3 weeks to the laboratory conditions, the rats were used for the study under constant observation and were exposed to 12/12 h of light/darkness cycle. The Institutional Animal Ethics Committee (IAEC) of our university (IAEC–862/GO/Re-s/ReBi-L/04/CPCSEA, SKUAST-Jammu, India) approved the protocols for the experiments vide proposal no 3/IAEC/2020. While DM was administered through oral gavage (3.10 mg/kg body weight and 1/10th dose of median lethal dose (LD_{50})) [41], sodium fluoride was provided at the rate of 4.5 ppm (9.945 mg/L of drinking water which is 3-fold the upper level recommended by WHO, i.e., 1.5 ppm of fluoride).

2.4. Sample Collection and Analysis. The cervical dislocation method was employed to sacrifice the animals, and the brain samples were collected for histopathological examination and antioxidant biomarker studies using 10% formalin and ice-cold 0.5 M phosphate buffer (pH 7.4), respectively. Before the animals were sacrificed, blood samples (3-4 mL) were directly collected from the hearts of the animals in heparinized tubes. A Teflon-coated homogenizer was used (1000 rpm, 5–7 min, 4°C) to prepare a tissue homogenate (10%). While reduced glutathione (GSH) levels were determined using whole blood, the erythrocyte sediment and plasma were separated from the blood samples by centrifugation method (3000 rpm, 15 min, 4°C) for further analysis. The erythrocyte sediment thus obtained was diluted in the ratio of 1 : 1 (v/v) using normal saline solution (NSS) and centrifuged (10 min) to obtain a buffy coat after discarding the supernatant. This was followed by the addition of NSS to the RBCs on a v/v basis. The process was repeated three times. The activities of different antioxidant enzymes were determined using 1% hemolysate prepared by mixing 100 μL of washed RBCs and 9.9 mL of 0.1 M PBS (pH 7.4).

2.5. Fluoride Estimation. The F level in the brain and plasma was measured (w/v wet basis) following a standard method of extraction [42, 43].

2.6. Determination of Antioxidant Biomarkers in Brain and Blood. Phenylacetate was used as a substrate to measure the activity (U/mL, where one unit equals μmol of phenol formed per min) of arylesterase (AE) [44]. Reduced glutathione (GSH), total thiols (TTH), and total antioxidant status (TAS) were determined following well-known standard methods [45–47]. The glutathione peroxidase (GPx) and catalase (CAT) activity levels were determined as per the methods of Hafeman et al. [48] and Aebi [49], respectively. The activities of glutathione reductase (GR) and superoxide dismutase (SOD) were evaluated following the methods elaborated elsewhere [50, 51], respectively. Similarly, advanced oxidation protein product (AOPP) and malondialdehyde (MDA) levels in brain tissue were evaluated following standard methods [52, 53].

2.7. Histopathology. The samples from the cerebrum and cerebellum of animals from various groups were collected in formalin, and the paraffin-embedded sections were stained with hematoxylin and eosin after proper processing (washed, dehydrated, and cleared) [54]. The sections were evaluated for various histomorphological alterations.

2.8. Statistical Analysis. The results expressed as mean ± standard errors are presented in tables and figures and were obtained by analyzing the data using ANOVA ($p \leq 0.05$) and Duncan's multiple range tests (SPSS 21.0).

3. Results

3.1. Alterations in Antioxidant Status of Blood. Alterations in the activities of AChE, AE, nonenzymatic (TAS, TTH, and GSH), and enzymatic (CAT, SOD, GPx, and GR) components of antioxidant system and cellular damage indicators (oxidation of protein and lipids) in erythrocytes of Wistar rats are presented in Tables 2 and 3.

3.1.1. Levels of GSH, TAS, and TTH. When given alone, DM and F did not affect TAS but caused a significant ($p < 0.05$) reduction in GSH and TTH levels; however, their simultaneous administration caused a significant ($p < 0.05$) fall in GSH, TTH, and TAS when compared to control levels. ZO restored decreased levels of TTH in all the intoxicated groups and also corrected the fall in TAS values in dual intoxicated rats. Similarly, quercetin also restored both TAS and TTH levels in the combined toxicity group (Table 2).

3.1.2. Activities of AE and AChE. Even though all groups treated with toxicants underwent a significant ($p < 0.05$) decline in AE values as compared to the control values, dual toxicant exposure created a significant reduction ($p < 0.05$) in AE as compared to the respective single toxicant exposure. AChE values were found to be significantly affected in the case of dual toxicant exposure as well as F exposure. ZO remediated depletion in AE and AChE after single toxicant exposure. ZO and quercetin caused a complete recovery of AChE but only a partial amelioration of AE levels. The mean values along with the standard error are presented in Table 2.

3.1.3. Activities of CAT and SOD. Both DM and F alone significantly ($p < 0.05$) reduced SOD but not CAT levels when compared to control. Their concomitant exposure, however, caused significant ($p < 0.05$) reductions in both CAT and SOD levels. Supplementation of quercetin and ZO was effective in complete recovery of CAT levels in coexposed rats, and on the other hand, SOD levels in these rats showed significant improvement upon ZO supplementation only (Tables 2 and 3).

Table 1: Treatment regimen followed for the experiment.

Groups	Treatments	Dose and route of administration
I	Control	1 mL/day/rat, PO (per os), drinking water
II	Dimethoate (DM)	31.0 mg/kg (1/10 LD50), PO
III	Fluoride (F^-)	4.5 ppm in drinking water
IV	DM + F^-	1/10 of LD50 (PO) + 4.5 ppm in drinking water
V	*Zingiber officinale* (ZO)	300 mg/kg BW (body weight), PO
VI	ZO + DM	300 mg/kg (PO) + 1/10 of LD50 (PO)
VII	ZO + F^-	300 mg/kg (PO) + 4.5 ppm
VIII	ZO + DM + F^-	300 mg/kg (PO) + 1/10 of LD50 + 4.5 ppm
IX	Quercetin + DM + F^-	100 mg/kg (PO) + 1/10 of LD50 + 4.5 ppm

Table 2: Effect of hydroalcoholic extract of *Z. officinale* on toxicity induced by fluoride and dimethoate alone and in combination with erythrocyte antioxidant system in *Wistar rats*.

Groups	TAS	GSH	TTH	AE	AChE	CAT
Control	$15.71^{b} \pm 1.23$	$3.37^{bc} \pm 0.27$	$0.31^{c} \pm 0.01$	$2.32^{c} \pm 0.35$	$6065.75^{c} \pm 468.70$	$35.30^{b} \pm 1.74$
1/10 LD50 DM	$13.12^{ab} \pm 0.62$	$1.83^{d} \pm 0.05$	$0.11^{b} \pm 0.02$	$0.67^{a} \pm 0.05$	$5227.38^{c} \pm 307.73$	$37.31^{b} \pm 1.99$
Fluoride (4.5 ppm)	$16.46^{b} \pm 1.64$	$1.66^{a} \pm 0.07$	$0.13^{ab} \pm 0.03$	$0.82^{a} \pm 0.09$	$2361.50^{a} \pm 219.33$	$35.08^{b} \pm 4.78$
DM (1/10) + fluoride (4.5 ppm)	$12.09^{a} \pm 0.81$	$1.12^{a} \pm 0.02$	$0.06^{a} \pm 0.03$	$0.37^{d} \pm 0.04$	$2178.88^{a} \pm 397.49$	$12.42^{a} \pm 0.87$
ZO extract (300 mg/kg)	$18.98^{bc} \pm 1.24$	$2.92^{b} \pm 0.12$	$0.29^{c} \pm 0.02$	$0.75^{a} \pm 0.08$	$5864.75^{c} \pm 158.83$	$40.29^{b} \pm 2.41$
ZO extract + DM	$13.85^{a} \pm 1.36$	$2.03^{d} \pm 0.18$	$0.27^{c} \pm 0.03$	$1.95^{c} \pm 0.67$	$4096.38^{b} \pm 149.51$	$33.25^{b} \pm 3.50$
ZO extract + F^-	$16.33^{b} \pm 0.89$	$1.66^{a} \pm 0.07$	$0.25^{c} \pm 0.02$	$1.62^{b} \pm 0.16$	$4490.13^{b} \pm 493.95$	$53.40^{c} \pm 7.65$
ZO extract + DM + F^-	$21.00^{c} \pm 0.42$	$3.41^{b} \pm 0.57$	$0.30^{c} \pm 0.02$	$1.34^{b} \pm 0.22$	$6480.25^{c} \pm 497.72$	$40.97^{bc} \pm 3.01$
Quercetin (100 mg/kg) + DM + F^-	$18.37^{b} \pm 1.17$	$3.15^{cb} \pm 0.13$	$0.35^{c} \pm 0.01$	$1.29^{b} \pm 0.36$	$7163.75^{c} \pm 652.17$	$37.05^{b} \pm 3.22$

ZO: *Zingiber officinale* and DM: dimethoate ($n = 6$). Values having different superscripts (a, b, and c) in a column are statistically different from one another at a 5% level of significance. Values of TAS (total antioxidant status) are expressed in mM. Values of TTH (total thiols) are expressed in μM. Values of reduced glutathione (GSH) are expressed in mM. Values of SOD (superoxide dismutase) and GPx (glutathione peroxidase) are expressed in units/mg of Hb. Values of CAT (catalase) are expressed in μmol H_2O_2 decomposed/min/mg of Hb. Acetylcholinesterase (AChE) activity is expressed in nmol of thiol produced/min. Activities of arylesterase (AE) are expressed in U/mL.

Table 3: Effect of hydroalcoholic extract of *Z. officinale* on toxicity induced by fluoride and dimethoate alone and in combination with erythrocyte antioxidant system in *Wistar rats*.

Groups	SOD	GPx	GR	AOPP	MDA
Control	$54.57^{b} \pm 3.81$	$7.34^{b} \pm 0.38$	$2.89^{c} \pm 0.47$	$0.53^{c} \pm 0.04$	$2.36^{a} \pm 0.13$
1/10 LD50 DM	$31.57^{c} \pm 0.71$	$4.11^{a} \pm 0.53$	$1.92^{b} \pm 0.20$	$0.52^{c} \pm 0.08$	$5.17^{b} \pm 0.56$
Fluoride (4.5 ppm)	$34.09^{c} \pm 0.37$	$4.66^{a} \pm 0.70$	$1.63^{ab} \pm 0.33$	$0.62^{d} \pm 0.11$	$5.74^{b} \pm 1.76$
DM (1/10) + fluoride (4.5 ppm)	$32.85^{ca} \pm 0.82$	$4.03^{a} \pm 0.23$	$1.42^{a} \pm 0.10$	$0.68^{d} \pm 0.14$	$6.02^{c} \pm 0.19$
ZO extract (300 mg/kg)	$46.92^{b} \pm 3.43$	$8.57^{b} \pm 0.27$	$2.63^{c} \pm 0.38$	$0.52^{c} \pm 0.03$	$2.21^{a} \pm 0.51$
ZO extract (300 mg/kg) + DM (1/10 LD50)	$33.25^{a} \pm 3.50$	$6.16^{ab} \pm 2.46$	$5.08^{d} \pm 0.37$	$0.35^{a} \pm 0.05$	$2.05^{ab} \pm 0.62$
ZO extract (300 mg/kg) + F^- (4.5 ppm)	$53.40^{b} \pm 7.65$	$5.30^{ab} \pm 2.17$	$4.98^{d} \pm 0.42$	$0.44^{a} \pm 0.02$	$3.89^{a} \pm 0.18$
ZO extract (300 mg/kg) + DM (1/10 LD50) + F^- (4.5 ppm)	$40.97^{d} \pm 3.01$	$5.33^{b} \pm 2.74$	$5.39^{d} \pm 0.47$	$0.47^{c} \pm 0.07$	$2.43^{a} \pm 0.42$
Quercetin (100 mg/kg) + DM (1/10 LD50) + F^- (4.5 ppm)	$37.05^{ad} \pm 3.22$	$7.07^{b} \pm 2.87$	$4.23^{cd} \pm 0.60$	$0.58^{c} \pm 0.04$	$2.45^{a} \pm 0.41$

Values are given as mean ± SE of 6 animals unless otherwise stated. Values having different superscripts (a, b, and c) in a column are statistically different from one another at a 5% level of significance. Values of SOD (superoxide dismutase) are expressed in units/g of tissue. Values of GR (glutathione reductase) are expressed in nmol of NADPH/min. GPx (glutathione peroxidase) is expressed in units/g of tissue. Values of the advanced oxidation protein product (AOPP) are expressed in μM of chloramine-T. Values of malondialdehyde (MDA) are expressed in nmol of MDA formed/g/h.

3.1.4. Activities of GPx and GR. All types of toxicant exposure caused a significant ($p < 0.05$) decrease in activities of GPx and GR, but the decline was steepest in the case of dual intoxication. ZO and quercetin showed comparable effectiveness in the complete resurrection of the levels of GPx and GR reduced upon coexposure to F and DM (Table 3).

3.1.5. Levels of AOPP and MDA. The levels of AOPP were significantly ($p < 0.05$) elevated only after F or combined exposure whereas MDA levels were significantly increased by each toxicant when administered individually. F along with DM yielded significantly ($p < 0.05$) higher MDA concentration as compared to these toxicants when given alone. ZO and

quercetin manifested similar protective abilities by providing a complete safeguard to AOPP and MDA activities in the face of dual toxicant administrations (Table 3).

3.2. Alterations in Antioxidant Status of the Brain. Alterations in the activities of AChE, antioxidant system (TAS, TTH, AE, CAT, SOD, GPx, and GR), and cellular damage indicators (protein and lipids) in the brain tissue of Wistar rats were accessed to determine the extent of damage and its amelioration with ginger and quercetin.

3.2.1. Effects on Activities of AChE and AE in the Brain. Administration of toxicants (groups II, III, and IV) led to a significant ($p < 0.05$) decrease in values of AChE and AE. ZO extract brought about a complete amelioration of the levels of both enzymes in all toxicant-administered groups including the combination group (Table 4). In contrast, quercetin only significantly ($p < 0.05$) improved AChE levels in the combined toxicant-administered rats with no significant impact on the reduced AE activities.

3.2.2. Effects on the Levels of TAS and TTH in the Brain. The groups II, III, and IV which were intoxicated with DM, F, and their combination, respectively, exhibited significantly ($p < 0.05$) decreased levels of TAS and TTH as compared to control. Supplementation with ZO completely restored changes in TAS and TTH values in the dual intoxicated rats while quercetin could only significantly ($p < 0.05$) circumvent TTH levels. The mean values along with the standard error are presented in Table 4.

3.2.3. Effects on Activities of CAT and SOD in the Brain. A significant decline in SOD and CAT values was seen after the administration of toxicants. In addition, dual administration of toxicants caused a significantly steeper depreciation in the values of both these enzymes when compared to their respective single toxicant administration. ZO administration successfully upgraded SOD and CAT levels which fell in response to F or DM alone. Meanwhile, quercetin and ZO caused complete amelioration in the levels of these antioxidants in dual intoxicated groups (Tables 4 and 5).

3.2.4. Effects on Activities of GPx and GR in the Brain. Toxicants singly or in combination significantly ($p < 0.05$) lowered GPx and GR contents in comparison to the control levels. Dual toxicant insult triggered a significantly higher depreciation in levels of the GPx and GR as compared to the respective values of individual toxicant-treated rats. ZO administration in all toxicant groups caused complete restoration of GPx and GR levels including the group receiving both the toxicants simultaneously. Quercetin also restored GPx levels completely to the levels of control. While significant improvements in GR levels in comparison to the rats treated with both the toxicants were observed,the GR values in the quercetin group were significantly lower than those in the control group (Table 5).

3.2.5. Effects on the Levels of AOPP and MDA in the Brain. A significant ($p < 0.05$) elevation in AOPP and MDA levels was recorded after toxicant administration (groups II, III, and IV) as compared to the control group. Simultaneous administration of F and DM caused a further significant ($p < 0.05$) rise in their respective levels as compared to their corresponding values after a single toxicant administration. ZO treatment enabled significant ($p < 0.05$) and complete amendment in AOPP and MDA levels in all groups treated either with single or combined toxicants. On the other hand, quercetin fully corrected only AOPP levels while MDA levels could only be partially repaired upon its supplementation (Table 5).

3.2.6. Fluoride Levels in Plasma and Brain Tissue. Alterations in F levels in plasma and brain induced by F and DM alone as well as in combination and effect of hydro-alcoholic extract of ZO in Wistar rats are presented in Table 6. Rats subjected to only DM treatment did not show altered F levels in plasma or blood whereas animals given F-only treatment caused a significant ($p < 0.05$) increment in F levels in plasma as well as in brain tissue. Interestingly, DM administration along with F manifested significantly ($p < 0.05$) higher F levels in plasma as well as in brain as compared to control; however, their levels were significantly reduced as compared to F-only treatment. ZO supplementation significantly ($p < 0.05$) reduced increased F levels in brain as well as in plasma of NaF-only intoxicated rats. Quercetin completely restored plasma and brain F contents to baseline levels; in contrast, ZO could completely restore F levels in brain but not in plasma in rats with combined administrations of toxicants.

3.3. Histopathological Alterations in Brain (Cerebrum and Cerebellum)

3.3.1. Cerebrum. Cerebral sections in group I did not show any pathological changes. Neuronal morphology was normal, and glial cells were finely distributed throughout the neuropil and meningeal lining also showed no pathological abnormalities (Figure 1(a)). In group II rats, mild perineuronal edema as well as perivascular edema, hemorrhage, and spongiosis were noticed besides mild neuronal degeneration (Figure 1(b)). Group III rats revealed moderately severe spongiosis along with neuronal degeneration, pyknosis, and perineuronal as well as perivascular edema (Figure 1(c)). In group IV rats, the cerebrum had pathological changes of the most severe degree. The presence of randomly distributed multifocal glial aggregates was recorded apart from neuronal degeneration and neuronophagia (Figure 1(d)). Also, perivascular edema, mild diffuse gliosis with severe vacuolation in neuropil leading to formation of microcavitation, was present in group IV (Figure 1(e)). Congestion of meningeal vessels along with hemorrhage and lymphocytic infiltration in the meninges was also recorded in some rats of group IV. Group V rats did not have any significant changes in their cerebral cortex and their architecture mirrored that of group I rats (Figure 1(f)).

Table 4: Effect of hydroalcoholic extract of *Z. officinale* on toxicity induced by fluoride and dimethoate alone and in combination with brain antioxidant system in *Wistar rats*.

Groups	TAS	TTH	AE	AChE	CAT
Control	$20.58^{b} \pm 0.14$	$1.99^{a} \pm 0.49$	$2.71^{b} \pm 0.15$	$22141.75^{c} \pm 1284.82$	$3120.53^{cd} \pm 77.09$
1/10 LD50 DM	$13.55^{a} \pm 0.58$	$0.99^{b} \pm 0.26$	$1.81^{a} \pm 0.09$	$16256.88^{b} \pm 1482.37$	$1358.58^{a} \pm 101.80$
Fluoride (4.5 ppm)	$14.27^{a} \pm 0.94$	$1.19^{b} \pm 0.27$	$1.67^{a} \pm 0.05$	$12661.50^{a} \pm 813.21$	$1009.41^{a} \pm 43.60$
DM (1/10) + fluoride (4.5 ppm)	$12.13^{a} \pm 0.56$	$0.91^{b} \pm 0.19$	$1.50^{a} \pm 0.07$	$6845.63^{a} \pm 349.30$	$801.87^{e} \pm 50.35$
ZO extract (300 mg/kg)	$17.23^{ab} \pm 2.08$	$1.66^{a} \pm 0.43$	$2.58^{b} \pm 0.31$	$18315.63^{c} \pm 744.51$	$3373.89^{d} \pm 175.66$
ZO extract (300 mg/kg) + DM (1/10 LD50)	$14.57^{a} \pm 0.87$	$1.38^{a} \pm 0.68$	$3.82^{c} \pm 0.29$	$18278.13^{bc} \pm 675.17$	$2851.13^{bc} \pm 153.44$
ZO extract (300 mg/kg) + F^{-} (4.5 ppm)	$17.71^{ab} \pm 1.41$	$1.19^{b} \pm 0.27$	$2.82^{b} \pm 0.17$	$18946.75^{bc} \pm 2068.48$	$2564.56^{b} \pm 109.09$
ZO extract (300 mg/kg) + DM (1/10th LD50) + F^{-} (4.5 ppm)	$19.84^{b} \pm 0.57$	$1.46^{a} \pm 0.13$	$2.69^{b} \pm 0.46$	$18640.00^{bc} \pm 1202.75$	$2972.76^{bcd} \pm 141.25$
Quercetin (100 mg/kg) + DM (1/10th LD50) + F^{-} (4.5 ppm)	$14.54^{a} \pm 1.00$	$1.87^{a} \pm 0.33$	$1.91^{a} \pm 0.23$	$18024.00^{bc} \pm 1774.67$	$2485.08^{bc} \pm 209.13$

Values are given as mean ± SE of 6 animals unless otherwise stated. Values having different superscripts (a, b, and c) in a column are statistically different from one another at a 5% level of significance. Values of TAS (total antioxidant status) are expressed in mM. Values of TTH (total thiols) are expressed in μM. Acetylcholinesterase (AChE) activity is expressed in nmol of thiol produced/min/mg of tissue. Activities of arylesterase (AE) are expressed in U/mL. Values of CAT (catalase) are expressed in μmol H_2O_2 decomposed/min/g of tissue.

Table 5: Effect of hydroalcoholic extract of *Z. officinale* on toxicity induced by fluoride and dimethoate alone and in combination with brain antioxidant system in *Wistar rats*.

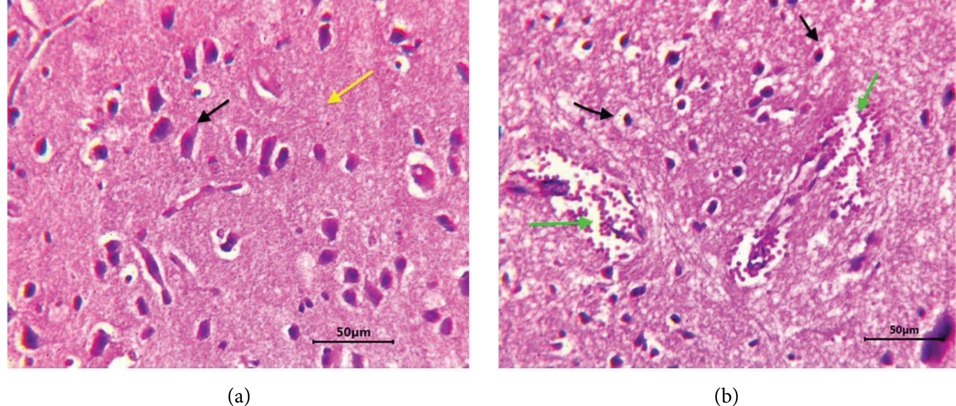
Groups	SOD	GPx	GR	AOPP	MDA
Control	$846.48^{b} \pm 26.99$	$236.12^{b} \pm 14.84$	$32.30^{b} \pm 3.84$	$1.36^{b} \pm 0.05$	$50.53^{b} \pm 2.32$
1/10 LD50 DM	$386.44^{a} \pm 8.16$	$125.63^{a} \pm 4.29$	$18.74^{a} \pm 1.18$	$1.77^{c} \pm 0.22$	$253.41^{d} \pm 17.09$
Fluoride (4.5 ppm)	$358.61^{a} \pm 10.49$	$115.35^{a} \pm 3.64$	$16.82^{a} \pm 0.66$	$1.92^{c} \pm 0.21$	$143.21^{c} \pm 8.52$
DM (1/10) + fluoride (4.5 ppm)	$237.08^{c} \pm 10.61$	$68.09^{d} \pm 4.66$	$12.52^{d} \pm 0.49$	$2.88^{d} \pm 0.21$	$374.33^{e} \pm 28.51$
ZO extract (300 mg/kg)	$793.57^{b} \pm 29.71$	$249.82^{bc} \pm 18.39$	$28.68^{b} \pm 3.02$	$1.07^{a} \pm 0.08$	$46.60^{ab} \pm 7.22$
ZO extract (300 mg/kg) + DM (1/10 LD50)	$802.56^{b} \pm 40.45$	$278.47^{c} \pm 17.28$	$35.38^{c} \pm 5.16$	$1.21^{ab} \pm 0.12$	$54.05^{b} \pm 3.70$
ZO extract (300 mg/kg) + F^- (4.5 ppm)	$828.83^{b} \pm 45.92$	$232.84^{bc} \pm 11.62$	$30.91^{bc} \pm 3.36$	$1.16^{ab} \pm 0.07$	$69.64^{b} \pm 4.69$
ZO extract (300 mg/kg) + DM (1/10 LD50) + F^- (4.5 ppm)	$801.05^{b} \pm 18.75$	$234.62^{bc} \pm 7.42$	$30.43^{bc} \pm 2.88$	$1.13^{ab} \pm 0.04$	$47.04^{ab} \pm 3.00$
Quercetin (100 mg/kg) + DM (1/10 LD50) + F^- (4.5 ppm)	$706.67^{b} \pm 26.96$	$253.87^{bc} \pm 12.62$	$20.33^{a} \pm 1.67$	$1.39^{ab} \pm 0.08$	$37.21^{a} \pm 2.99$

Values are given as mean ± SE of 6 animals unless otherwise stated. Values having different superscripts (a, b, and c) in a column are statistically different from one another at a 5% level of significance. Values of SOD (superoxide dismutase) are expressed in units/g of tissue. Values of GR (glutathione reductase) are expressed in nmol of NADPH/min. GPx (glutathione peroxidase) is expressed in units/g of tissue. Values of the advanced oxidation protein product (AOPP) are expressed in μM of chloramine-T. Values of malondialdehyde (MDA) are expressed in nmol of MDA formed/g/h.

Table 6: Fluoride levels in plasma and brain on subacute exposure to fluoride alone and in combination with dimethoate (DM) and *Z. officinale* in *Wistar rats*.

Groups	Plasma	Brain
Control	$0.17^{a} \pm 0.005$	$0.32^{b} \pm 0.028$
1/10th LD50 DM	$0.19^{a} \pm 0.008$	$0.32^{b} \pm 0.019$
Fluoride (4.5 ppm)	$0.36^{d} \pm 0.045$	$0.98^{e} \pm 0.077$
DM (1/10th) + fluoride @4.5 ppm	$0.26^{bc} \pm 0.005$	$0.67^{cd} \pm 0.025$
ZO extract (300 mg/kg)	$0.18^{ab} \pm 0.003$	$0.33^{b} \pm 0.004$
ZO extract (300 mg/kg) ± DM (1/10 LD50)	$0.16^{a} \pm 0.001$	$0.33^{b} \pm 0.000$
ZO extract (300 mg/kg) + F^- (4.5 ppm)	$0.26^{c} \pm 0.029$	$0.47^{c} \pm 0.008$
ZO extract (300 mg/kg) + DM (1/10 LD50) + F^- (4.5 ppm)	$0.22^{b} \pm 0.012$	$0.33^{b} \pm 0.006$
Quercetin (100 mg/kg) ± DM (1/10t LD50) + F^- (4.5 ppm)	$0.19^{ab} \pm 0.006$	$0.27^{a} \pm 0.005$

Values are given as mean ± SE of 6 animals unless otherwise stated. Values have been measured in ppm. Values having different superscripts (a, b, c, d, and e) in a column are statistically different from one another at a 5% level of significance. ZO: *Zingiber officinale*.



(a) (b)

Figure 1: Continued.

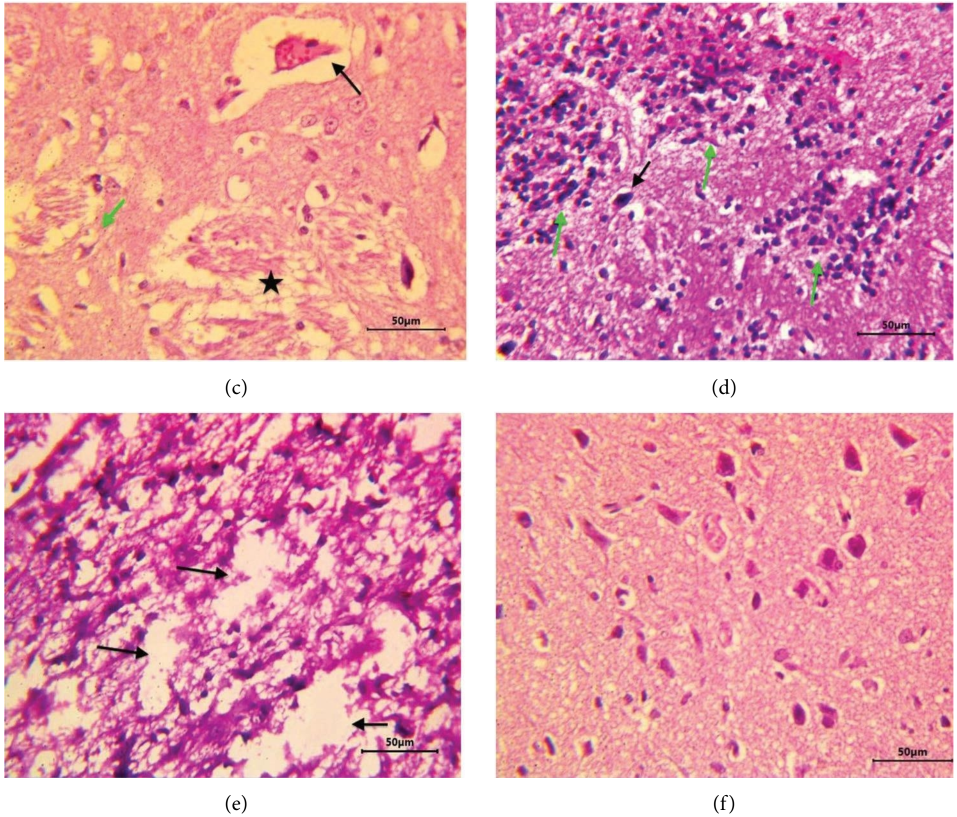
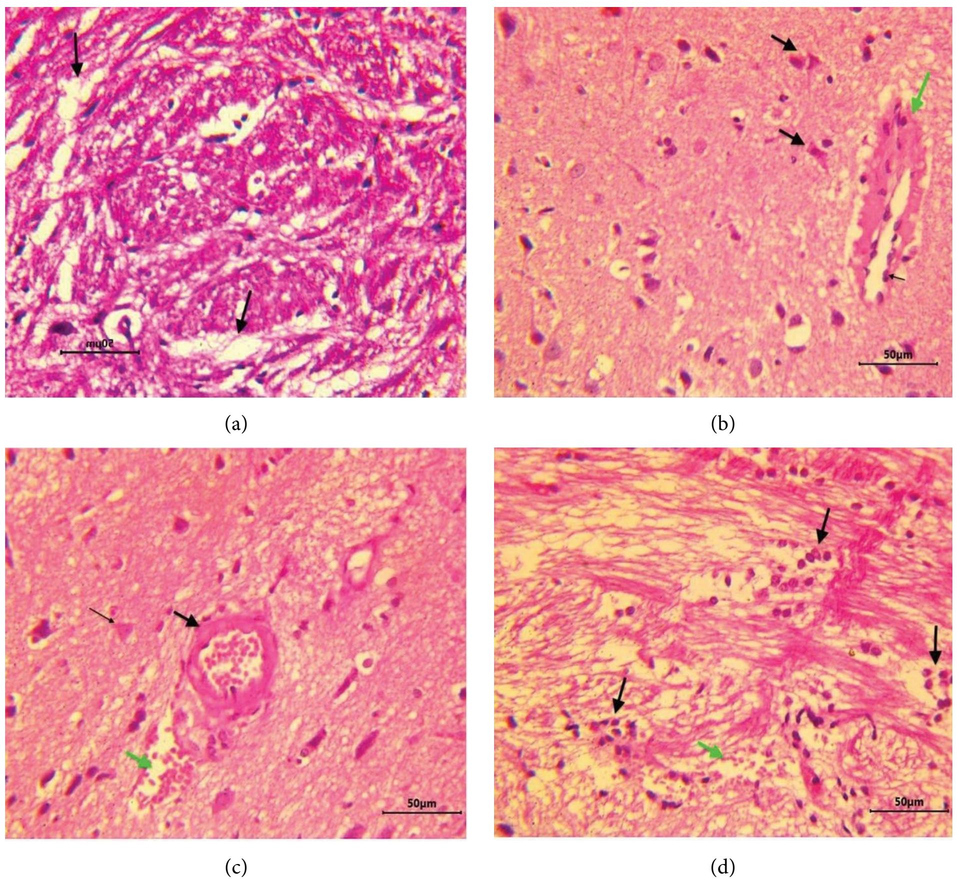


Figure 1: (a–f) Normal architecture of cerebrum of rats in group I with healthy neurons (arrow) and neuropil (arrow) (a), perivascular edema and hemorrhage (arrow), perineuronal edema, and neuronal degeneration (arrow) in group II (b), congestion, perivascular edema (black arrow), shrunken, and pyknotic neurons (green arrow) alongside spongiosis (star) in group III (c), large aggregates of glial cells (green arrows) in neuropil with severe neuronal degeneration (black arrow) (d) with severe spongiosis leading to microcavitation (arrow) in group IV (e), and no pathological lesions seen in the cerebrum of group V (f). H&E 400x.

Group VI rats showed mild spongiosis and neuronal degeneration (Figure 2(a)). Cerebrum of group VII had endothelial cell hypertrophy and perivascular edema (Figure 2(b)). Also, occasionally neurons multifocally were shrunk with hypereosinophilic cytoplasm and pyknotic nucleus. Satellitosis wherein glial cells encircled around the degenerated neurons was also observed (Figure 2(b)).

In group VIII, rats had less severe changes when compared to those seen in group IV. Focal glial aggregation was absent. However, mild congestion and fibrinoid necrosis of the vascular wall leading to perivascular edema and hemorrhage were recorded. Overall, spongiosis was also subdued (Figure 2(c)). However, in comparison to group VIII, group IX rats had more severe lesions consisting of multifocal small aggregates of glial cells which were randomly distributed in the neuropil (Figure 2(d)). Moreover, spongiosis and neuronal degeneration were also evident (Figure 2(d)). Lesions in group IX were, however, less intense when compared to the changes observed in group IV.

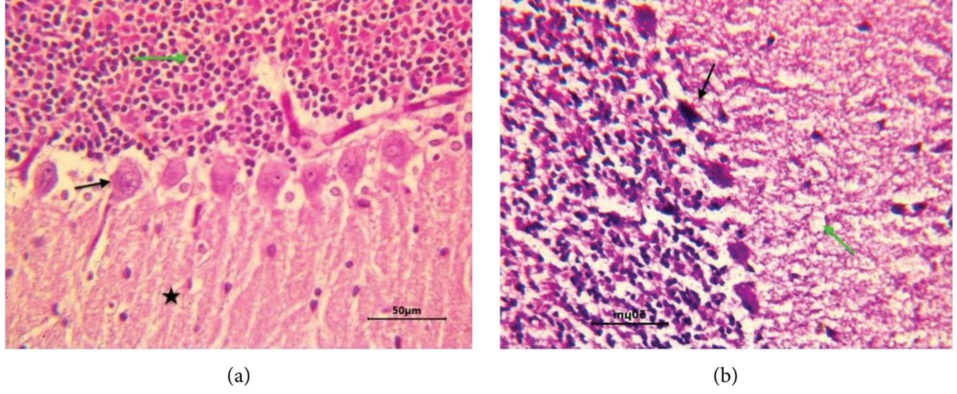
3.3.2. Cerebellum. The cerebellum of control rats showed normal cerebellar architecture with healthy Purkinje cells, granule cell layer, and molecular layer (Figure 3(a)). In group II, mild degenerative changes in Purkinje cells and neurons of the granule cell layer were discernible along with spongiosis in the molecular layer (Figure 3(b)). Similarly, in group III, Purkinje cell degeneration, atrophy, and loss were seen but spongiosis was minimal (Figure 3(c)). Most severe changes in the cerebellar cortex and white matter were noted in group IV and included necrosis and loss of Purkinje cells as well as granule layer cells (Figure 3(d)). Moderately severe congestion, gliosis, and perivascular edema with severe spongiosis characterized by extensive dilatation of myelin sheaths could be appreciated in white matter and molecular layer (Figures 3(d) and 3(e)). Hemorrhage and gliosis were also observed in white matter in rats of this group. In contrast, no pathological alterations were appreciated in the cerebellum of group V animals (Figure 3(f)). However, mild Purkinje cell degeneration was observed in group VI rats (Figure 4(a)). The cerebellum from group VII also showed mild degeneration of Purkinje cells and mild spongiosis in white matter tracts (Figure 4(b)). Group VIII cerebellum (Figure 4(c)) revealed only mild neuronal degeneration and congestion in comparison to the severe pathological lesions seen in the cerebellum of group IV rats. The degree of spongiosis was also very mild in group VIII. Group IX had more severe lesions in their cerebellum (Figure 4(d)) as compared to those in group VIII including congestion, spongiosis in the white matter along with degeneration, and



(a) (b)

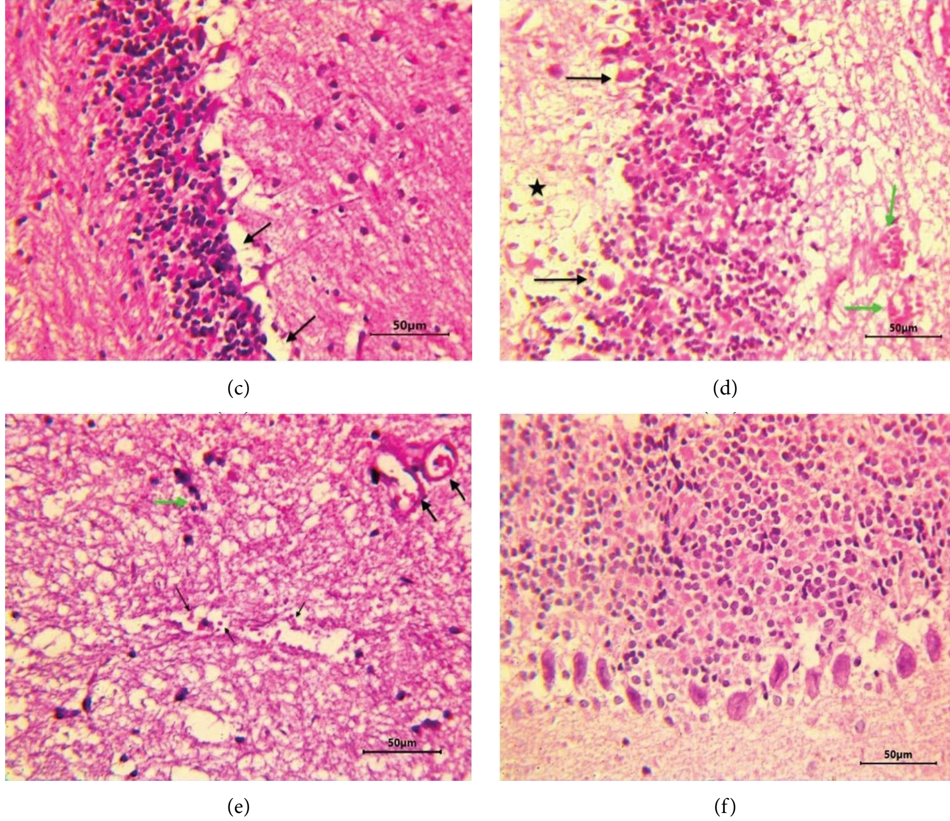
(c) (d)

Figure 2: (a–d) Mild neuronal degeneration, gliosis, and vacuolation in the neuropil (arrows) in group VI (a), perivascular edema (green arrow), endothelial hypertrophy (thin black arrow), and neuronal degeneration (black arrows) in group VII (b), hyalinisation/fibrinoid necrosis in the blood vessel wall (black arrow) with perivascular hemorrhage (green arrow) and neuronal degeneration (thin black arrow) in group VIII (c), and glial aggregation (black arrows) and moderately severe spongiosis with hemorrhage (green arrow) in group IX rats (d). H&E 400x.



(a) (b)

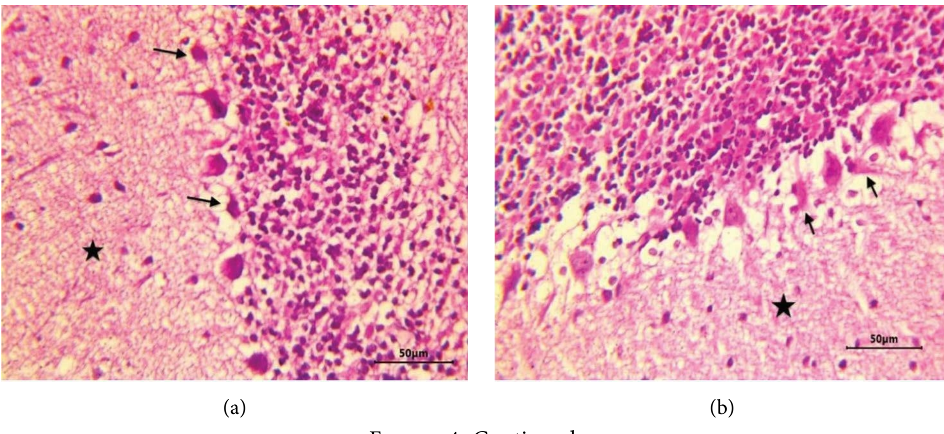
Figure 3: Continued.



(c) (d)

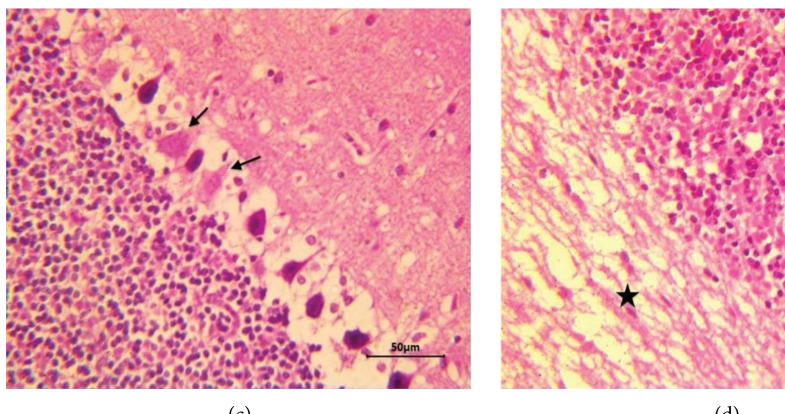
(e) (f)

Figure 3: (a–f) Normal Purkinje cells (black arrow), granule layer cells (green arrow), and molecular layer (star) in group I rats (a), degeneration in Purkinje cell layer (black arrow) along with mild spongiosis in the molecular layer (green arrow) (b), loss of Purkinje cells in group III rats (arrow) (c), vacuolation (spongiosis) and congestion in white matter (green arrow), necrosis of Purkinje cells (black arrow) alongside their disappearance and vacuolation in the molecular layer (star) in group IV rats (d), congestion (black arrow), spongiosis, gliosis (green arrow), and hemorrhage (thin arrows) in group IV rats (e), and normal cerebellar architecture in group V (f). H&E 400x.



(a) (b)

Figure 4: Continued.



(c) (d)

Figure 4: (a–d) Degeneration of Purkinje cells (black arrow) and mild spongiosis (star) in group VI (a), Purkinje layer cell degeneration (arrow) and mild spongiosis (star) VII (b), neuronal degeneration and phagocytosis of dying neurons by glial cells (neuronophagia) (black arrows) in group VIII (c), and degeneration and loss of Purkinje cells (arrow) and severe spongiosis (star) in cerebellar white matter in group IX (d).

loss of Purkinje cells; however, in contrast to lesions observed in cerebellum of group IV, spongiosis and neuronal degeneration were of much less severity.

4. Discussion

The incidence of neurological disorders, such as Parkinson's disease, Alzheimer's disease, dementia, and brain tumors, among the general public is on the rise. Likewise, an unprecedented increase in the number of kids being diagnosed with developmental disorders, such as autism, dyslexia, and slow learning, has been witnessed during the last few decades. Evidence is emerging that rising levels of environmental degradation and omnipresent pollution are major incriminating factors for the significant spurt in cases of mental health diseases around the world [55]. Since inherent high lipid concentration in nervous tissue makes it especially susceptible to oxidant damage, pollutants, such as F and OP pesticides such as DM which can cross the blood-brain barrier, may induce oxidative brain injury and neuroinflammation [29, 56, 57]. Erythrocytes transport gases and maintain systemic redox equilibrium but are among the first to bear the brunt of oxidative damage in the event of excessive generation of reactive oxygen species owing to exposure to environmental pollutants, which not only damages their structure and reduces their finite lifespan but also can impinge systemic oxygen delivery causing anemia and hypoxia [58, 59]. Antioxidant machinery in erythrocytes is apt to counteract oxidative damage but injury occurs when an overload of free radical generation overpowers this antioxidant response system causing peroxidation of membrane phospholipids reducing erythrocytic deformability and expediting their premature removal from the general circulation [60]. Reduction in levels of enzymatic and nonenzymatic antioxidants, such as SOD, CAT, GSH, GPX, GR, TTH, and TAS, is indicative of exhaustion incumbent upon increased catabolism of free radicals such as H_2O_2. Membrane integrity of erythrocytes is also affected by AChE activity alterations, and the latter is a marker for assessment of anemia [61]. Moreover, since NO is produced by endothelial cells in the presence of ACh, fluctuations in AChE in response to poisoning can affect vascular dilation and leukocytic adhesion during inflammation [62]. Thiols are sulfhydryl group-containing compounds with exuberant antioxidant activity which assist in maintaining a reducing environment inside cells. Examples include oxidative stress busters such as GSH, a tripeptide rich in cysteine, and glycine and glutamic acid, which apart from being a potent guard against free radical damage and a mediator of detoxification reactions, also serves as a cofactor for GP_X. Enzyme GR regenerates GSH from its oxidized form, so excess free radical generation can crash GR values.

Low-dose long-term exposure to a mixture of toxicants can overwhelm the antioxidant capacity of erythrocytes due in part to acceleration in auto-oxidation of hemoglobin, accumulation of fluorescent heme degradation products, oxidative stress-induced premature eryptosis, and sequestration of injured nondeformable RBCs out of circulation leading to diminishment in oxygen transfer to vital organs such as brain [63, 64]. Since oxidative phosphorylation serves as the only means of energy generation for neurons, an adequate oxygen supply to brain is quintessential and toxicant-induced oxidative damage to erythrocytes can also indirectly impact brain functioning or exacerbate primary oxidative brain damage.

The present work studied the impact of simultaneous F and DM exposure on brain and blood antioxidation apparatus. While DM is a well-researched cholinergic toxin [1, 13], repeated exposure to F also induces neuron apoptosis and is suspected to contribute to developmental delays and impaired memory [65, 66]. Thus, a combined F and DM exposure may be associated with furtherance in neurobehavioral alterations, cognitive deficits, and structural alterations in brain histology. Scientific literature has presented clear evidence that F induces oxidative injury and neurotoxicity [60, 67, 68] while DM poisoning can cause AChE inhibition and toxicity in a wide variety of species including aquatic fauna and birds [13]. Here, we assessed the

induction of neurotoxicity and hematotoxicity after their individual as well as combined exposure. F-only exposure was toxic to erythrocytic antioxidant machinery as it brought a significant drop in levels of SOD, GPx, GR, GSH, TTH, and AChE and significant augmentation in values of MDA and AOPP which validates the findings of earlier workers who have also documented role of F in oxidative erythrocytic damage [58, 69, and 27]. Results of the present work also showed oxidative damage-induced significant fall in levels of erythrocytic GSH, TTH, AE, SOD, GPx, and GR and a significant increment in MDA after DM-only administration which agreed with the previously published results [60, 70]. It was noteworthy that TAS levels in RBCs were significantly reduced only after the coexposure to the toxicants. In brain, individual F and DM treatment significantly reduced the levels of TAS, TTH, AE, AChE, CAT, SOD, GPx, and GR besides significantly stepping up the contents of MDA and AOPP. Notably, all animals exposed to DM and F in concomitance had a more significant decrease in GP_X, GR, AE, CAT, and SOD alongside a higher significant increase in MDA and AOPP in brain. Increased MDA and AOPP levels suggested greater oxidative damage to polyunsaturated lipids and proteins, respectively, after combined toxicity [70, 71]. Significantly higher alterations in AE, CAT, and MDA concentrations in blood were also observed in the combination group as compared to those receiving any single toxicant exposure, which is similar to the findings of Okediran et al. [72]. Escalation of lipid and protein peroxidation after toxic interaction also caused far greater damage in brain parenchyma visible as severe neuronal degeneration, perivascular vacuolation in neuropil, gliosis, and multifocal glial aggregate formation alongside congestion and hemorrhage in brain. Existing literature concurs with the current research that subacute exposure to F or OP compounds alone or in combination with metalloids can effectuate significant devitalization of protective antioxidant enginery in brain with significant alterations in SOD, CAT, GPx, and MDA levels besides incurring pathological damage to brain [73–75]. Earlier researchers showed that coexposure to deltamethrin and F inhibited AChE activity, increased hepatic oxidative stress, and altered biochemical parameters indicative of hepatic damage in rats [76, 77]. Additionally, Dec et al. [78] suggested pre- and postnatal exposure to F-induced morphological changes in rat liver and brain by impairment of antioxidant defense mechanism and modulation in cyclooxygenase expression.

Unpremeditated exposure to a concoction of toxic agents, such as heavy metals and pesticides, is a widespread and common concern all over the world; therefore, researchers are trying to unearth inherent preventive properties in natural ingredients that can counteract ill effects of environmental pollutants on health of all organisms including humans. Ginger is commonly used in Indian ayurvedic and other traditional systems of medicine for the treatment of various disorders [16], and our research provided scientific evidence that it could serve as an excellent antioxidant supplement to mitigate dual F and DM neuro and hematotoxicity. In the present study, ginger subverted oxidative injury after combined F and DM exposure by significantly raising and completely restoring the altered levels of TAS, AE, AChE, CAT, GPx SOD, GSH, TTH, and AOPP and MDA in brain in contrast to quercetin which did not bring about restoration of TAS, AE, and GR. In blood, ginger displayed similar ameliorative potential as that of quercetin since both could bring about complete amelioration in TAS, GSH, TTH, AChE, CAT, GPx, GR, AOPP, and MDA but only partial correction was seen in the levels of AE and SOD which is in accordance with the previously published data [18]. Hydroalcoholic extract of ZO was found to contain phytochemicals, such as flavonoids, quercetin, and curcumin [18], which explains its slightly better effectivity in countering F and DM toxicity than quercetin in our study. Amara et al. [58] also advocated the use of antioxidants, such as selenium and vitamin E, to offset DM-induced oxidative stress-mediated erythrocytic damage. Umarani et al. [79] documented the protective properties of rutin against F-induced oxidative cardio and hematotoxicity. Sutalangka and Wattanathorn [80] propounded ZO as a neuroprotectant and cognitive ability enhancer when used in combination with *C. rotundus*. Boric acid manifested neuroprotective effects and engendered improvements in altered levels of MDA and SOD besides shielding from DNA damage caused by *in vitro* F toxicity on rat synaptosomes [81]. In a similar manner, Wattanathorn et al. [82] reported that alcoholic extract of ZO effectively improved cognitive functioning and neuron density in the hippocampus of rats who developed focal ischemic infarct after clamping of their right medial cerebral artery. Likewise, Shanmugam et al. [83] recommended ginger may be used to assuage hyperglycemia-induced oxidative stress-mediated neurotoxicity in streptozotocin-induced diabetic rats since treatment with ginger successfully lowered raised MDA levels and replenished SOD, CAT, GPx, GP, and GSH. In the current investigation, ginger significantly reduced pathological alterations in brain in rats given F or DM since neuronal degeneration, necrosis, gliosis, and spongiosis were significantly diminished in dually intoxicated rats treated with ZO. Interestingly, ZO seemed to confer greater amelioration than quercetin in rats administered both toxicants simultaneously as overall lesions in the brain of the ginger-treated group were less severe than those in quercetin-administered rats. Similarly, neurodegeneration and neuroinflammation in the cerebral cortex and cerebellum in streptozotocin-induced diabetic rats were inhibited by ginger, and the latter also reduced expression of TNF-α (tumor necrosis factor-α) and caspase-3 in the diabetic rats [84].

5. Conclusions

Present findings have shed light on the fact that F in synergy with DM has the potential to significantly elevate oxidative stress in blood as well as in brain, and the combination can perpetrate appreciably greater damage in cerebral and cerebellar histoarchitecture than that inflicted after exposure to any one toxicant. However, ginger extract supplementation not only significantly reversed the oxidative damage to the erythrocytic and brain antioxidant setup induced by

coexposure to F and DM but also alleviated dual exposure-induced pathological lesions in brain. Hence, ginger extract supplementation in diet can work as a prevention strategy to negate the occurrence of subacute neurotoxicity and hematotoxicity which can be an unwanted but unavoidable ramification of DM contamination in F endemic areas.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding authors on a reasonable request.

Ethical Approval

The experimental protocols were duly approved by the Institutional Animal Ethics Committee (IAEC), vide proposal no. 3/IAEC/2020 (Registration no. of IAEC–862/GO/Re-s/ReBi-L/04/CPCSEA).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Priyanka Sharma investigated the study, proposed the methodology, performed a formal analysis, and wrote the original draft. Pawan Kumar Verma conceptualized and supervised the study, curated the data, performed project administration, collected resources, and reviewed and edited the article. Shilpa Sood conceptualized and supervised the study, visualized the study, and curated the data. Rajinder Raina curated the data, proposed the methodology, and reviewed and edited the article. Sheen Tukra investigated the study, proposed the methodology, and reviewed and edited the article. Zuhaib F. Bhat curated the data, proposed the methodology, and reviewed and edited the article. Rana Muhammad Aadil curated the data, collected resources, and reviewed and edited the article.

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