# Oxidative renal damage induced by fluoride and dimethoate and its mitigation by Zingiber officinale in Wistar rats

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**Research Article**

**Keywords:** Fluoride, Dimethoate, Nephrotoxicity, Zingiber officinale, Wistar rats.

**Posted Date:** May 19th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1653918/v1

# Abstract

Simultaneous exposure of dimethoate (DM) and fluoride ($F^-$) is a natural phenomenon which may insidiously impact functioning of various organs including kidney. *Zingiber officinale* (ZO) is harbours excellent natural antioxidant properties. Therefore, the present investigation was aimed to determine the potential of ZO in alleviating renal oxidative damage induced by DM and $F^-$ alone and by their concurrent exposure in Wistar rats. Subacute toxicity study was conducted on fifty four Wistar rats randomly allocated to nine groups with six rats in each. Following sub-acute exposure of either $F^-$ or DM a significant rise in blood urea nitrogen, creatinine and uric acid along with a significant declined in plasma levels of reduced glutathione and nitric oxide occurred. Significantly declined total antioxidant status, total thiols, catalase, superoxide dismutase, glutathione peroxidase, aryl esterase and acetylcholinesterase in renal tissue with increased levels of lipid and protein peroxidation were recorded alongside pathological alterations in renal tissue of toxicant exposed rats as compared to control. However, more severe changes were observed in levels of the above biomarkers and renal histoarchitecture on co-administration of both the toxicants indicating enhanced toxicity. Concurrent exposures of ZO extract along with either or both the toxicants led to significant restoration of activities of the antioxidant apparatus, biochemical profile of kidney and renal histology. Hence, repeated administration of ZO extract alongside individual as well as dual toxicant treatment caused amelioration of oxidative damage, renal biomarkers and histopathological alterations in renal tissue of Wistar rats.


# Introduction

Rapid industrialization and urbanization has reduced percentage of land under cultivation impelling nonchalant application of pesticides for boosting food production to support a continuously growing population especially in the developing world which in turn has introduced a significant volume of pollutants into environment and aquatic ecosystems. Dimethoate (O, O- dimethyl- S-methylcarbamoylmethylphosphorothioate), an organophosphorus compound is rampantly used in agriculture as an insecticide [1, 2] to control arthropod infestation in agricultural products [3]. Dimethoate (DM), a class II agent as per the WHO classification, is a recognized neurotoxin, which inhibits acetylcholinesterase (AChE) with consequent interruption of cholinergic neurotransmission in the central and peripheral nervous systems apart from altering other metabolic enzymes in target and non-target species *viz.* birds, insects, mammals, etc [2]. In addition, continued exposure to low levels of DM induced oxidative stress spurred DNA damage and impaired functioning of membrane integrated ATPases leading to compromised renal functioning and altered renal morphology [4].

On the other hand, excessive intake of halogen fluorine, the most electronegative element naturally occurring in earth's crust and also abundant in air, water and soil due to its unabated emission via anthropogenic and geogenic activities, causes fluorosis which is a crippling disease affecting inhabitants in most parts of the globe. After exposure, fluoride ($F^-$) is readily absorbed in the body, reaches various organs like heart, kidney, muscle, liver, bone and brain and impairs tissue functions by inducing oxidative

damage [5, 6]. Exact mechanism of $F^-$ toxicosis is not known but it has been suggested that oxidative stress is an important player in $F^-$ mediated damage to various tissues. It binds calcium ions inducing hypocalcaemia and causes disruption of various physiological processes leading to cardiovascular disorders, developmental as well as behavioral neurological disorders and chronic renal diseases [7, 8]. It also harbours pro-oxidative capabilities, inhibits antioxidant enzymes at the forefront of fighting oxidative stress and its exposure impairs AChE activities and it may augment organophosphorus induced multi-systemic toxicity [9, 10]. Previous published reports have found an association between unabated low level $F^-$ exposure and significant alterations in hepato-renal health indices of human and animals [11–13]. In the contemporary scenario, animals and human populations are exposed to multiple number of toxicants simultaneously, many of which may act collusively to cause clandestine nephrotoxicity. Multiple lines of evidence from experimental studies substantiate that co-exposure to more than one environmental pollutant results in additive or synergistic toxicity interactions [14–16]. So, high and ever rising environmental contamination with DM along with pervasiveness of naturally elevated $F^-$ concentrations in ground water poses increased risk of renal toxicity in exposed people or animals. In-fact a recent study has determined occurrence of glomerular filtration deficits and increased odds of developing chronic kidney impairment among pesticide applicators [17]. The effect of combined toxicity not only can be exceptionally deleterious in people with pre-existing impinged renal functioning such as those suffering from chronic renal diseases but also it presents as a serious occupational hazard for people from endemic fluorosis areas employed in pesticide application business [18].

Newer sources of dietary antioxidants are being researched experimentally for their nephroprotection potential and are being increasingly used clinically for relieving stress of oxidant excess in patients with kidney impairment [19]. Previous research has shown that natural compounds have potential to protect against DM induced oxidative stress driven toxicity affecting various organs [20–22]. Specifically, workers have demonstrated that intake of herbal extracts shields from oxidative damage led nephrotoxicity created by DM [23] and antioxidants are also beneficial in reducing fluoride toxicity [24]. *Zingiber officinale* (family Zingiberaceae) commonly known as ginger, a common culinary spice, is a rhizome of a perennial plant that has been incorporated in different remedial formulations of Ayurvedic and traditional medicines for a wide range of acute and chronic illnesses since ancient times [25]. Due to presence of potent bioactive compounds, ginger has wide ranging pharmacological activities and hence possesses immense potential as a nutraceuticals [25]. A study found ginger to be protective against DM induced toxicity in male rabbits [26]. In-fact, use of other condiments previously granted protection against renal damage after $F^-$ intoxication in rats [27] but efficacy of ginger in amelioration of $F^-$ and DM combined toxicosis has not been elucidated yet. In this study we intended to evaluate ameliorative antioxidant abilities of ginger against nephrotoxicity induced by individual as well as concurrent exposure to $F^-$ and DM in Wistar rats.

# Materials And Methods

Extract preparation: Sufficient quantities of rhizome of *Zingiber officinale*, purchased from the local market and identified by the Taxonomists in University of Kashmir (Voucher specimen No 2921, dated 03/03/2020), were cleaned, dried and pulverized into fine powder using electric grinder. Powdered ZO was subjected to hydro-alcoholic extraction (1:1) in Soxhlet apparatus while keeping hot plate temperature between 65–70°C and finally dried in a rotatory evaporator (55–60°C, 15 rpm). The dried extract was stored in a glass jar under refrigeration and desiccation.

## Drugs and chemicals used

Quercetin, serine selicylate, 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS), was obtained from Sigma Aldrich, USA, while sodium fluoride, ethyl alcohol, Nicotinamide adenine dinucleotide sodium salt (NADPH), 5,5-Dithiobis (2-nitro benzoic acid) (DTNB) from Hi-Media, Mumbai, India. All other chemicals used in the study were analytical grade and obtained from SD-Fine Chemicals, Mumbai, India. Tafgor (Dimethoate, 30% EC) was obtained from AgroShopy, Rallis India Limited, Mumbai, India.

## Experimental design

A 28 day *in-vivo* experiment was conducted on healthy Wistar rats of either sex weighing 180–190 gm obtained from Indian Institute of Integrative Medicine (CSIR Lab), Jammu. The animals were provided standard managemental conditions *viz.* pelleted ration and clean drinking water *ad-libitum.* The pelleted food provided to Wistar rats was supplied by Animal Feed Unit, Department of Animal Nutrition, CSKHPKV, Palampur, Himanchal Pradesh INDIA (Table 1). The rats were acclimatized in the laboratory conditions for a period of 3 weeks prior to the start of experiment and were kept under constant observation during the entire study period. A daily cycle of 12 h of light and 12 h of darkness was provided to animals. The experimental protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) vide proposal no 3/IAEC/2020 (Registration No of IAEC – 862/ac/04/CPCSEA). Dimethoate (DM) used at the rate of 3.10 mg/kg body weight (1/10th dose of median lethal dose ($LD_{50}$) daily via oral gavage and fluoride 4.5 ppm (three times of the WHO recommended upper level of fluoride) in drinking water. In the present study, sodium fluoride was used as source of fluoride (9.945mg/L of drinking water) provide 4.5 ppm fluoride in drinking water. A total of 54 adult Wistar rats were randomly allocated in nine groups with six rats in each and details of the treatment regimen followed are presented as under

Table 1
Composition of pelleted feed provided to Wistar rats

| Ingredients | Percentage |
|---|---|
| Crushed maize meal | 55.00 |
| Soybean meal | 20.00 |
| Fish meal | 12.00 |
| Skim milk powder | 5.00 |
| Soya oil | 2.50 |
| Molasses | 3.00 |
| Salt | 0.40 |
| Dicalcium phosphate | 1.25 |
| Limestone powder | 0.50 |
| Mineral premix | 0.15 |
| Vitamin premix | 0.20 |

| Groups | Treatment | Dose and Route of administration |
|---|---|---|
| i. | Control | 1 ml /day/rat, PO, drinking water |
| ii. | *Zingiber officinale* (ZO) | 300 mg/kg BW, PO |
| iii. | Dimethoate (DM) | 31.0 mg/kg (1/10th $LD_{50}$), PO |
| iv. | Fluoride ($F^-$) | 4.5 ppm in drinking water |
| v. | DM + $F^-$ | 1/10th of $LD_{50}$ (PO) + 4.5 ppm in drinking water |
| vi. | ZO + DM | 300 mg/kg (PO) + 1/10th of $LD_{50}$ (PO) |
| vii. | ZO + $F^-$ | 300 mg/kg (PO) + 4.5 ppm |
| viii. | ZO + DM + $F^-$ | 300 mg/kg (PO) + 1/10th of $LD_{50}$ + 4.5 ppm |
| ix. | Quercetin + DM + $F^-$ | 100 mg/kg (PO) + 1/10th of $LD_{50}$ + 4.5 ppm |

Blood and tissue samples collection: At the end of the experiment, 3-4ml heart blood from each rat was collected in heparinized tubes and animals were then sacrificed by cervical dislocation and kidneys were collected in ice-cold 0.5 M phosphate buffer (pH 7.4) for antioxidant biomarker studies and in 10% formalin for histopathological examination. Tissue homogenate (10%) was prepared by homogenizing

the tissue using Teflon-coated homogenizer at 1000 rpm for 5–7 minutes at 4°C. Whole blood is used for the estimation of hemoglobin and reduced glutathione (GSH). Plasma for biochemical analysis was obtained after centrifuging blood at 3000 rpm for 15 min and was kept at 4°C for further analysis. Plasma renal biomarkers viz. blood urea nitrogen (BUN), creatinine (CR), uric acid (UA) and minerals (calcium and phosphorus) were determined using standard kits supplied by Transasia Bio-Medicals Ltd, India using Chemistry Analyzer (CHEM-7, Mannheim, Germany). Levels of nitric oxide (NO) were measured in plasma of all the groups by spectrophotometrically using copper-cadmium alloy [28]. The erythrocyte sediment remaining after the harvest of plasma was diluted with normal saline solution (NSS) in the ratio of 1:1 (v/v) basis by gentle thorough mixing. The diluted erythrocytes were centrifuged for 10 min and after discarding the supernatant along with buffy coat, again NSS was added to the RBC on v/v basis and the process was repeated three times. A final 1% haemolysate was prepared for determination of different antioxidant enzymes by adding 9.9 ml of 0.1M PBS (pH 7.4) to 100 µl washed RBC.

### Determination of antioxidant biomarkers

The activity of arylesterase (AE) was measured by using phenyl acetate as a substrate and was expressed in unit per ml (U/ml), where one unit corresponds to µmol phenol formed per minute [29] Reduced glutathione (GSH), total antioxidant status (TAS) and total thiols (TTH) were estimated using standard methods [30–32]. The enzymatic parameters *viz*., catalase (CAT) and glutathione peroxidase (GPx) were determined as per the methods of Aebi, [33] and Hafeman et al. [34], respectively. The activities of superoxide dismutase (SOD) and glutathione reductase (GR) were determined as per the method described by Marklund and Marklund [35] and Carlberg and Mannervik [36], respectively. Similarly, malondialdehyde (MDA) and advance oxidation protein product (AOPP) levels in renal tissue were determined using standard methods [37, 38].

### Histopathology

Representative samples from kidneys of rats in different groups collected in formalin were washed, dehydrated, cleared, paraffin embedded, sectioned and stained with hematoxylin and eosin. The prepared sections were examined for presence of histomorphological changes.

### Statistical analysis

The data was presented as mean ± standard error and was analyzed for analysis of variance at 5% level of significance using the Duncan Multiple Range test (SPSS 21.0).

## Results

### Plasma levels of renal biomarkers

The mean ± SE values of BUN, CR and UA of control and different treatment groups are presented in the Table 2. A significant rise in BUN and CR was seen in group III and V as compared to control. $F^-$ or DM didn't differ in their ability to raise BUN or CR levels. Dual toxicant administered groups however had significantly higher BUN and CR values as compared to that in rats receiving only one toxicant. Co-administration of ZO with either toxicant failed to cause any significant alterations in BUN but led to a significant decrease in CR values. A significant reduction in BUN as well as CR levels was however seen after dual toxicant administration in combination with ZO or quercetin. ZO was as effective as quercetin in amelioration of dual toxicant induced high BUN and CR levels. Toxicity induced by DM but not F triggered significant elevation in UA concentrations. Further, combined exposure to F and DM also caused significant increase in UA levels but these levels were not significantly higher than that in DM alone group. While, addition of ZO completely prevented any alteration in UA in DM or dual toxicant groups, quercetin was only partially successful in protecting UA concentrations in dual toxicant exposed rats. Alterations recorded in plasma calcium and phosphorus levels in different experimental rats are presented in Table 3. Plasmatic Ca and P values displayed a significant drop in all toxicant groups in comparison to the corresponding levels in control animals but no further collapse was noted upon using the toxicants simultaneously. ZO however corrected the plummet in these values in all toxicant groups and after its supplementation, no significant difference was noticed as compared to the corresponding levels in control animals. Effect of quercetin in bringing back the plasma concentration of Ca and P was analogous to that of ZO.

Table 2

**Effect of hydro-alcoholic extract of** Zingiber officinale **(ZO) on toxicity induced by fluoride ($F^-$) and dimethoate (DM) alone and in-combination on plasma renal biomarkers and minerals in Wistar rats.**

| Groups | BUN | CR | Uric acid | Calcium | Phosphorus |
|---|---|---|---|---|---|
| Control | $42.84^{a} \pm 2.40$ | $0.43^{a} \pm 0.03$ | $2.57^{a} \pm 0.41$ | $10.99^{c} \pm 0.56$ | $9.72^{c} \pm 0.47$ |
| ZO Extract (300 mg/kg) | $38.54^{a} \pm 3.64$ | $0.49^{a} \pm 0.07$ | $3.49^{a} \pm 0.97$ | $10.34^{c} \pm 0.93$ | $8.28^{ab} \pm 0.67$ |
| Dimethoate (DM -1/10th $LD_{50}$) | $56.45^{b} \pm 4.42$ | $1.11^{c} \pm 0.12$ | $4.52^{bc} \pm 0.60$ | $5.08^{a} \pm 0.79$ | $5.56^{a} \pm 0.43$ |
| Fluoride ($F^-$ - 4.5 ppm) | $55.04^{b} \pm 2.74$ | $1.05^{c} \pm 0.11$ | $3.89^{abc} \pm 0.49$ | $5.55^{a} \pm 0.53$ | $6.70^{b} \pm 0.81$ |
| DM (1/10th $LD_{50}$) + $F^-$ (4.5 ppm) | $112.99^{d} \pm 3.69$ | $1.50^{d} \pm 0.08$ | $4.82^{c} \pm 0.47$ | $5.21^{a} \pm 0.84$ | $5.32^{ab} \pm 0.59$ |
| ZO Extract + DM (1/10th $LD_{50}$) | $43.35^{a} \pm 3.70$ | $0.63^{b} \pm 0.08$ | $3.20^{a} \pm 0.35$ | $9.27^{bc} \pm 0.43$ | $9.27^{abc} \pm 0.43$ |
| ZO Extract + $F^-$ (4.5 ppm) | $44.44^{a} \pm 3.14$ | $0.60^{b} \pm 0.08$ | $3.30^{ab} \pm 0.39$ | $10.16^{bc} \pm 0.32$ | $10.16^{bc} \pm 0.32$ |
| ZO Extract + DM (1/10th $LD_{50}$) + $F^-$ (4.5 ppm) | $81.37^{c} \pm 6.58$ | $0.53^{ab} \pm 0.03$ | $2.91^{ab} \pm 0.58$ | $12.40^{cd} \pm 0.79$ | $10.40^{c} \pm 0.79$ |
| Quercetin (100 mg/kg) + DM (1/10th $LD_{50}$) + $F^-$ (4.5 ppm) | $72.28^{c} \pm 9.98$ | $0.56^{ab} \pm 0.02$ | $3.19^{b} \pm 0.52$ | $10.63^{cd} \pm 0.72$ | $10.63^{cd} \pm 0.72$ |

Values are given as mean ± SE of 6 animals unless otherwise stated

Values having different superscripts (a,b,c,d) in a column are statistically different from one another at 5% level of significance

Values of BUN (blood urea nitrogen), CR (creatinine) and uric acid are expressed in mg/dl

Values of Calcium and phosphorus is expressed in mg/dl

Table 3
Effect of hydro-alcoholic extract of *Zingiber officinale* on toxicity induced by fluoride and dimethoate alone and in-combination on renal antioxidant system in Wistar rats.

| Groups | TAS | TTH | AE | AChE | CAT |
|---|---|---|---|---|---|
| Control | $20.26^{a}$ ± 0.35 | $1.96^{c}$ ± 0.42 | $3.41^{b}$ ± 0.36 | $17241.00^{c}$ ± 890.71 | $3112.69^{b}$ ± 172.21 |
| ZO Extract (300 mg/kg) | $21.68^{a}$ ± 1.25 | $1.98^{c}$ ± 0.36 | $2.96^{b}$ ± 0.25 | $12907.63^{bc}$ ± 448.89 | $3469.04^{b}$ ± 105.89 |
| Dimethoate (DM -1/10th $LD_{50}$) | $16.01^{b}$ ± 0.19 | $1.07^{b}$ ± 0.28 | $0.80^{a}$ ± 0.06 | $8269.13^{b}$ ± 302.86 | $3072.02^{b}$ ± 166.92 |
| Fluoride ($F^{-}$ - 4.5 ppm) | $15.12^{b}$ ± 1.13 | $1.25^{ab}$ ± 0.54 | $0.79^{a}$ ± 0.04 | $8158.13^{b}$ ± 709.49 | $3187.47^{b}$ ± 586.52 |
| DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $10.68^{c}$ ± 0.87 | $0.58^{d}$ ± 0.16 | $0.51^{d}$ ± 0.16 | $6574.50^{a}$ ± 434.04 | $1854.48^{a}$ ± 240.79 |
| ZO Extract (300 mg/kg) + DM (1/10th $LD_{50}$) | $15.71^{b}$ ± 0.98 | $0.80^{a}$ ± 0.08 | $2.72^{b}$ ± 0.23 | $12079.63^{bc}$ ± 565.97 | $4372.77^{d}$ ± 898.00 |
| ZO Extract (300 mg/kg) + $F^{-}$ (4.5 ppm) | $16.13^{b}$ ± 1.49 | $1.19^{a}$ ± 0.54 | $2.70^{b}$ ± 0.40 | $14446.50^{ba}$ ± 442.64 | $2968.29^{b}$ ± 114.96 |
| ZO Extract (300 mg/kg) + DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $19.62^{b}$ ± 0.32 | $1.97^{c}$ ± 0.35 | $2.92^{b}$ ± 0.52 | $16436.88^{bc}$ ± 714.71 | $3276.49^{b}$ ± 133.96 |
| Quercetin (100 mg/kg) + DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $15.53^{b}$ ± 0.86 | $0.90^{a}$ ± 0.24 | $1.47^{a}$ ± 0.28 | $17569.75^{c}$ ± 962.12 | $3007.47^{b}$ ± 490.10 |

Values are given as mean ± SE of 6 animals unless otherwise stated

Values having different superscripts (a, b, c) in a column are statistically different from one another at 5% level of significance

Values of TAS (Total antioxidant status), expressed in mM

Values of TTH (Total thiols)expressed in µM

Activities of arylesterase (AE) expressed in U/ml

Acetylcholinesterase (AChE) activity expressed in nmole of thiols produced/min/mg of tissue

Values of CAT (Catalase) are expressed in µmol $H_2O_2$ decomposed/ min/ g of tissue

## Effect on plasma GSH and NO

Alterations in plasma GSH and NO levels in different treated groups and control rats were shown in Fig. 1. Plasma GSH and NO levels registered significant reduction in all groups given toxicants either alone or in combination (Group II, IV and V) as compared to the levels in control group. Additionally, levels in group V

were significantly lower as compared to the levels in group III and group IV showcasing significant greater suppression in NO production after dual toxicant exposure. Administration of ZO raised NO and GSH levels in groups VI, VII and VIII as compared to that in groups III, IV and V respectively. Quercetin also significantly improved GSH and NO levels in group IX as compared to group V but NO contents in group IX were significantly lower as compared to that seen in group VIII indicating better protection from ZO as compared to that from quercetin. Overall, the herbal agents could not completely restore the NO level but the level of GSH are comparable to the concentrations in control.

### Antioxidant biomarkers in renal tissue

Table 3 represents the data of various antioxidant enzyme activities in renal tissue of control and treated groups. In group III, IV and V after exposure of toxicants either alone or in combination, the levels of TAS, TTH, AE, AChE were significantly decreased as compared to control. Moreover, a significantly greater plunge in TAS, TTH and AChE contents was encountered in rats after concurrent exposure to F and DM as compared to that seen in either F or DM given rats. While, in group VI, VII and VIII, ZO led to significant enhancement in TTH and AE levels as compared to the respective values in III, IV and V even though the TAS, AChE levels in VI and VII did not differ significantly as compared to group III and IV concentrations. Further, TTH didn't differ between group IV and group VII. ZO as well as quercetin caused significant increase in group VIII and IX AChE levels as compared to the respective values in group V. Complete amelioration was seen in levels of TAS, AE, TTH, AChE in dual toxicant administered rats supplemented with ZO whereas quercetin could not completely restore fall in TTH and AE in combined toxicity group. As far as CAT was concerned, individual toxicant administration did not cause a significant decrease in its concentration as compared to the levels in control group but levels in dual toxicant group were significantly reduced as compared to the concentrations after either toxicant administration. Intake of ZO significantly uplifted the CAT contents in the compound toxicity group. In the same vein, quercetin also significantly amended the fallen CAT values after dual chemical toxicosis.

Variations in concentration of SOD, GPx, GR, AOPP and MDA in different experimental groups are depicted in Table 4. Compared to control rats, a significant reduction was seen in SOD, GPx, GR levels where as a significant elevation was noted in AOPP and MDA concentrations after individual or dual toxicosis. Furthermore, SOD levels were significantly reduced and the levels of AOPP and MDA were significantly inflated in animals receiving both the toxicant as compared to rats enduring single toxicant exposure. Incorporation of ZO reversed the trend of depletion in antioxidant parameter values of SOD and GR in all toxicant groups whereas GPx values were significantly corrected only in individual toxicant administered rats. In rats given F and DM simultaneously, additional ZO or quercetin supplementation could not ameliorate the depreciation in GPx concentrations. In the same way, ZO as well as quercetin completely reinstated the AOPP levels in all poisoning groups. In contrast ZO and quercetin could only partially restore MDA contents in the respective toxicity groups.

Table 4
Effect of hydro-alcoholic extract of *Zingiber officinale* on toxicity induced by fluoride and dimethoate alone and in-combination on antioxidant system of renal tissue in Wistar rats.

| Groups | SOD | GPx | GR | AOPP | MDA |
|---|---|---|---|---|---|
| Control | $736.82^{b} \pm 26.88$ | $262.21^{c} \pm 21.38$ | $50.04^{bc} \pm 3.26$ | $1.42^{a} \pm 0.14$ | $42.03^{a} \pm 4.55$ |
| ZO Extract (300 mg/kg) | $869.32^{b} \pm 16.46$ | $273.16^{c} \pm 13.08$ | $47.30^{b} \pm 3.41$ | $1.51^{a} \pm 0.05$ | $52.15^{a} \pm 8.98$ |
| Dimethoate (DM -1/10th $LD_{50}$) | $348.06^{a} \pm 22.81$ | $125.63^{a} \pm 6.78$ | $27.99^{b} \pm 3.47$ | $1.75^{b} \pm 0.27$ | $315.69^{c} \pm 20.35$ |
| Fluoride ($F^{-}$ - 4.5 ppm) | $329.59^{a} \pm 21.55$ | $128.66^{a} \pm 5.72$ | $17.26^{a} \pm 1.74$ | $1.87^{b} \pm 0.21$ | $385.97^{c} \pm 48.91$ |
| DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $252.88^{c} \pm 11.52$ | $193.67^{a} \pm 1.46$ | $14.52^{a} \pm 1.49$ | $2.65^{d} \pm 0.32$ | $492.83^{d} \pm 15.35$ |
| ZO Extract (300 mg/kg) + DM (1/10th $LD_{50}$) | $705.58^{b} \pm 35.99$ | $252.26^{bc} \pm 29.04$ | $36.17^{ab} \pm 4.05$ | $1.49^{a} \pm 0.05$ | $159.95^{b} \pm 9.64$ |
| ZO Extract (300 mg/kg) + $F^{-}$ (4.5 ppm) | $781.95^{b} \pm 35.79$ | $232.84^{ab} \pm 18.99$ | $45.12^{bc} \pm 12.74$ | $1.38^{a} \pm 0.10$ | $158.08^{b} \pm 9.52$ |
| ZO Extract (300 mg/kg) + DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $738.11^{b} \pm 24.28$ | $205.83^{ab} \pm 13.99$ | $52.58^{bc} \pm 6.87$ | $1.44^{a} \pm 0.04$ | $155.74^{b} \pm 8.06$ |
| Quercetin (100 mg/kg) + DM (1/10th $LD_{50}$) + $F^{-}$ (4.5 ppm) | $921.80^{d} \pm 32.34$ | $197.89^{abc} \pm 14.14$ | $27.14^{a} \pm 2.22$ | $1.46^{a} \pm 0.13$ | $66.80^{e} \pm 7.84$ |

Values are given as mean ± SE of 6 animals unless otherwise stated

Values having different superscripts (a, b, c) in a column are statistically different from one another at 5% level of significance

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

Values of GR (glutathione reductase) are expressed nmol of NADPH/min

Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T

Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr

## Histopathological alterations in renal tissue

Histomorphology of kidney from rats in different groups is presented in Fig. 2. Kidney sections of group I revealed normal architecture in which renal tubules were lined by cuboidal epithelium and presence of healthy glomeruli with tuft of capillaries contained in Bowman's capsule was noticed (Fig. 2a). Figure 2b

depicts the microscopic sections of kidney from rats in which only ZO extract was administered, which appeared similar to those from control group without any pathological changes. Group III rats revealed mild congestion along with mild degeneration of glomeruli and tubular epithelium (Fig. 2c). In group IV, pathological changes such as tubular degeneration, intertubular congestion, haemorrhage and edema were seen (Fig. 2d). Lesions in group V were most severe and included widespread tubular degeneration, randomly distributed multifocal areas of necrosis of tubular epithelium and tubulorrhexis along with congestion, haemorrhage and edema in the interstitium (2e and 2f). Group VI however revealed mild pathological changes including degeneration of tubular epithelium, casts in tubular lumen alongside congestion (Fig. 3a). Likewise, microscopic lesions in group VII were also mild comprising of tubular degeneration and casts, congestion besides mild interstitial edema (Fig. 3b). The changes in group VIII and IX were significantly subdued as compared to those seen in group V rats and consisted of mild congestion, mild degenerative changes in tubular lining (Fig. 3c). Additionally, hemorrhage and edema were apparent at few places in group IX (Fig. 3d). Nonetheless, when compared with the intensity of changes seen in group VIII, the lesions in group IX were appreciably less severe.

# Discussion

Proper functioning of the kidney is essential for homeostasis and elimination of toxic metabolites through urine [39, 40]. In current scenario, renal disorder is a global public health concern and a multi-factorial disease entity with both unmitigated excessive generation of oxidants, reduced antioxidant capacity and diminished clearance of pro-oxidant radicals [13, 41]. Climate change coupled with intensive agriculture practices involving non-judicious applications of pesticides such as DM as well as increased volume of elemental pollutants such as $F^-$ in soil and ground water cumulatively impose heavy impact on terrestrial as well as aquatic life forms [42, 43]. Kidney cells face very high $F^-$ ions concentrations making them susceptible to $F^-$ toxicity [44, 45] and exposure to DM causes pronounced ill effects on kidney [46]. Simultaneous exposure of $F^-$ and DM potentiated the redox imbalance induced renal damage when compared to exposure to either chemical alone [47, 48]. Urea is the end product of protein metabolism formed in liver whereas CR is formed from creatine after breakdown of muscle protein but both are excreted via kidneys and their elevated levels indicate diminished renal functioning. Uric acid on the other hand is the final breakdown metabolite of purine processing and its high serum levels point towards renal malfunction. Disturbances in electrolyte homeostasis including disruption in concentration of Ca and P and increased levels of BUN, CRE and UA are considered sensitive indicators of renal malfunctioning. In the current study all the above biochemical renal markers were elevated in all toxicant administered rats which are concurrent with the previous findings [5, 46, 49]. Alterations in biochemical parameters were also recorded upon fluoride and arsenic exposure in earlier studies [5, 50]. Analogous findings were registered during a study by Mahjoubi-Samet et al. [46] in which DM induced nephrotoxicity in adult rats and their suckling pups. Further, in the present study, levels of CR, BUN and UA in co-exposed groups were significantly higher from that seen in individual toxicant exposed rats indicating greater abnormalities in renal physiology in rats co-exposed to $F^-$ and DM. Interestingly, our results also highlight that continuous co-exposure can lead to significant renal impairment. However, it was found that with ZO

extract supplementation, the toxicant administration led changes in levels of renal biomarkers were significantly reversed. Nitric oxide is a vasodilator which relaxes muscles of blood vessel walls leading to increase in blood circulation. Its levels alter in response to oxidative stress and inflammation in body. Clinically, it has been observed that lower levels of NO predispose to various cardiovascular diseases like arteriosclerosis and hypotension [51, 52].

Significant reduction in NO levels was observed after administration of $F^-$ and DM alone. Moreover, combination toxicant group recorded further depletion in NO levels. Similar findings were documented by Miranda et al.[53]. Also, ZO addition ameliorated toxicant brought about significant reduction in NO levels in contrast quercetin was less effective in restoring NO levels in the combination toxicant group. Exposure to $F^-$ stimulates ROS production and the ensuing oxidative stress hampers antioxidant enzyme activity, energy metabolism and ion transport across plasma membranes [54]. Acute fatal $F^-$ toxicity diminishes calcium ionic concentration in blood which affects contractability of cardiac cells causing cardiac arrest [55], whereas prolonged exposure to $F^-$ leads to electrolyte imbalance with significant reduction of Ca and magnesium levels in plasma [56]. In the present study, after administration of DM or $F^-$, the plasma levels of both ions (Ca and P) were decreased and the depreciation was more significant after the co-exposure of DM and F. However, diazinon administration in rats led to a fall in calcium levels without effecting phosphorus concentrations [57]. Derangements in blood calcium and phosphorus levels were also registered by Sharma et al. [5] in rats after $F^-$ intoxication. Rat ameloblasts witnessed increased calcium influx raising intracellular calcium levels upon exposure to high $F^-$ concentrations [58] which may lead to imbalance in Ca and P ratio. Another study revealed $F^-$ induced influx of calcium inside cells causing drop in calcium levels in plasma [56]. Further, kidney being primarily responsible for $F^-$ excretion, damaging effects due to $F^-$ induced alteration in renal antioxidant enzymes as appreciated in the present work might have also interfered with absorption and renal clearance of phosphate. Since latter's altered concentration affects Ca levels, it may have also contributed towards steep fall in plasmatic calcium concentration. Taken together, all these could have resulted in a sharp drop in Ca levels in blood in our work.

In the present study, administration of $F^-$ and DM alone and in combination induced reduction in concentrations of both enzymatic and non-enzymatic antioxidants but increased lipid and protein peroxidation. However, these changes were significantly dampened after ginger administration. GSH performs various functions in living organisms. Apart from acting as a carrier of active thiols group such as cysteine residues, it also acts as a co-factor for GST and GPx [59, 60]. A decrease in the TAS spurs decrease in parameters such as TTH, AE and AChE. Also, there is strong evidence that compounds having oxidant activity can not only modulate AChE activity but also repress its gene expression [61]. Exposure to herbicidal agent clomazone has been shown to decreased AChE and antioxidant levels but at the same time increased lipid peroxidation in erythrocytes [62]. Moreover, upsurge in AOPP and MDA concentrations portend oxidative damage to cellular proteins and lipids respectively. Additionally, in the event of oxidative insult, activity of AE, which grants protection to lipids against their peroxidation, may be severely curbed. Large scale fluctuations in lipid and protein peroxidation along with depletion of

oxidant scavengers may unfold into frank tissue damage. Similar to the depletion in levels of TAS, TTH, AE, AChE, CAT, SOD, GPx, GR and rise in MDA and AOPP our study. Concurrent findings were also reported by Khan et al. [10], who studied toxic effects of deltamethrin and $F^-$ in rats. Reduction in CAT, GSH and NO levels in rat were also were reported by Miranda et al.[53], after $F^-$ toxicity. Likewise, various studies reported decrease in CAT, SOD and GSH in response to toxicant pesticides and metals administrations in response to oxidative damage [14, 50, 63].

A wide variety of alterations such as degeneration, necrosis, and haemorrhage in renal parenchyma were noticed histologically in our study which is most likely the result of toxicant induced oxidative damage. Histopathological changes were most severe in the dual toxicant administered group receiving maximum doses of both the extraneous compounds. Similar changes have been observed by [46, 64, 65]. Similarly, Sharma et al. [5] also found that chronic exposure to F induced significant changes in oxidative stress parameters and histomorphology of hepatic, renal and cardiac tissues in Wistar rats. Various experimental studies have indicated that simultaneous exposure to phytochemicals (flavonoids) and toxicants reduces toxicant induced renal damage [39, 60, 66]. Correspondingly, ginger administration bolstered antioxidant profile in our experimental rats. In the present work, ginger extract supplementation reversed deviations in plasma biomarkers of renal injury, renal TAS, AE, AChE, GPx, GR, AOPP and MDA levels alongside renal histoarchitecture in all toxicant groups of our study thereby authenticating the presence of antioxidant, anti-apoptotic and anti-inflammatory properties in ginger as suggested in earlier *in-vitro* and *in-vivo* studies [67]. Nephroprotection was also imparted by essential oils of ZO and turmeric rhizomes against cadmium-induced toxicity in rats in a prior report [68]. Furthermore, commendable antioxidant and chelating properties of ZO against cadmium nephrotoxicity were also propounded in a study done by Gabr et al.[69]. Maghsoudi et al.[70], also promulgated ginger as a therapeutic remedy for renal dysfunctions. Correspondingly, essential oils from ginger were found to mitigate renal damage induced by administration of cadmium and acetaminophen [68, 71]. Similarly, studies revealed that supplementation of ginger extract effectively countered toxic changes such as sloughing of tubular epithelium, dilatation of renal tubules and interstitial fibrosis in renal parenchyma induced by fructose consumption [68, 71]. 6-gingerol an active component of ginger efficiently alleviated kidney dysfunctions, oxidative stress and histopathological changes induced by mercuric chloride in male rats [72]. In a nutshell, use of ZO in the present investigation conferred noticeable protection against the oxidative damage unleashed by dual toxicant exposure which was better than that seen following quercetin treatment. Taken together, the results of this study highlight the potential perils of simultaneous intake of toxic dose of DM and $F^-$ on kidney functioning in animals and humans. It is particularly concerning for people and animals in places where DM is being used in agricultural practices without much caution and at the same time naturally $F^-$ contaminated ground water is utilized for irrigation and drinking. Besides, use of ginger must be encouraged among populations in such areas to counter harmful effect of instantaneous exposure to $F^-$ and DM.

# Conclusions

Observations of present study indicated that repeated co-exposure of $F^-$ and DM produced significant alterations in plasma renal biomarkers, oxidative alterations and histopathological changes in renal tissues of Wistar rats. These changes were significantly are severe as compared to those produced by exposure to individual toxicant alone. Co-administration of toxicants either alone or in combination along with ZO in Wistar rats accorded significant protection against these alterations as signified by considerable improvements in plasmatic renal function indices as well as presence of formidable resistance to oxidative damage and histomorphological alterations in renal tissues of these animals.

# Declarations

All authors are agreed to publication of article in this form

**Funding:** This work is supported by the grant provided by the Indian Council of Medical Research (ICMR), New Delhi, India (Sanction order No. 36/6/2020/TOXI/BMS, dated 16/11/2021) for conducting the research work is greatly acknowledged.

**Conflict of Interests:** The author(s) declare(s) that they have no competing interests

**Availability of data and materials:** All data generated or analyzed during this study are included in this published article

**Code availability:** NA

**Author contribution:** All authors contributed to the study conception and design. Dr Priyanka Sharma and Dr Pawan Kumar Verma contributed to conception, design and execution of the research work to generate the basic data. Dr Shilpa Sood carried out histopathological work and final editing of the manuscript. Dr Rajinder Raina and Dr Rasia contributed to statistical analysis of data.

**Ethics approval:** The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) vide letter no. 03/IAEC/2020 dated 22/10/2020.

**Consent to participate:** Not applicable

**Consent for publication:** All authors are agreed to publication of article

**Acknowledgements:** Authors thank the Dean, Faculty of Veterinary Science and Animal Husbandry, R S Pura, Jammu for providing necessary facilities for conducting the research.

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# Figures

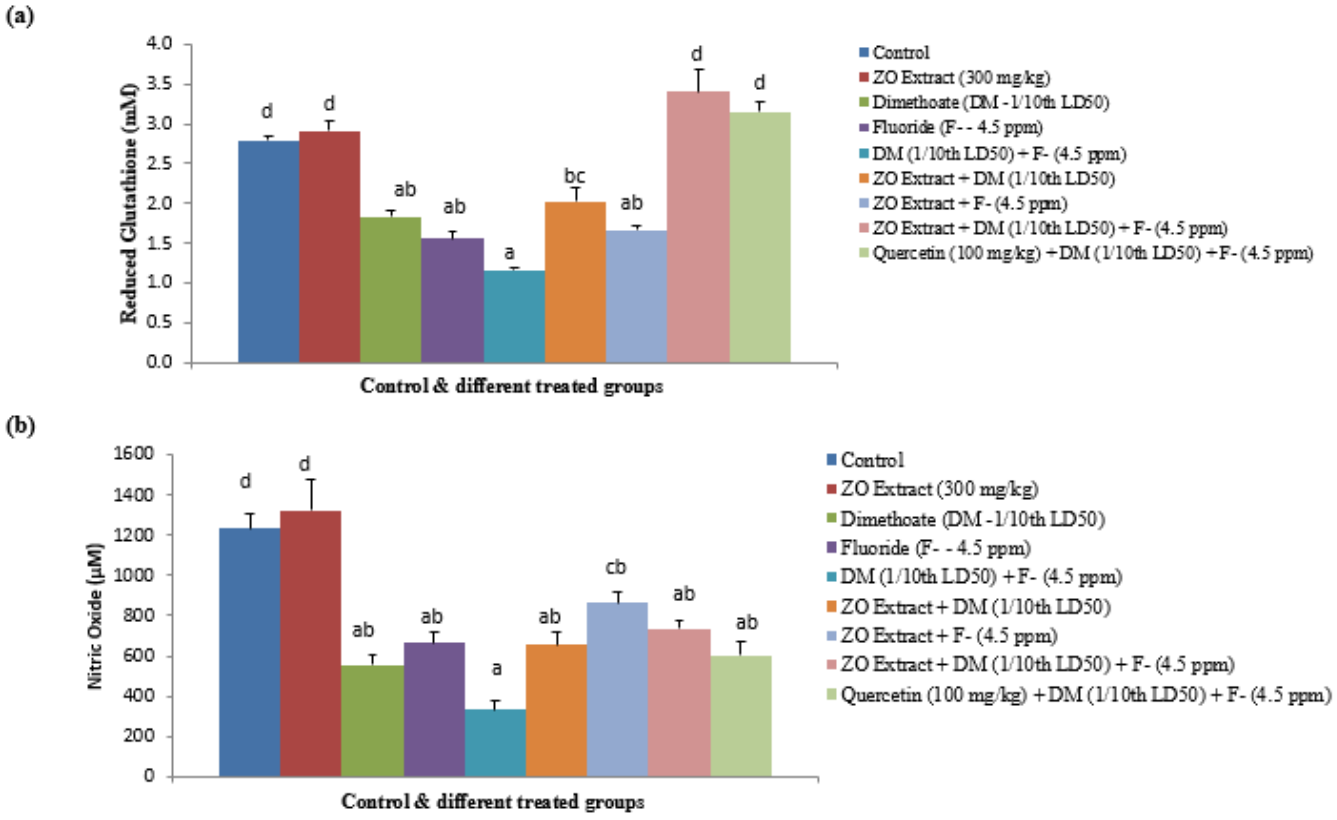
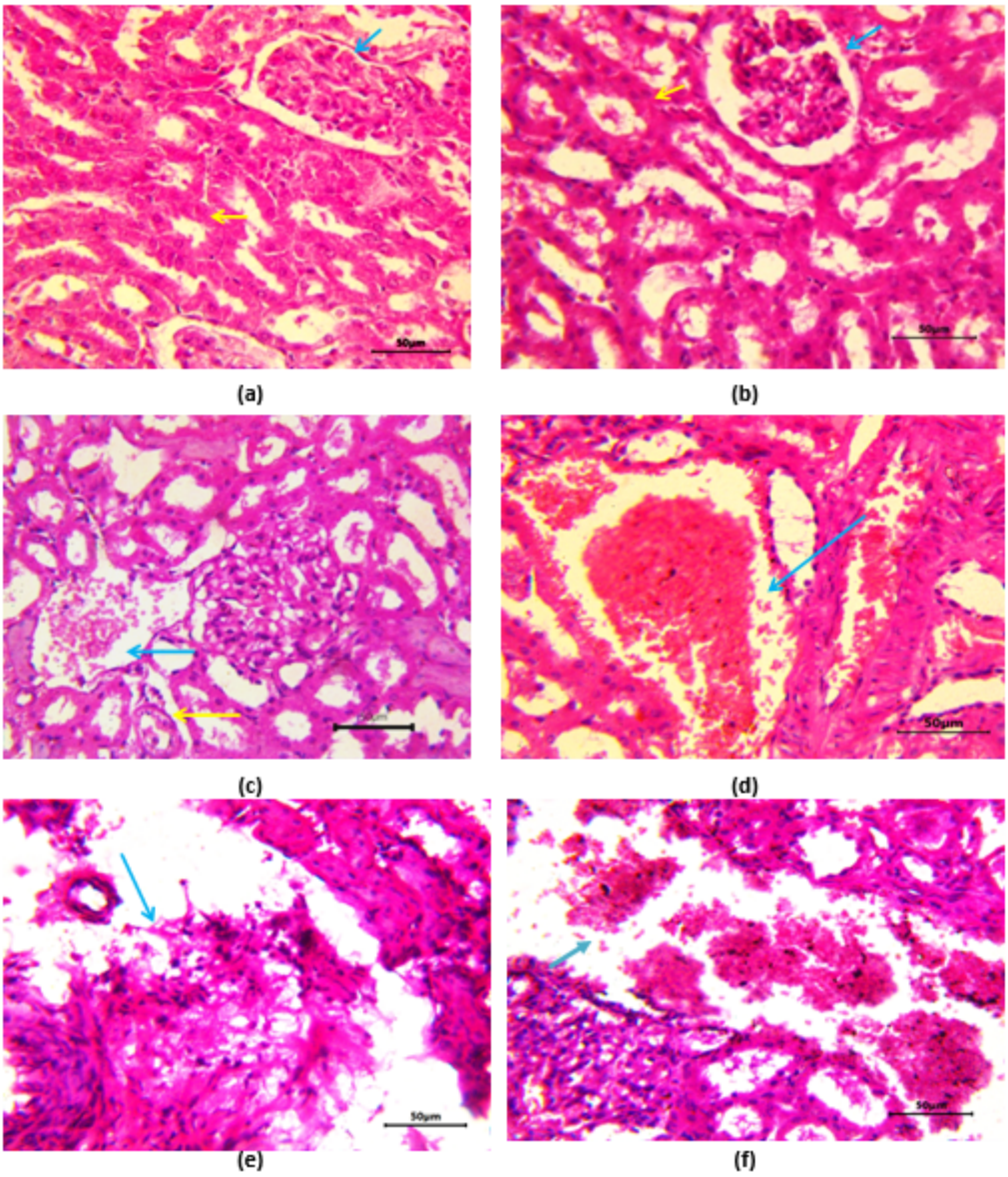


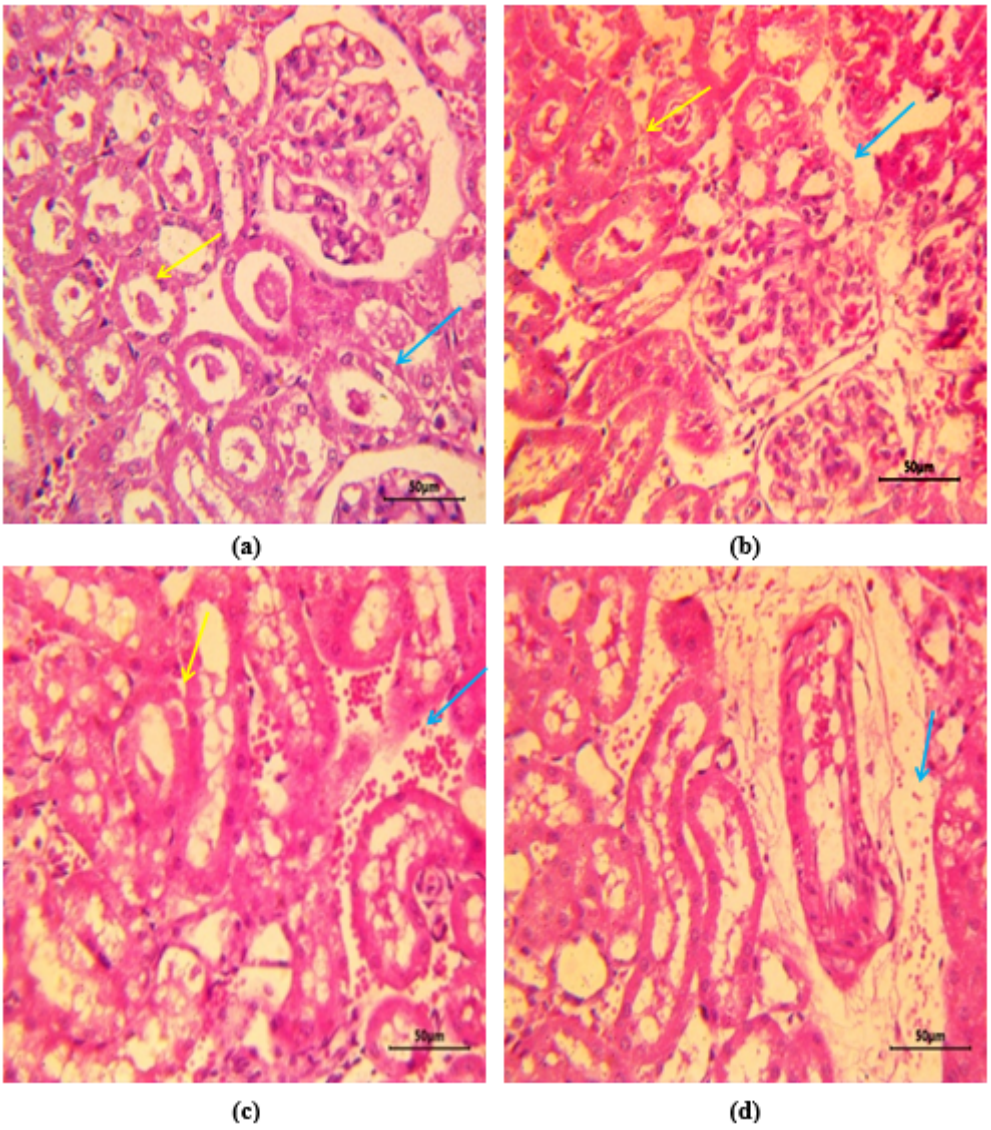

**Figure 1**

**Effect of hydro-alcoholic extract of *Zingiber officinale* (ZO) on levels of reduced glutathione (GSH) and nitric oxide (NO) in plasma of rats following subacute exposure of fluoride ($F^-$) and dimethoate (DM) alone and in-combination (**Values having different superscripts (a,b,c,d) in a column are statistically different from one another at 5% level of significance)




## Figure 2

Alterations in the histomorphology of kidney in different exposed groups; (a) Group 1: control kidney section from with normal glomerulus (blue arrow) and tubules (yellow arrow) lined with cuboidal epithelium; (b) normal architecture of kidney with healthy glomerulus (blue arrow) and tubules (yellow arrow) in group II; (c) group III showed mild congestion (blue arrow) and mild tubular degeneration (yellow arrow) and interstitial congestion and haemorrhage (arrow) in group IV (d). Exposure to both the toxicants (group V) leads to severe tubular necrosis, haemorrhage and edema (arrow) (e) and severe intestinal hemorrhage (f) in renal tissue of rats (H&E 400X).




## Figure 3

Mild vacuolar degeneration (blue arrow) in tubular epithelium and tubular casts (yellow arrow) seen in group VI (a) and group VII epithelium of renal tubules showing presence of mild tubular degeneration (blue arrow) and casts in tubular lumen (yellow arrow) (b). Group VIII showed mild inter-tubular congestion (blue arrow) and mild degenerative changes of tubular epithelium (yellow arrow) (c) whereas in group IX indicated interstitial edema mixed with haemorrhage (arrow) (d) in kidney of Wistar rats. (H&E 400X).