

Mechanisms of Action of Diallyl Disulfide Against Trichloromethane-Induced Renal Toxicity is Via Inhibitions of Oxidative Stress, NFκB Activation, and Apoptosis in Rats

Oluwatobi T. Somade* Adeyinka H. Adedokun Idowu K. Adeleke
Muslimot A. Taiwo Mulikat O. Oyeniran

Department of Biochemistry, College of Biosciences, Federal University of Agriculture Abeokuta, Nigeria

Abstract

The renal-protective effect of diallyl disulfide (DADS) on trichloromethane (CHCl₃)-induced renal toxicity was investigated. Twenty five rats, divided into five groups of five animals each were used. CHCl₃ at the dose of 200 mg/kg was orally administered, and concomitantly treated with DADS (50 mg/kg), 5 days/week for a period of 3 weeks. Compared with control, there was no significant increase in kidney malondialdehyde (MDA), but a significant increase in levels of nuclear factor kappa B (NFκB) expressions, TUNEL positive cells (apoptosis), as well as hydrogen peroxide (H₂O₂), nitric oxide (NO) and reduced glutathione (GSH) concentrations. In addition, a significant decrease in expressions of kidney p53 and catalase (CAT) activity, and a non-significant decrease in glutathione peroxidase (GPx) activity were recorded following CHCl₃ administration. Conversely, following DADS treatment, there was a significant increase in the expressions of p53, and a significant and non-significant decrease in apoptotic positive cells and NFκB expressions respectively. Administration of DADS significantly reduced the levels of H₂O₂ and NO, but did not have effect on the level of GSH, while CAT and GPx activities were significantly improved. Protection by DADS against TCM-induced renal-toxicity may therefore be via suppressions of NFκB activation, oxidative stress and apoptosis in rats.

Keywords: Apoptosis, Diallyl disulfide, NFκB, Oxidative stress, p53, Trichloromethane

Introduction

CHCl₃ is a colorless and volatile liquid with a distinct odor. CHCl₃ is nonflammable, slightly soluble in water and is readily miscible with most organic solvents (Lewis 1993). CHCl₃ is the most predominant by-product of water disinfection with chlorine-based chemicals (Craun 1993), also formed in large quantities as a consequence of chlorination of cooling water in power plants and in the process of bleaching paper (Deinzer et al. 1978). CHCl₃ has a widespread use in industry as solubilizer, diluent, and dispersant (Abbassi et al. 2010). It is one of the major environmental toxicants (HazDat 2001), classified as a group 2B carcinogen (IARC 1999). At low concentrations, metabolism occurs majorly through cytochrome P₄₅₀-2E1 (Constan et al. 1999). The level of P₄₅₀-2E1, and hence the rate of chloroform metabolism, is induced by a variety of alcohols such as ketones and ethanol. Also at high chloroform concentrations, metabolism is via cytochrome P₄₅₀-2B1/2 (ILSI 1997; U.S. EPA 1997, 1998c).

In the presence of oxygen, the predominant product is trichloromethanol, which spontaneously and effectively dehydrochlorinates to form phosgene. In the absence of oxygen, the major metabolite is dichloromethyl free radical (U.S. EPA 1997; ILSI 1997). Virtually all tissues of the body have capacity to metabolize CHCl₃, but the metabolic rate is greatest in kidney cortex, liver, and nasal mucosa (ILSI 1997). These tissues are also the major sites of CHCl₃ toxicity, pointing the importance of metabolism in the mode of action of CHCl₃ toxicity.

Phosgene and dichloromethyl free radical, the products of oxidative metabolism and reductive metabolism respectively, are both highly reactive. The rate of phosgene hydrolysis is highly rapid, with a half-time of less than 1 second (De Bruyn et al. 1995). Phosgene also reacts with nucleophiles, including hydroxy groups, thiols, primary and secondary amines (Schneider and Diller 1991). For example, phosgene reacts with the thiol group of GSH, forming S-chloro-carbonyl glutathione, which in turn can either interact further with glutathione to form diglutathionyl dithiocarbonate, or form glutathione disulfide and carbon monoxide (ILSI 1997). The major reaction is hydrolysis by water, forming carbon dioxide and hydrochloric acid.

Phosgene also undergoes attack by nucleophilic groups such as -NH₂, -OH, and -SH in cellular macromolecules including the polar heads of phospholipids, proteins or enzymes, resulting in generation of covalent adducts (Noort et al. 2000; Pereira and Chang 1981; Pereira et al. 1984; Pohl et al. 1977, 1980, 1981). These molecular adducts can interfere with molecular functions such as loss of enzyme activity, which may lead to loss of cellular activities and subsequently cell death (WHO 1998; ILSI 1997).

Pharmacological properties of phytochemicals are well documented and their underlying mechanisms of action are gradually being understood. Garlic (*Allium sativum* L.) is one of the most used spices in food, and as remedy for a variety of illnesses (Shin et al. 2014). In garlic, over 20 different kinds of organo-sulfur compounds have been identified from sulfur-containing amino acids, and is considered a cancer preventive agent. Previous

studies have shown inhibition of chemically induced carcinogenesis in different organs by certain sulfur-containing compounds (Ariga and Seki 2006). One of these compounds is DADS, a major component of the secondary metabolites that has been well documented as a potent compound to prevent cancer, urotoxicity, genotoxicity, nephrotoxicity, and hepatotoxicity (Nakagawa et al. 2001; Guyonnet et al. 2002; Manesh and Kuttan 2002; Pedraza-Chaverri et al. 2003; Fukao et al. 2004; Kim et al. 2016).

DADS inhibits the promotion phase of DMBA-induced skin tumors in mice (Belman et al. 1989), the growth of canine mammary neoplastic CMT-13 cells (Sundaram and Milner 1993), rat and human CYP2E1 and rat CYP2A3 expressions (Morris et al. 2004). DADS affects histone acetylation and this may be attributed to its protective properties in colon cancer (Druesne et al. 2004). DADS induces apoptosis in neuroblastoma cells (Filomeni et al. 2003) and HepG2 hepatoma (Wen et al. 2004), suggesting it as an anti-proliferative agent in cancer therapy (Filomeni et al. 2003).

Evaluation of biochemical and molecular mechanism by which CHCl_3 causes renal toxicity in rats and the search for alternatives that ameliorate this toxicity may provide substantial information with clinical implications. Based on the aforementioned, the present study investigated the chemo-therapeutic effects of DADS on CHCl_3 -induced renal toxicity in male wistar rats.

2. Materials and Methods

2.1. Chemicals and test substances

CHCl_3 (98% purity), is a product of AD Chemicals Limited, Great Britain. DADS (98 % purity) was purchased from Sigma Chemical Co., Saint Louis, MO, USA. Rat NF κ B and p53 monoclonal primary antibodies were purchased from ABCAM UK; rat monoclonal secondary antibodies were purchased from Dako (Agilent Technologies, USA). Promega DeadEnd™ Colorimetric TUNEL System, for detection of apoptosis in tissue sections was purchased from Promega Corporation (Madison, WI, USA). Pure (100 %) cholesterol free Mazola corn oil is a product of ACH Food Companies, Inc., Memphis, TN, USA. All other chemicals and reagents were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England.

2.2. Experimental animals and study design

Twenty five (25) male wistar albino rats of an average weight of 150 g used for this study were obtained from the animal house of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. They were housed in steel metal cages in the animal house of our department and were served food and water *ad libitum*. Permission to use the animals was approved by the Institution's Animal Ethical Committee. After 3 weeks of acclimatization, the rats were divided randomly into five groups of five animals each. Group I animals serve as normal control, group II animals served as vehicle control and were orally administered corn oil, groups III and IV animals were orally administered 200 mg/kg CHCl_3 (Larson et al. 1995) and 50 mg/kg DADS (Kim et al. 2016) respectively, while groups V animals were orally administered CHCl_3 (200 mg/kg) and concomitantly treated with DADS (50 mg/kg).

2.3. Sample collections and preparations

Administrations lasted for fifteen (15) days (5 days/week for 3 weeks), and 24 h after, animals were sacrificed. They were handled and used in accordance with the international guide for the care and use of laboratory animals (NRC 1996). The right kidneys were harvested, washed in ice-cold saline (0.9% w/v) solution, blotted dry, weighed and then fixed in 10 % phosphate buffered formalin for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, immunohistochemistry, and histopathology. The rest was suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) for homogenization. Homogenization was followed by centrifugation at 5000 rpm for 10 min. The resulting supernatant was aliquoted into Eppendorf tubes and used for other biochemical analyses.

2.4. Kidney immunohistochemistry

This was performed as described by Ajayi et al. (2016). Poly-L-lysine charged slides were rehydrated in xylene as well as decreasing concentration of ethanol (100 to 50 %). Heat-induced epitope retrieval was done in citrate buffer (pH 6.0) for 20 minutes followed by immersion in cold water for 10 minutes. The sections were marked with PAP pen and the endogenous peroxidase activities in the tissues were blocked with 5% hydrogen peroxide for 5 minutes in a dark cupboard. The sections were subsequently incubated overnight at 4°C with anti-NF κ B or anti-p53 primary monoclonal antibodies. The slides were thereafter washed with Tris buffer saline and further incubated with Horse-radish peroxidase labeled anti-rabbit monoclonal secondary antibodies (Dako, Agilent Technologies, US). Immune complexes were visualized using 0.05 percent 3, 3-diaminobenzidine (DAB), counterstained with hematoxylin and the slides were visualized under light microscope. The percentage of tissue stained positive cells was scored.

2.5. TUNEL assay

This was carried out based on the manufacturer's protocol (Promega DeadEnd™ Colorimetric TUNEL System). Briefly, paraffin embedded tissue sections were washed in xylene for 5 minutes, followed by immersion in 100 % ethanol for 5 minutes, rehydrated in decreasing concentrations of ethanol (100 to 50 %) for 3 minutes, and washed by immersion in 0.85 % sodium chloride and phosphate buffer saline (PBS) for 5 minutes each. Apoptosis was detected by fixing slides in 4 % paraformaldehyde in PBS for 15 minutes, addition of protein K solution and incubation at room temperature for 10 – 30 minutes. Equilibration buffer was added to equilibrate at room temperature for 5 – 10 minutes, followed by addition of recombinant terminal deoxynucleotidyl transferase (rTdT) reaction mixture to the tissue sections on the slides and then incubated for 60 minutes at 37°C. Reaction was stopped by immersion in 2X saline-sodium citrate (2X SSC) buffer for 15 minutes, blocked by immersion of slides in 0.3 % hydrogen peroxide for 3 – 5 minutes. Streptavidin HRP was added to slides, incubated for 30 minutes at room temperature, stained with diaminobenzidine (DAB) and developed until a light brown background appeared. Visualization of stained slides was done using Nikon E100 light microscope (Novel Optics, NanJing, China) for apoptotic cells.

2.6. Estimation of MDA concentration

MDA concentration, a marker of lipid peroxidation (LPO) was determined by the method of Buege and Aust (1978). In this procedure, 0.1 mL of the supernatant was added to 2 mL of trichloroacetic acid–thiobarbituric acid–hydrochloric acid (TCA/ TBA/HCl) (1:1:1 ratio) reagent, boiled at 100°C for 15 minutes, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against blank. MDA concentration was calculated using the molar extinction coefficient for MDA-TBA complex of $1.55 \times 10^6 \text{ M}^{-1}\text{cm}^{-1}$.

2.7. Estimation of H₂O₂ level

H₂O₂ level was estimated according to the method of Wolff (1994). Reaction mixture contains 2.5 mL of buffer, 250 μL Ammonium ferrous sulfate, 100 μL sorbitol, 100 μL xylenol orange, 25 μL sulfuric acid, and 50 μL of the sample. The mixture was thoroughly mixed till it foamed, followed by incubation for 30 minutes at room temperature. The pale pink color developed was read against blank at 560 nm.

2.8. Estimation of NO level

NO concentration in kidney was estimated using Griess Reagent (Green et al. 1982). The reaction mixture was made up of 150 μL sulfanilamide, 100 μL distilled water and 50 μL of sample. The mixture was incubated for 10 minutes, and then addition of 150 μL N-naphthylethylenediamine, followed by incubation for another 10 minutes. The concentration of NO was measured at 540 nm.

2.9. Estimation of GSH concentration

Reduced glutathione (GSH) level was determined by the method of Moron et al. (1979), where the color developed was read at 412 nm.

2.10. Determination of GPx activity

GPx activity was determined by the method of Rotruck et al. (1973). Color developed was read at 412 nm.

2.11. Estimation of CAT activity

Catalase (CAT) activity was determined by the method of Sinha (1972). The reaction mixture (1.5 mL) contained 0.01 M phosphate buffer, pH 7.0, tissue homogenate (0.1 mL) and 2M H₂O₂ (0.4 mL). The reaction was stopped by the addition of 2 mL dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios), followed by heating in boiling water for 10 minutes, and then cooled at room temperature. The absorbance was read at 570 nm.

2.12. Determination of total protein concentration

Kidney total protein concentration was determined by the method of Gornall et al. (1949), and used for the estimations of MPO, CAT, SOD, GPx, and GST activities.

2.13. Histopathological analysis

Briefly, kidney sections were fixed in phosphate buffered formaline solution for 48 hours. After dehydration in an increasing concentration of alcohol and cleared twice in xylene, the tissues were embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye, and finally observed at x 400 magnification under a Nikon light microscope.

2.14. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences program version 17.0. Data were expressed as mean \pm standard error of mean. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of CHCl_3 administration and DADS treatment on relative kidney weight

Following CHCl_3 administration, there was no significant difference ($p > 0.05$) in the relative kidney weight compared with control group and/or DADS treated groups (Fig. 1).

3.2. Effect of CHCl_3 administration and DADS treatment on hepatic expressions of NF κ B

As represented in figure 2, expressions of renal NF κ B significantly increased ($p < 0.05$) following CHCl_3 administration compared with the control. However, treatment with DADS did not have any significant effect ($p > 0.05$).

3.3. Effect of CHCl_3 administration and DADS treatment on hepatic expressions of p53

Expressions of kidney p53 were depicted in figure 3. Following CHCl_3 administration, level of p53 expressions significantly decreased ($p < 0.05$) compared with control. The expressions were however significantly increased ($p < 0.05$) following DADS treatment.

3.4. Effects of DADS on CHCl_3 -induced hepatic cellular apoptosis

Administrations of CHCl_3 significantly increased ($p < 0.05$) the number of apoptotic cells (TUNEL positive cells) compared with control (Fig. 4). However, following DADS concomitant treatment, the number of apoptotic cells (TUNEL positive cells) was significantly ($p < 0.05$) reduced when compared with CHCl_3 group.

3.5. Effect of CHCl_3 administration and DADS treatment on kidney MDA concentration

No significant difference ($p > 0.05$) in kidney MDA was recorded following administration of CHCl_3 compared with control and/or DADS treated groups (Fig. 5).

3.6. Effect of CHCl_3 administration and DADS treatment on kidney H_2O_2 level

CHCl_3 administration significantly ($p < 0.05$) increase the level of H_2O_2 (Fig. 6), but was restored to level comparable with the control when concomitantly treated with DADS (Fig. 6).

3.7. Effect of CHCl_3 administration and DADS treatment on kidney NO concentration

NO concentration was also significantly ($p < 0.05$) increased by CHCl_3 administration (Fig. 7) compared with control, and DADS co-administration was able to significantly reduced the concentration (Fig. 7).

3.8. Effect of CHCl_3 administration and DADS treatment on level of kidney GSH

A significant increase ($p < 0.05$) in the level of GSH was recorded following CHCl_3 administration (Fig. 8). DADS intervention was however ineffective (Fig. 8).

3.9. Effect of CHCl_3 administration and DADS treatment on activity of kidney GPx

For GPx (Fig. 9), administration of CHCl_3 did not have significant ($p > 0.05$) effect on the enzyme compared with control, but co-administration of DADS was effective as it significantly ($p < 0.05$) increased its activity (Fig. 9).

3.10. Effect of CHCl_3 administration and DADS treatment on activity of kidney CAT

CAT activity was significantly decreased ($p < 0.05$) by the administration of CHCl_3 compared with control (Fig. 10). Concomitant treatment with DADS significantly increased ($p < 0.05$) the activity to level comparable with control (Fig. 10).

3.11. Effects of DADS treatment on kidney histopathology of rats administered CHCl_3 and treated with DADS

For the control, corn oil, and DADS only groups, no visible lesions were seen (Fig.11). Following CHCl_3 administration, there was a severe interstitial congestion of the kidney especially at the renal cortex, while treatment with DADS revealed few foci of mild interstitial hemorrhage (Fig. 11).

4. Discussion

The present study investigated the chemotherapeutic effect of DADS in CHCl_3 -induced nephrotoxicity in male

wistar rats. Following the administration of 200 mg/kg CHCl_3 five days a week for three weeks, no significant effect was seen on the relative kidney weight compared with control and other groups (Fig. 1). Similarly, in a study conducted by Larson et al. (1995), the relative kidney weight only increased after three weeks of administration of 180 mg/kg/day of CHCl_3 .

NF κ B is a transcription factor that has been reported to promote carcinogenesis by regulating the expression of genes that are involved in cell proliferation, metastasis and apoptosis (Erstad and Cusack 2013; Hoesel and Schmid 2013; Jing and Lee 2014). In the cytoplasm, NF κ B is rendered inactive by being sequestered to its inhibitor called I κ B. Upon exposure of the cell to toxicants, a cascade of reactions ensue which leads to the phosphorylation of I κ B by IKK, a kinase, and subsequent ubiquitination and degradation, causing the separation of NF κ B and its translocation into the nucleus where it coordinate the expressions of genes that participate in inflammation and carcinogenicity. While NF- κ B has become a promising target for anticancer therapy, the complexity of NF κ B regulation has made it challenging to develop agents to suppress its activation (Saud et al. 2016). In this study, the significant increase in the expressions of renal NF κ B is an indication of TCM-induced activation, thus suggesting its role in renal toxicity.

p53 is a transcription factor that activates genes that function in cellular responses to DNA damage. It plays a critical role in controlling cell cycle arrest and apoptosis (Vousden and Lane 2007). p53 gene stops cell cycle in G1 phase when there is DNA damage or a base mismatch. Failure of the damage to repair itself causes the cell to undergo apoptosis, which may occur at any stage of the cell cycle (Wei et al. 2001). Apoptosis is a form of cell death where cells actively participate in their own destruction, a process characterized by specific morphological and biochemical changes and also by alterations of genomic expression (Kajta 2004; Philchenkov 2004). Being a key component in the response to cellular stress, p53 serves as a major obstruction for carcinogenesis (Haupt et al. 2003). Significant decrease in the expressions of p53 recorded in this study following TCM administration may be attributed to a role in kidney cancer (NCI 1976) and excessive apoptosis, which agrees with the studies of Zhu et al. (2016) who reported low p53 expressions in cisplatin-induced nasopharyngeal cancer, and Ahmed et al. (2014) who also reported low expressions following nicotine administration. Furthermore, increase in the expressions of p53 by DADS co-administrations can be attributed to its renal-protective and anticancer properties as reported by Nakagawa et al. (2001), Guyonnet et al. (2002), and Pedraza-Chaverri et al. (2003).

Apoptosis, a process often referred to as programmed cell death, occurs when cells have developed irreparable defects that can ultimately lead to cancer. p53 is a well-known tumor suppressor protein that can prevent cells from becoming malignant via initiation of cell cycle arrest (Streuli 2006; Vogelstein and Kinzler 2004). It is a key regulator of apoptosis and carcinogenesis (Levine et al. 1991; Parant and Lozano 2003; Chen et al. 2005; Vousden and Prives 2005; Pelengaris and Khan 2006). The significant increase in apoptosis (TUNEL positive cells) following CHCl_3 administration is an indication of damage to the kidney cells (ILSI 1997), causing the renal cells to undergo apoptosis in a failed attempt to repair the cells. This may also explain the low expressions of p53 recorded in this study, which may have been overwhelmed in an attempt to ameliorate CHCl_3 toxicity. In similar findings by Lee et al. (2014), administration of carbon tetrachloride (CCl_4) to rats significantly increased the number TUNEL positive cells. Also, in this study, the resulting significant decrease following DADS co-administration with CHCl_3 explains its antioxidant, anti-apoptotic and renal-protective effects as also reported by Lee et al. (2014), as well as Kim et al. (2014) and Kim et al. (2016).

Quantification of thiobarbituric acid (TBARS) is majorly used to assess lipid peroxidation and indirectly, oxidative stress *in vitro* and *in vivo* (Beltowski et al. 2000). Oxidation of lipids causes disruption of the membrane bilayers that are rich in unsaturated fatty acids, following their attack by free radicals in an attempt to become paired and stable, compromising cell integrity, and leading to leakage of cellular content from the damaged organ into the blood stream (Somade et al. 2016, 2017). In this study, no significant effect was recorded for kidney MDA (Fig. 2) following administration of CHCl_3 . The capability of the kidney antioxidant defense system to protect against free radical attack may be responsible for this.

The hazardous chemical effects of H_2O_2 molecules are as a result of direct activity, emanating from their oxidizing properties, and the indirect activity where they serve as a source for more toxic species, like hydroxyl radicals and hypochlorous acid (Kohen and Nyska 2002). The significant high level of H_2O_2 (Fig. 3) is an indication of tissue damage (Farombi et al. 2013). However, the significant decrease following DADS intervention is an indication of its antioxidant and free radical mopping properties (Lee et al. 2014).

NO is a potent pro-inflammatory mediator, a low level is known to play an important role in cellular signaling under normal physiological condition. On the other hand, excessive production in the cell can lead to generation of peroxynitrite which subsequently damage the tissue (Ignarro et al. 1999; Ajayi et al. 2016). Thus, high level of hepatic NO reported in this study (Fig. 4) may be an indication of nitrosative stress and renal inflammation following CHCl_3 administration. Following DADS co-treatment, the resulting significant decrease in the level of NO (Fig. 4) suggests its anti-inflammatory and anti-nitrosative potentials (Indhira et al. 2012; Chiang et al. 2006; Chang and Chen 2005).

Oxidative stress is characterized by increase in the levels of free radicals due to insufficient antioxidant defense (Mittler 2002). It has been documented in clinical and experimental studies to play a key role in the etiology of many diseases including cancer, rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, and neurological disorders such as Parkinson and Alzheimer (Valko et al. 2007). The body has its antioxidant systems to prevent the production of reactive oxygen or nitrogen species and the ravaging and destructive effects they can cause. The most important intracellular antioxidant defense enzymes are catalase, glutathione peroxidase superoxide dismutase (Altan et al. 2006) and non-enzymatic antioxidants like reduced glutathione (Tomlin 1994). The significant increase in the level of kidney GSH as well as significant and non-significant decrease in CAT and GPx activities respectively following CHCl_3 administration is an indication of free radical generation (H_2O_2), which the endogenous antioxidant enzymes were responsive to. Antioxidant potentials of DADS was exerted which resulted to the restoration of the renal CAT (Lee et al. 2014; Kim et al. 2015; Khatua et al. 2016; Ko et al. 2017a) and GPx (Ko et al. 2017b; Pedraza-Chaverri et al. 2003; Liu et al. 2012; Lee et al. 2014) activities.

The severe interstitial congestion of the kidney especially at the renal cortex is an evidence of CHCl_3 toxicity on the renal cells. However, the few foci of mild interstitial hemorrhage following DADS treatment are indications of conferred protection to some extent, against the toxicity.

In conclusion, protection by DADS against TCM-induced renal-toxicity may therefore be via suppressions of NFkB activation, oxidative stress and apoptosis in rats.

Conflict of interest

None

References

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FIGURES

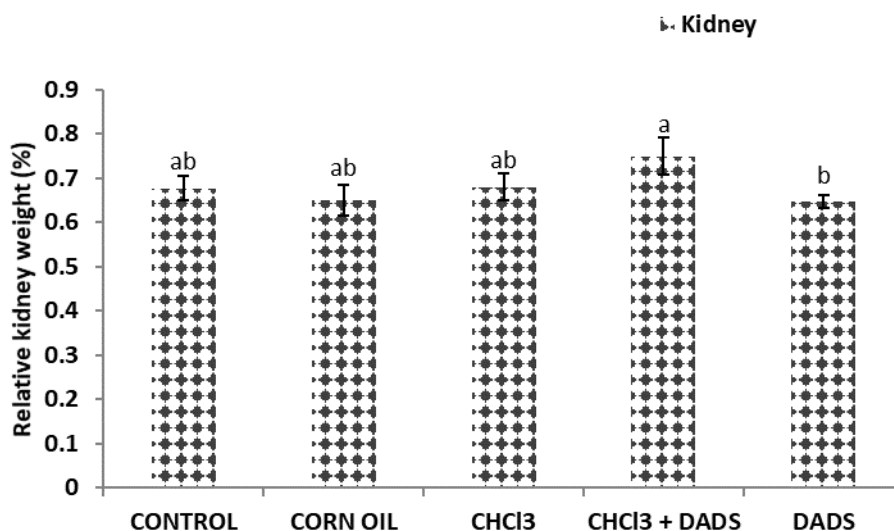


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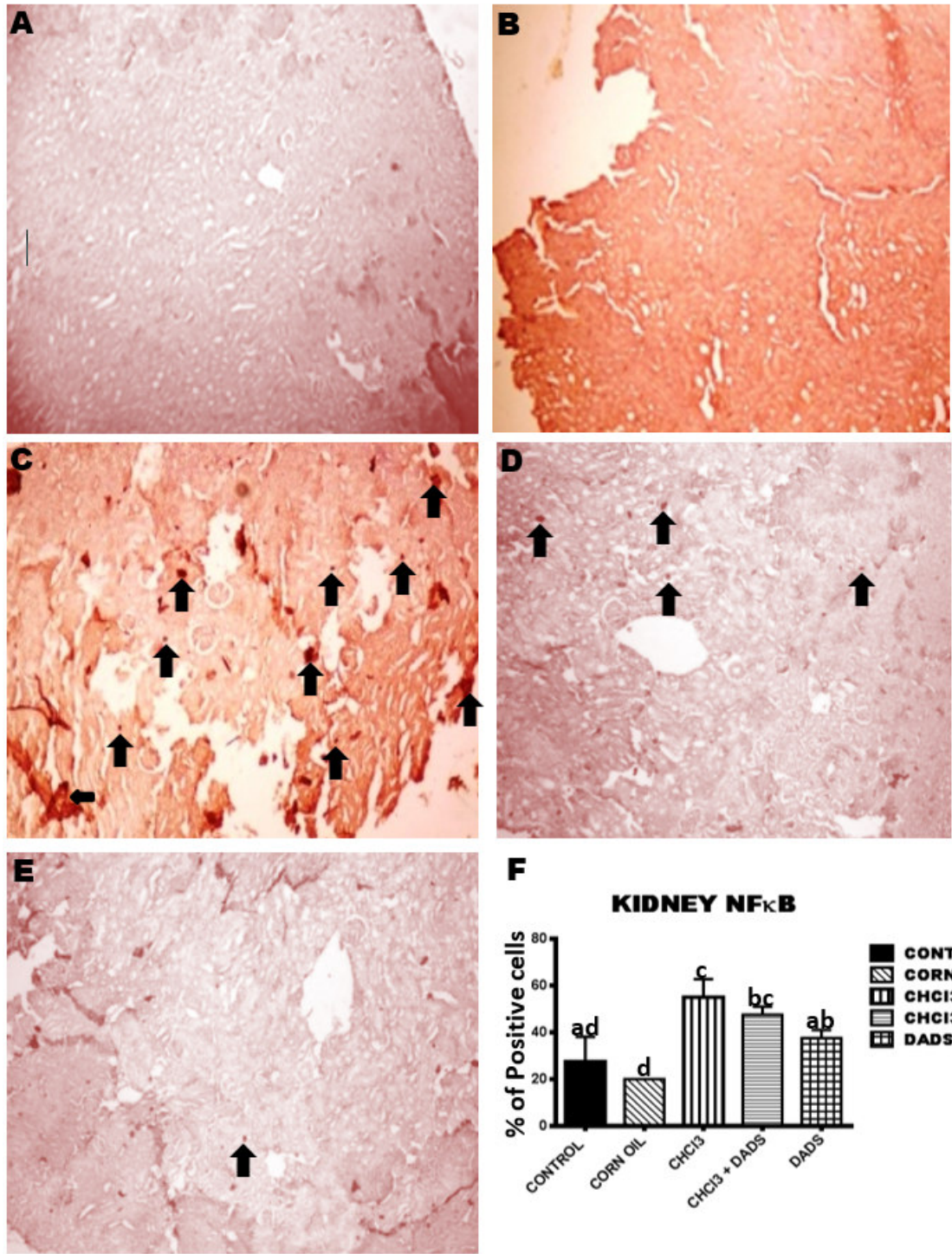


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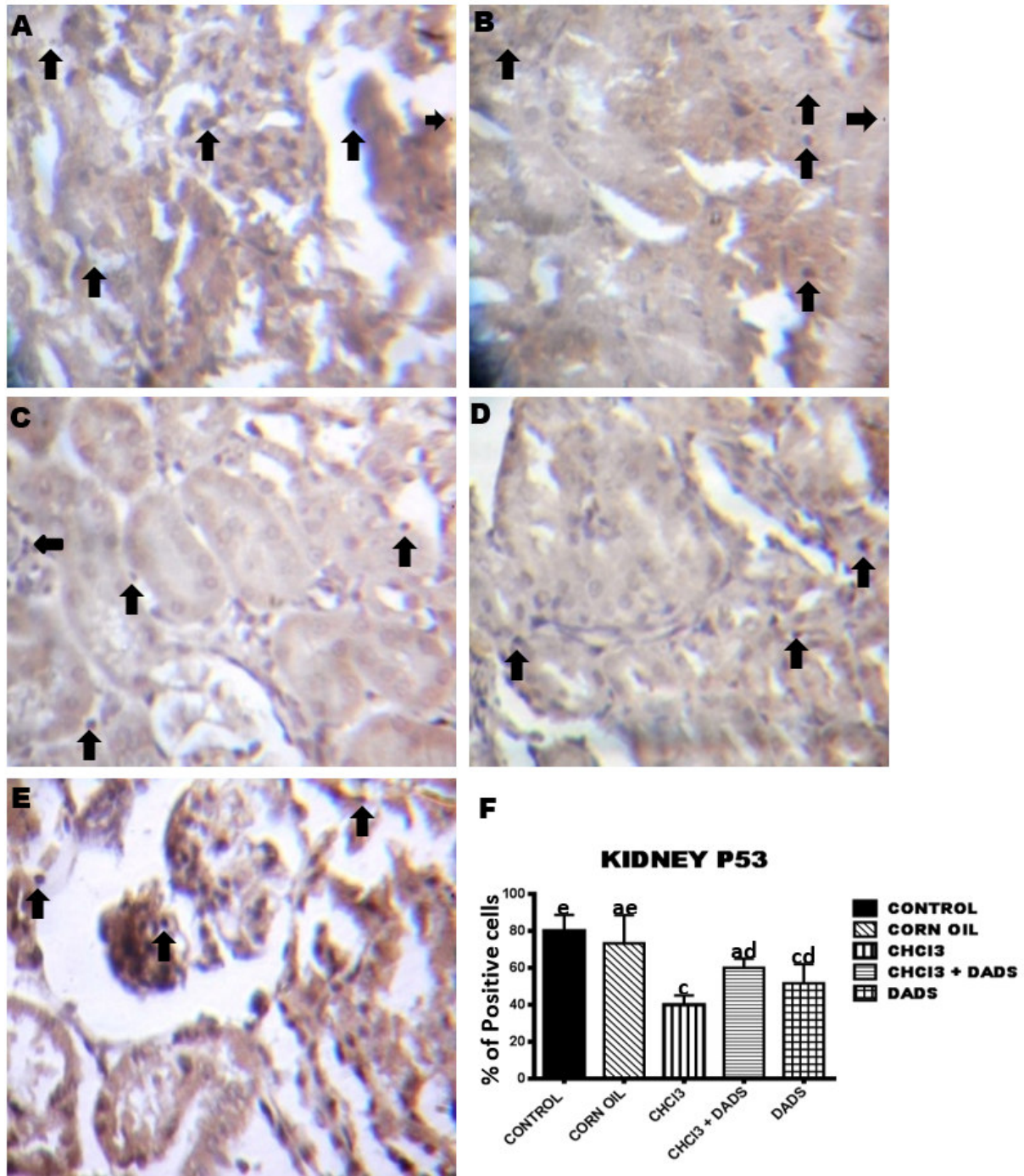


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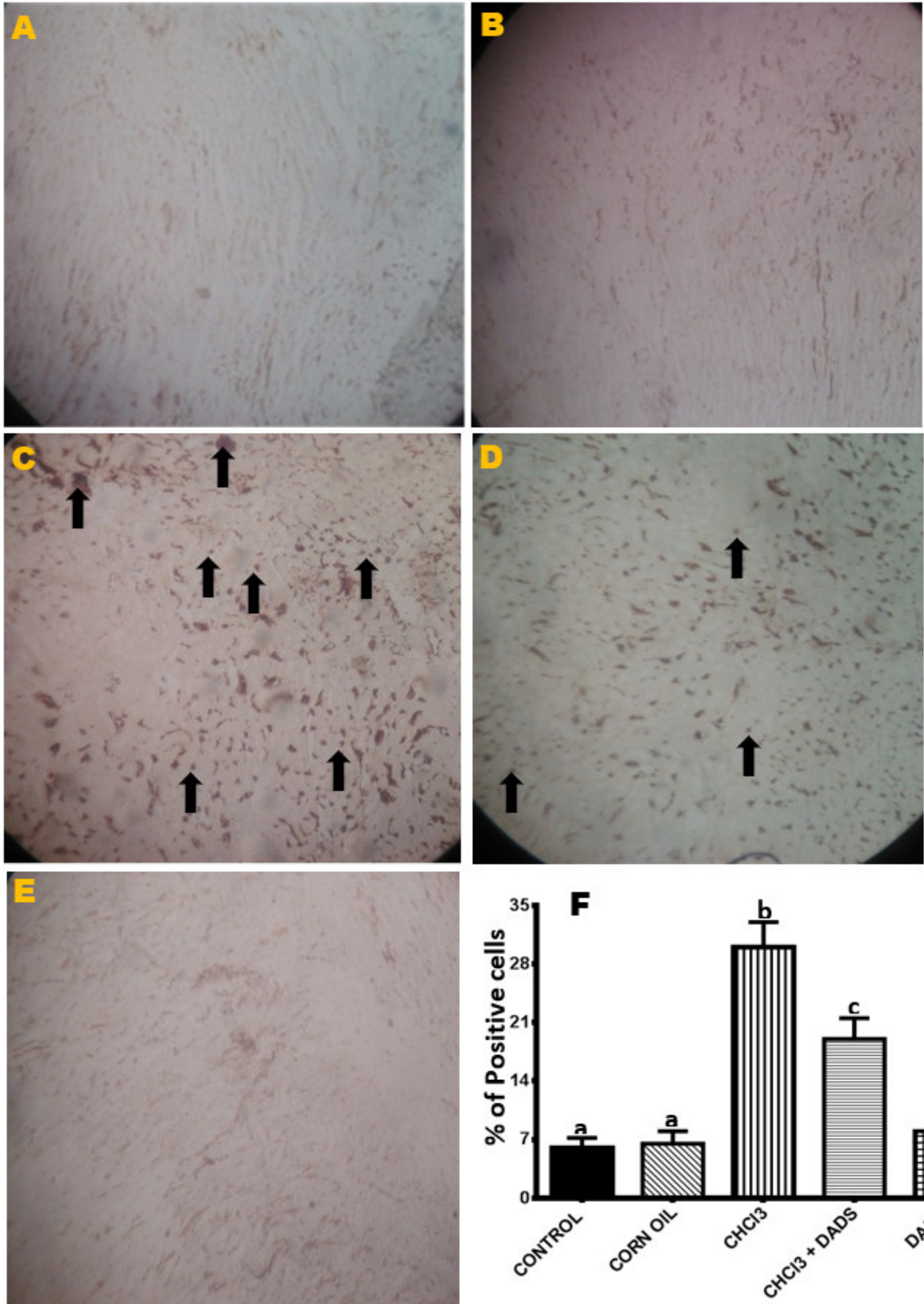


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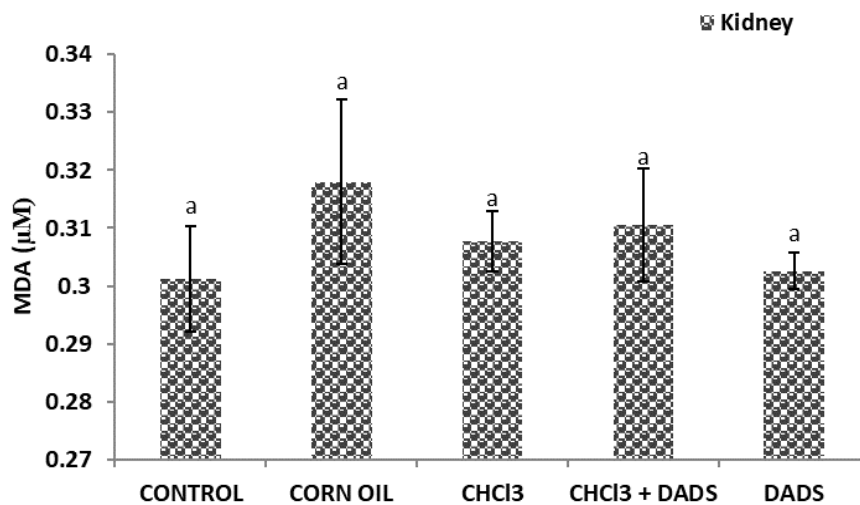


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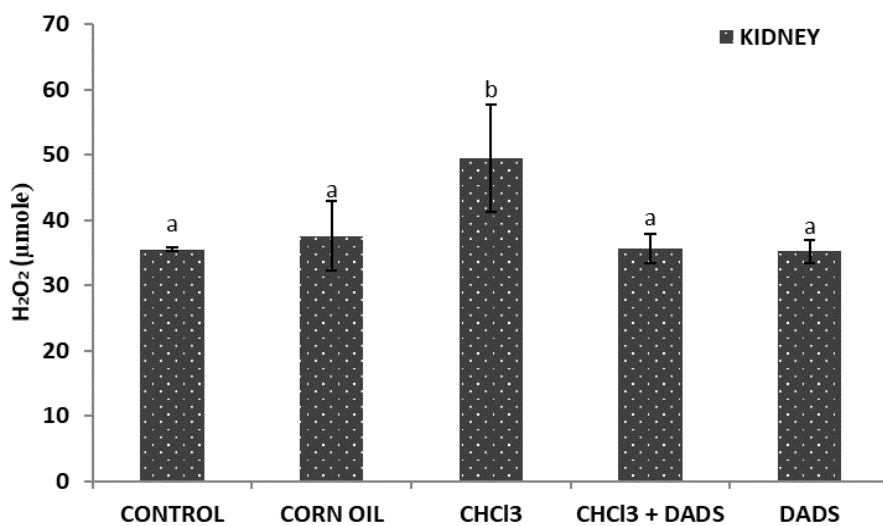


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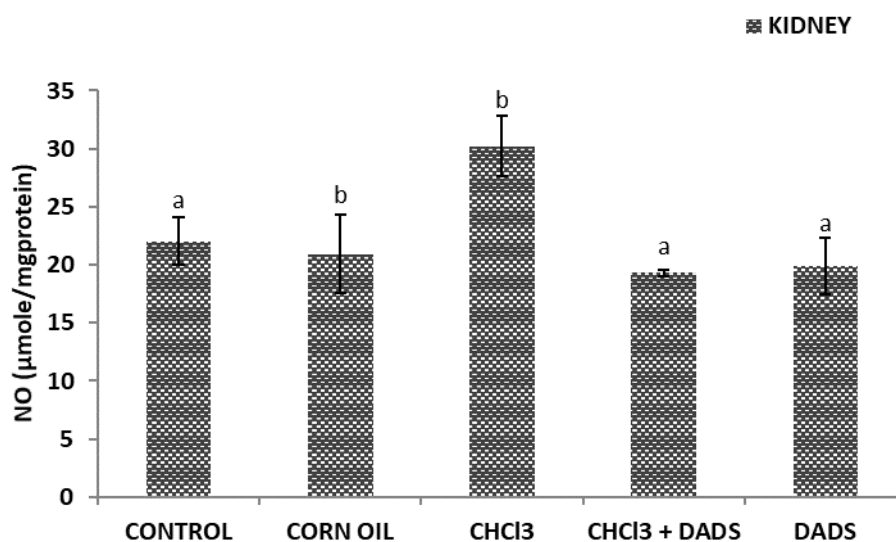


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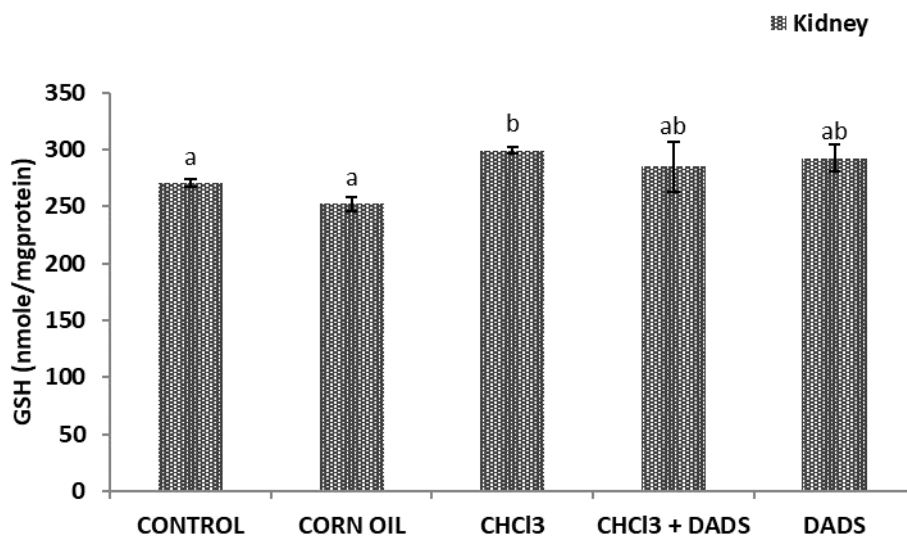


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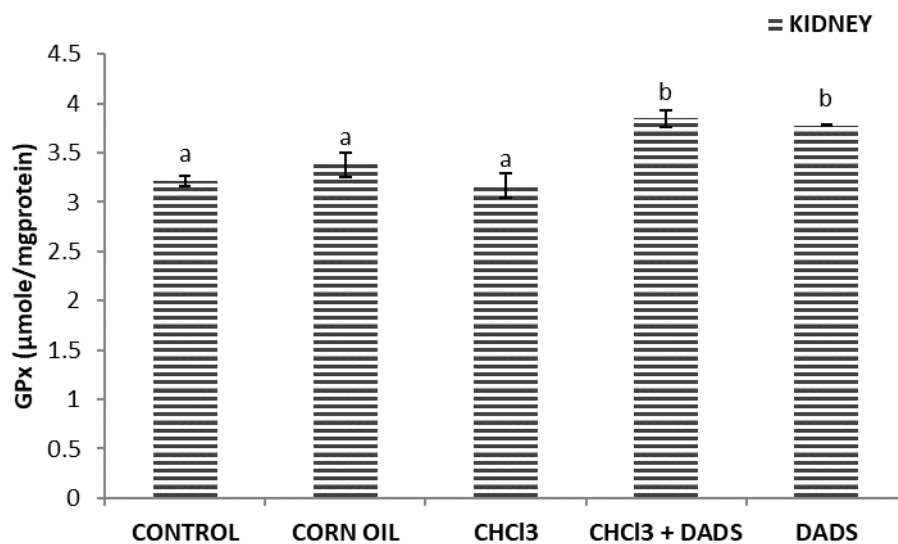


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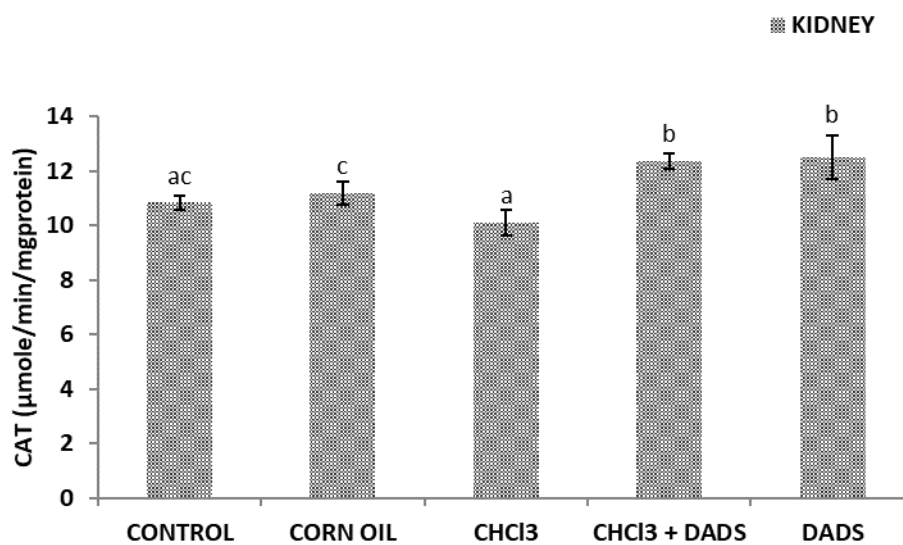


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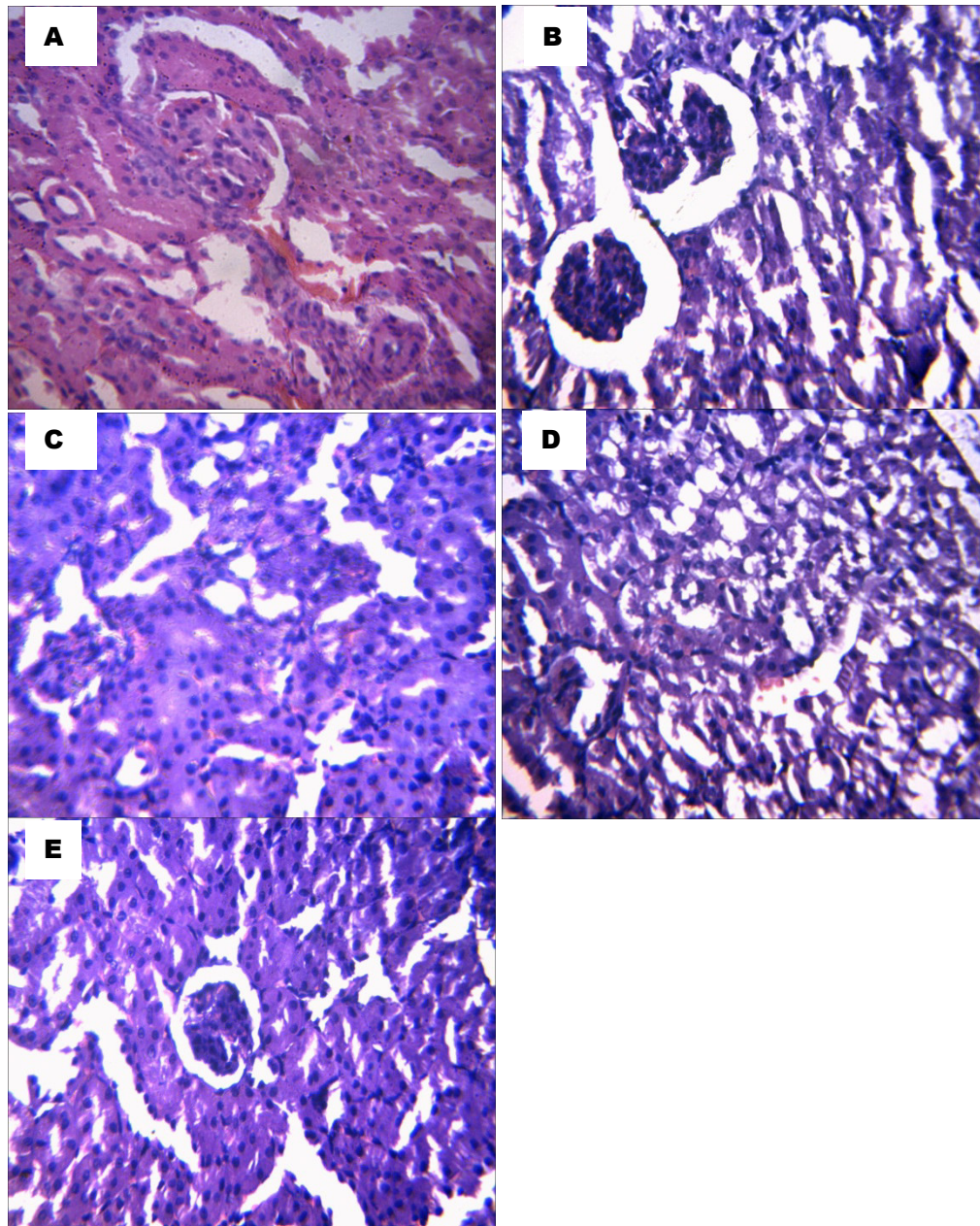


Fig. 11.

Legends to figures

Fig. 1. Effect of CHCl_3 administration and DADS treatment on relative kidney weight. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 2. Effects of DADS on CHCl_3 -induced NF κ B activation. Representative photographs of immunohistochemical analysis of NF κ B in kidney sections of (A) control, (B) cornoil, (C) CHCl_3 , (D) CHCl_3 + DADS, (E) DADS. The black arrows point to NF κ B positive cells. (F) Each bar represents mean \pm SEM of three (3) rats per group. Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 3. Effects of DADS on CHCl_3 -induced p53 expressions. Representative photographs of immunohistochemical analysis of p53 in kidney sections of (A) control, (B) cornoil, (C) CHCl_3 , (D) CHCl_3 + DADS, (E) DADS. The black arrows point to p53 positive cells. (F) Each bar represents mean \pm SEM of three (3) rats per group. Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 4. Effects of DADS on CHCl_3 -induced renal cellular apoptosis. Representative photographs of TUNEL

assay performed on kidney sections of (A) control, (B) cornoil, (C) CHCl_3 , (D) CHCl_3 + DADS, (E) DADS. The black arrows point to the apoptotic cells (TUNEL-positive cells). (F) Values are expressed as mean \pm standard error of mean (n = 3). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 5. Effect of CHCl_3 administration and DADS treatment on kidney MDA concentration. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 6. Effect of CHCl_3 administration and DADS treatment on kidney H_2O_2 concentration. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 7. Effect of CHCl_3 administration and DADS treatment on kidney NO concentration. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 8. Effect of CHCl_3 administration and DADS treatment on kidney GSH concentration. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 9. Effect of CHCl_3 administration and DADS treatment on kidney GPx activity. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 10. Effect of CHCl_3 administration and DADS treatment on kidney CAT activity. Values are expressed as mean \pm standard error of mean (n = 5). Bars labeled with different superscript are statistically significant ($p < 0.05$). CHCl_3 = trichloromethane; DADS = diallyl disulfide

Fig. 11. Effects of DADS on CHCl_3 -induced kidney histopathological alterations (100x). Control (A): No visible lesion, Corn oil (B): No visible lesion, CHCl_3 (C): There is a severe interstitial congestion of the kidney especially at the renal cortex, CHCl_3 + DADS (D): There are few foci of mild interstitial hemorrhage, DADS (E): No visible lesion. CHCl_3 = trichloromethane; DADS = diallyl disulfide