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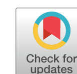
Methyl cellosolve-induced renal oxidative stress and time-dependent up-regulation of pro-inflammatory cytokines, apoptotic, and oncogenic markers in rats



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ABSTRACT

Methyl cellosolve (MC) is used in production of textile, paints, stains, inks, surface coatings, and anti-icing additive in hydraulic fluids and jet fuel. Consequently, the present study investigated its effect on renal cells, in a time-course study in male Wistar rats. Animals were orally administered 50 mg/kg body weight of MC for a period of 7, 14, and 21 days. Following 7 days of administration of MC, there was a significant increase in the levels of K-Ras, c-Myc, TNF-α, IL-6 and NO, while GSH level and SOD activity were significantly reduced compared with control. At the end of 14 days exposure, RKW, GSH, NO, and Bcl-2 levels were significantly decreased, while levels of K-Ras, c-Myc, p53, Bax, caspase-3, TNF-α, IL-1β, IL-6, MDA and GPx activity were significantly increased compared with control. After 21 days of MC administration, RKW, GSH, NO, IL-10 and Bcl-2 levels were significantly decreased, while levels of K-Ras, c-Myc, p53, Bax, caspase-3, TNF-α, IL-1β, IL-6, MDA and GST activity were significantly increased compared with control. Exposures to MC in any way should be strictly avoided as it could trigger renal damage through the disorganization of the antioxidant system, up-regulation of inflammatory, apoptotic, and oncogenic markers in rats.

1. Introduction

Glycol ethers are generally used in the production of textile dyes, printing inks, varnishes, and anti-icing additives in jet fuels. Many glycol ethers are endocrine disruptive agents that interfere with hormonal signaling and cause toxicities in different animal species, including humans [1–4]. Amongst various glycol ethers investigated, methyl cellosolve (MC), ethyl cellosolve (EC) and their derivatives are majorly harmful to organs with increased rates of respiration and energy metabolism, including fetus, testis, bone marrow, and thymus [5]. Established toxicities include leucopenia, thymocyte degradation, developmental toxicity, and testicular degradation [6]. MC is produced at $> 10^6$ pounds/year in the United States and elicits adverse effects at much lower exposure levels than EC [7]. Previously, reviews focusing on the dosage, routes, metabolism and pharmacokinetic models of exposure and toxicity of MC have been published [2,8–10].

Oxidative metabolism of MC to methoxyacetic acid (MAA) is a key pre-requisite for MC teratogenicity and embryotoxicity [11–13]. MC is initially oxidized by alcohol dehydrogenase to 2-methoxyacetaldehyde. This short-lived intermediate is oxidized by aldehyde dehydrogenase to MAA, a more stable metabolite [10]. MAA is readily seen in blood and other body fluids of exposed persons and is eliminated *via* urine [14]. Intracellularly, MAA is bio-activated to a thioester by coupling with coenzyme A to form 2-methoxyacetyl ~ CoA, followed by entry into the citric acid cycle [10]. Toxicity of MAA can be ameliorated by co-treatment with D-glucose, glycine, formate, acetate, serine and certain tricarboxylic acid pathway metabolites [10,15,16].

Many industrial products of everyday use including laminators, pesticides, and diverse kinds of plastic products, many heavy metals, flame retardants, and phytoestrogens may act as endocrine disruptors [17]. Many chemicals that are also produced in high volumes are also suspected and known endocrine disruptors that can lead to obesity and related diseases [17]. MC elicits developmental, hematopoietic, and reproductive toxicities in numerous animal species including humans

Abbreviations: MDA, malondialdehyde; GSH, reduced glutathione; NO, nitric oxide; GPx, glutathione peroxidase; GST, glutathione S-transferase; SOD, superoxide dismutase; CAT, catalase; RKW, relative kidney weight; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; p53, tumor suppressor protein; Bax, Bcl-2 associated X; Bcl-2, B-cell lymphoma 2; c-Myc, myelocytomatosis; K-Ras, Kirsten rat sarcoma viral oncogene

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[1,2,12]. Testicular destruction is a hallmark of acute MC exposure [1,3,18]. A significant decrease in relative testicular weight occurs following exposure to MC at a dose of 500 mg/kg/day for 2 or more days, while 100 mg/kg/day results in the degeneration of pachytene spermatocytes within 24 h [19]. Continuous dosing led to progressive depletion of spermatocytes and early spermatid populations [19] *via* an apoptotic mechanism [20]. Two calcium channel blockers, verapamil and diltiazem prevent MC-induced toxicity in rats *in vivo*, suggesting their role in calcium deregulation in MC toxicity [21]. Increased prevalence of oligospermia and azoospermia, as well as lower sperm counts per ejaculate, were observed in 73 shipyard painters exposed to 2.6 mg/m^3 MC [16]. Lowest observed adverse effect level (LOAEL) for testicular toxicity of 167 mg/m^3 was recorded in rats during an MC dose-response studies [22] compared to 2.6 mg/m^3 in humans, suggesting a much greater sensitivity in humans. Both rat and human seminiferous tubules respond to MAA at the same dosage and in a similar manner, when treated *in vitro* with MAA, but the morphology of the dying spermatocytes in the two species differs [3].

In the light of the above, the present study investigated the time course effect of MC on renal markers of lipid peroxidation (MDA), oxidative stress (CAT, SOD, GPx, GST, GSH, and NO), inflammation (TNF-α, IL-1β, IL-6, and IL-10), apoptosis (caspase 3, p53, Bax, and Bcl-2) and proto-oncogenic markers (c-Myc and K-Ras) in male Wistar rats.

2. Materials and methods

2.1. Chemicals and kits

MC ($C_3H_8O_2$; CAS# 109-84-4; 99.5 % purity), is a product of BDH Laboratory Supplies, Poole, BH15 1TD, England. Rats TNF-α, IL-1β, IL-6, IL-10, caspase 3, p53, Bax, Bcl-2, c-Myc, and K-Ras enzyme-linked immunosorbent assay (ELISA) kits are products of Cusabio Technology Llc, Houston, TX, USA. Reagents/chemicals used in this study were of accepted grade, products of BDH Chemical Ltd, Poole, England or Sigma Chemical Co., Saint Louis, MO, USA.

2.2. Experimental animals and study design

Male Wistar albino rats (20) that weighed 150 g and above were used for this study. The rats were sheltered in cages in the animal house of the Department, where food and water were adequately served. The guidelines of the Institutional Animal Care and Use Committee were followed after approval by the Animal Ethical Committee of the Department of Biochemistry, FUNAAB, Nigeria. After one week of acclimatization, the rats were separated randomly into four groups containing five animals each. Group I animals served as control and were served only rat chow and water throughout the study, while groups II, III and IV animals were orally administered 50 mg/kg MC ($1/20^{th}$ of LD_{50}) for 7, 14, and 21 days respectively, based on calculated mean lethal dose (LD_{50}) conducted in this study. The periods of study were selected based on the findings of Pomierny et al. [23], that some ethylene glycol ethers induced brain oxidative stress when administered to rats five days a week for four weeks.

2.3. Sample collections and preparations

Group 1 animals were sacrificed on day 0 before the commencement of MC administration. MC was administered orally for 7, 14, and 21 days, and 24 h after each of these days (days 7, 14, and 21); animals were sacrificed by cervical dislocation. They were handled following the international guidelines for the care and use of experimental animals [24]. The kidney was excised, washed in ice-cold saline (0.9 % w/v) solution, blotted dry, and weighed. Kidney sections were suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) for disruption using a tissue homogenizer. Homogenized tissues were centrifuged at 5000 rpm for 10 min. The resulting supernatant was kept in Eppendorf tubes and used for the quantifications of biochemical parameters.

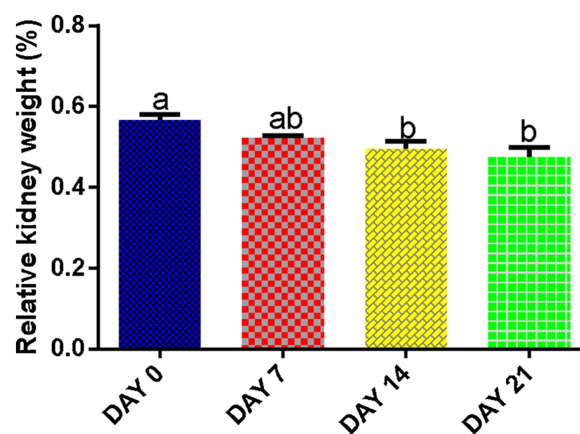

Fig. 1. Time course effect of MC on relative kidney weight. Values are expressed as mean ± standard error of the mean (n = 5). Bars labeled with different letters are statistically significant ($p < 0.05$).

2.4. Estimation of MDA concentration

Kidney concentration of malondialdehyde (MDA), a lipid peroxidation marker was estimated using the method of Buege and Aust [25]. In this method, 0.1 mL of the supernatant was added to 2 mL (1:1:1) of trichloroacetic acid-thiobarbituric acid-hydrochloric acid (TCA/ TBA/ HCl) reagent, The mixture was boiled at 100 °C for 15 min, and allowed to cool on ice. Flocculent materials were separated by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against a blank. MDA level was calculated using the molar extinction coefficient for the MDA-TBA complex of $1.55 \times 10^6\ M^{-1}\ cm^{-1}$.

2.5. Estimation of NO level

NO concentration was determined by using Griess Reagent [26]. In this method, 150 mL of sulfanilamide and 100 mL distilled water were added to 50 mL of sample, followed by incubation for 10 min. After incubation, 150 mL of N-naphthyl ethylenediamine was added followed by incubation for another 10 min and thereafter, NO concentration was measured at 540 nm.

2.6. Estimation of GSH concentration

GSH level was estimated using the method of Moron et al. [27]. The color developed was read at 412 nm.

2.7. Determination of GPx activity

GPx activity was estimated using the method of Rotruck et al. [28].

2.8. Determination of GST activity

GST activity was estimated using the method of Habig et al. [29]. This was based on enzyme-catalyzed reaction of GSH with 1-chloro-2,4-dinitrobenzene. The product (2,4-dinitrophenylglutathione) that was formed was measured at 340 nm.

2.9. Determination of SOD activity

SOD activity was estimated using the method of Misra and Fridovich [30] that is based on the ability of SOD to stop the auto-oxidation of adrenaline to adrenochrome.

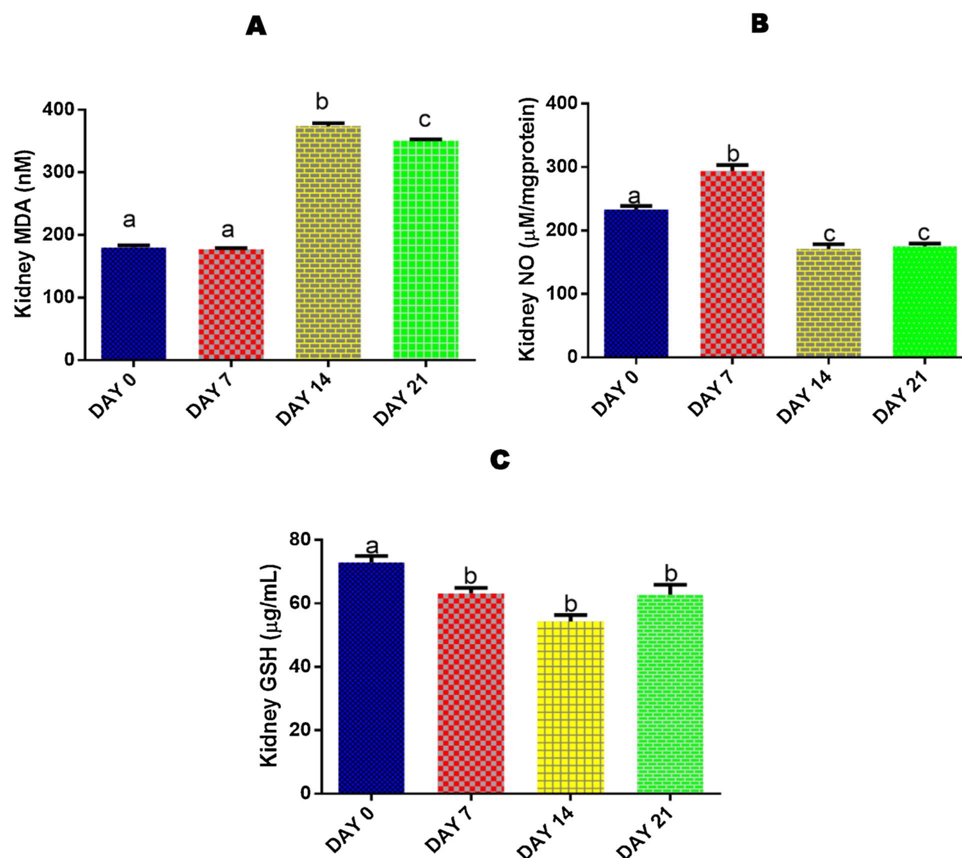

Fig. 2. Time course effect of MC on kidney MDA (2A), NO (2B), and GSH (2C) concentrations. Values are expressed as mean ± standard error of the mean (n = 5). Bars labeled with different letters are statistically significant ($p < 0.05$).

2.10. Estimation of CAT activity

CAT activity was estimated using the method of Sinha [31]. The reaction mixture (2.5 mL) contained 0.01 M phosphate buffer (pH 7.0), 0.25 mL of sample and 1 mL of 2 M hydrogen peroxide. The reaction was abruptly stopped by the addition of 0.5 mL dichromate-acetic acid reagent at 0, 1, 2, and 3 min, followed by heating in boiling water for 10 min, and then cooled at room temperature. The absorbance was read at 570 nm.

2.11. Estimations of kidney levels of TNF-α, IL-1β, IL-6, IL-10, caspase-3, p53, Bax, Bcl-2, c-Myc, and K-Ras

Procedures described in the purchased Cusabio ELISA kits (Cusabio Technology Llc, Houston, TX, USA) were followed. Briefly, 100 μL of samples and respective protein standards were added into the wells already pre-coated with an antibody specific for caspase-3, p53, Bax, Bcl-2, c-Myc, K-Ras, IL-1β, IL-6, TNF-α, or IL-10, and incubated for 2 h at 37 °C. Unbound substances were removed, and 100 μL of biotin-conjugated antibody specific for caspase-3, p53, Bax, Bcl-2, c-Myc, K-Ras, IL-1β, IL-6, TNF-α, or IL-10, was added to the well. At the end of washing, 100 μL of avidin conjugated Horseradish Peroxidase (HRP) was added and incubated for 1 h at 37 °C, and then addition of 90 μL of TMB substrate solution. A color proportional to the amount of IL-1β, IL-6, TNF-α, IL-10, caspase-3, p53, Bax, Bcl-2, c-Myc, or K-Ras bound in the initial step was obtained after incubation for 15–30 min at 37 °C. The stop solution was added to each well, mixed thoroughly, and color intensity was measured at 450 nm.

2.12. Determination of total protein concentration

Total protein concentration in the samples was determined using the method of Gornall et al. [32].

2.13. Histopathological analysis

Briefly, kidney sections were fixed in phosphate-buffered formalin solution for 48 h. 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 Tukey test for multiple comparisons among the groups of rats using Graph Pad Prism program version 6.0. Obtained results were expressed as mean ± standard error of the mean. P values lesser than 0.05 were considered statistically significant.

3. Results

3.1. Time course effect of MC on kidney relative weight

Compared with control and 7 days, only administrations of 50 mg/kg body weight of MC for 14 and 21 days significantly ($p < 0.05$) decreased kidney relative weight (Fig. 1).

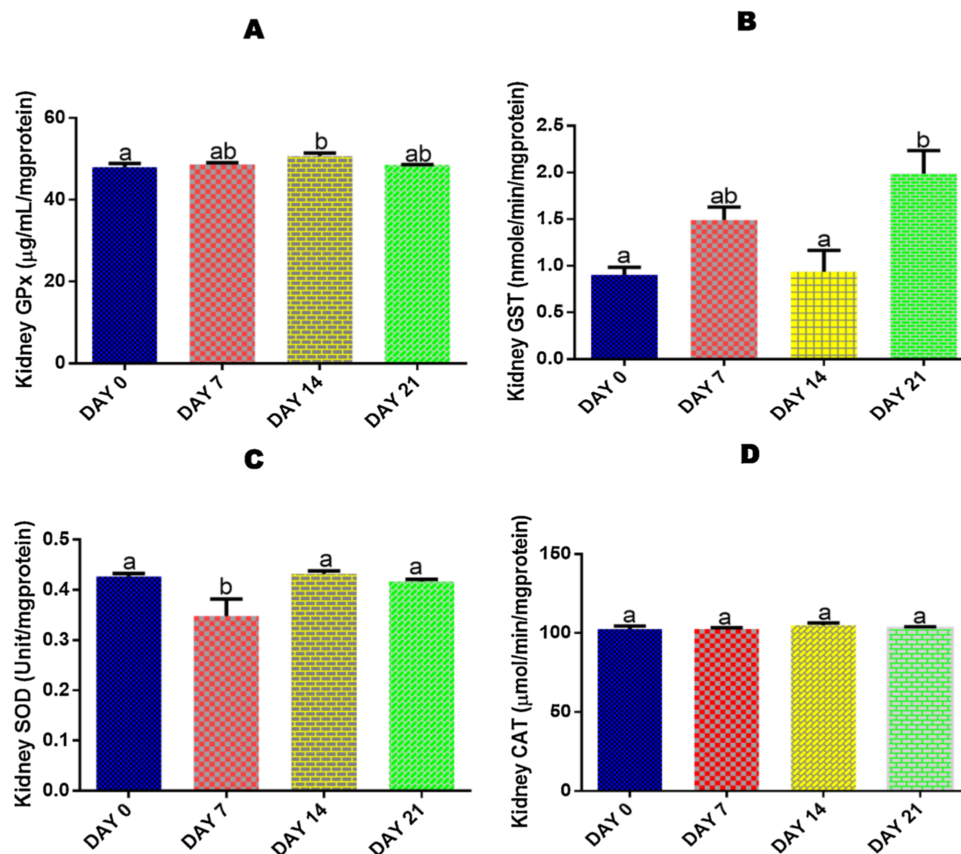

Fig. 3. Time course effect of MC on kidney GPx (3A), GST (3B), SOD (3C), and CAT (3D) activities. Values are expressed as mean ± standard error of the mean (n = 5). Bars labeled with different letters are statistically significant ($p < 0.05$).

3.2. Time course effect of MC on kidney MDA level

There was a significant ($p < 0.05$) increase in renal MDA level after 14 and 21 days of MC administration compared with control and 7 days of exposure (Fig. 2A).

3.3. Time course effect of MC on kidney NO level

Administrations of MC for 7 days resulted in a significant ($p < 0.05$) increase in renal NO level compared with control, while administrations for 14 and 21 days significantly ($p < 0.05$) decreased NO level compared with 7 days and control (Fig. 2B).

3.4. Time course effect of MC on kidney GSH level

Administrations of MC for 7, 14, and 21 days significantly ($p < 0.05$) decreased kidney level of GSH compared with control (Fig. 2C).

3.5. Time course effect of MC on kidney activity of GPx

Only administrations of MC for 14 days resulted in a significant ($p < 0.05$) increase in kidney activity of GPx compared with control (Fig. 3A).

3.6. Time course effect of MC on kidney GST activity

For GST, only 21 days of exposure to MC significantly ($p < 0.05$) increased the renal activity of the antioxidant enzyme compared with control, 7 and 14 days of exposure (Fig. 3B).

3.7. Time course effect of MC on kidney SOD activity

The kidney SOD activity was only significantly ($p < 0.05$) decreased following 7 days of MC administrations compared with control (Fig. 3C).

3.8. Time course effect of MC on kidney CAT activity

For kidney CAT activity, no significant ($p > 0.05$) effect was recorded after 7, 14, and 21 days of MC administrations compared with control (Fig. 3D).

3.9. Time course effect of MC on kidney TNF-α, IL-1β, IL-6, and IL-10 levels

Administrations of MC for 7, 14 and 21 days significantly ($p < 0.05$) increased renal levels of TNF-α (Fig. 4A) and IL-6 (Fig. 4B) compared with control. For IL-1β (Fig. 4C), only administrations for 14 and 21 days resulted in a significant ($p < 0.05$) increase, while IL-10 (Fig. 4D) was significantly ($p < 0.05$) decreased after 21 days only compared with control.

3.10. Time course effect of MC on kidney caspase-3, p53, Bax, and Bcl-2 levels

After 14 and 21 days of MC administrations, kidney levels of p53 (Fig. 5A), Bax (Fig. 5B), and caspase-3 (Fig. 5D) were significantly ($p < 0.05$) increased, while Bcl-2 (Fig. 5C) level was significantly reduced ($p < 0.05$) compared with control.

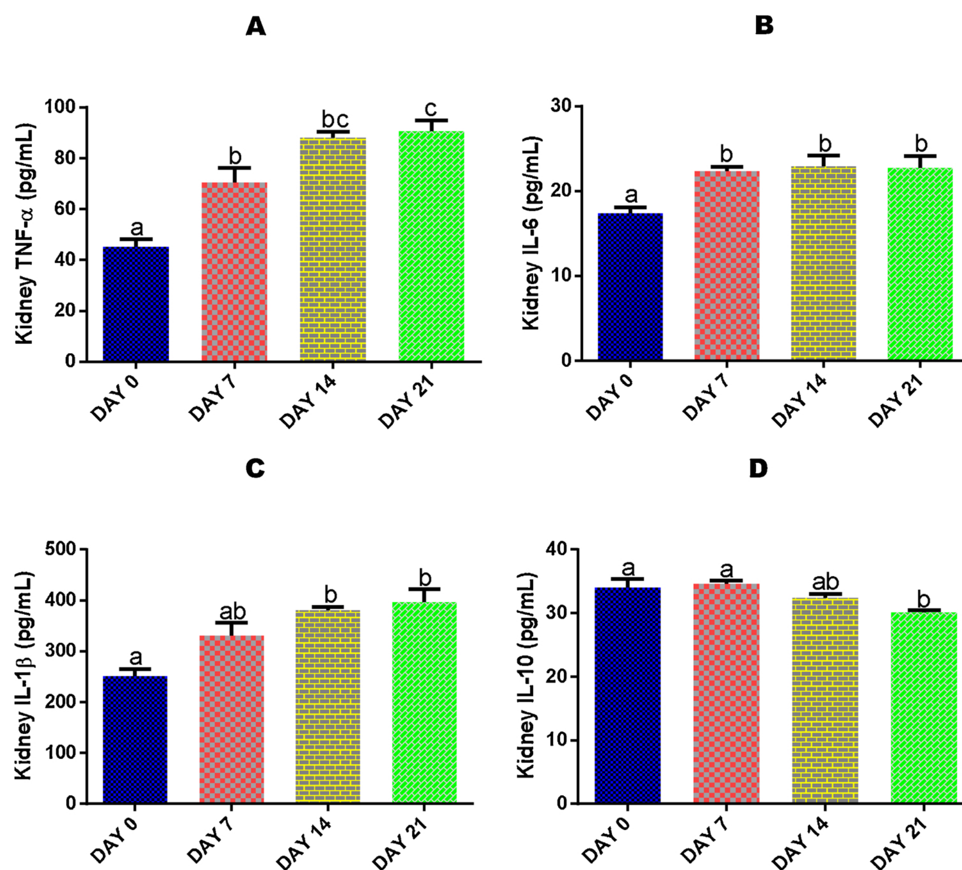

Fig. 4. Time course effect of MC on kidney TNF-α (4A), IL-6 (4B), IL-1β (4C), and IL-10 (4D) levels. Values are expressed as mean ± standard error of the mean. Bars labeled with different letters are statistically significant ($p < 0.05$).

3.11. Time course effect of MC on kidney levels of c-Myc and K-Ras

Both renal levels of c-Myc (Fig. 6A) and K-Ras (Fig. 6B) were significantly ($p < 0.05$) increased by MC after 7, 14 and 21 days of administrations compared with control. Also, 21 days of MC administrations resulted in a significant ($p < 0.05$) increase in c-Myc (Fig. 6A) level compared with control, 7 and 14 days of administrations.

3.12. Time course effect of MC on kidney histopathology

Kidney histopathology (Fig. 7) revealed erosion of bowman capsule, disseminated fatty infiltration of glomeruli and tubules with area of tubular degeneration, glomerular mesangialization, infiltration of bowman capsule with inflammatory cells, glomeruloscelorosis, as well as glomerular and tubular inflammation (very severe glomerulonephritis) after 7, 14 and 21 days of MC administrations compared with control that revealed intact glomeruli and tubules with no visible lesion.

4. Discussion

As a result of its high volatility, MC is expected to be present majorly in air. The half-life of MC in the atmosphere has been estimated to be in the range of 5.7–57 h, based on its reaction with hydroxyl radicals [33,34]. MC volatilizes swiftly from the water surface, with a calculated half-life of 2.8 h [35]. The environmental partitioning of MC when released into the air, water or soil was estimated by a Level III fugacity prototype [36]. When MC is discharged into the air, Equilibrium Criterion (EQC) Level III fugacity prototype [37] predicts that about 50 % would be present in the air, whereas approximately 25 % would be deposited in soil and about 25 % in water. When emitted into the water, more than 99 % would be present in water, and when released to soil, about 75 % would be present in the soil, whereas approximately 25 % would be present in water [36]. As a result, humans are unavoidably and inadvertently exposed to MC, and also chances of exposure are on the high side. This present study investigated the time course effect of MC administrations in male Wistar rats. Following exposures, relative kidney weight was significantly decreased after 14 and 21 days of MC administration, an indication of its renal toxic effect in the rats over time (Fig. 1).

Lipid peroxidation is a degenerative pathway of cell membrane components mediated through reactive oxygen species produced in the cell [38]. Measurement of thiobarbituric acid reactive substance (TBARS) is commonly used to check lipid peroxidation and indirectly, oxidative stress *in vivo* [39]. Lipid oxidation causes the disruption of the membrane bilayer, leading to the efflux of cellular content from the damaged organ into the bloodstream [40,41]. The significant increase in MDA concentration after 14 and 21 days of MC administrations (Fig. 2A) may be attributed to the disorganization of the antioxidant system and high production of reactive species that may have targeted the electron-rich unsaturated fatty acid components of cell membranes, leading to their oxidation and destruction, thereby undermining the cellular integrity and functions [41,42].

NO is a major molecule produced in the human body, acting as a major regulator in a vast array of important physiological functions, namely immune response, blood pressure and neural communication [43]. Over generation of cellular NO can lead to the production of peroxynitrite that can damage the tissues [44,45]. In this study, the significant increase in renal NO level (Fig. 2B) after 7 days of MC administrations compared with control may be due to MC-induced renal oxidative stress in the animals. Further exposures to MC for 14 and 21 days may have exacerbated the oxidative stress, leading to the

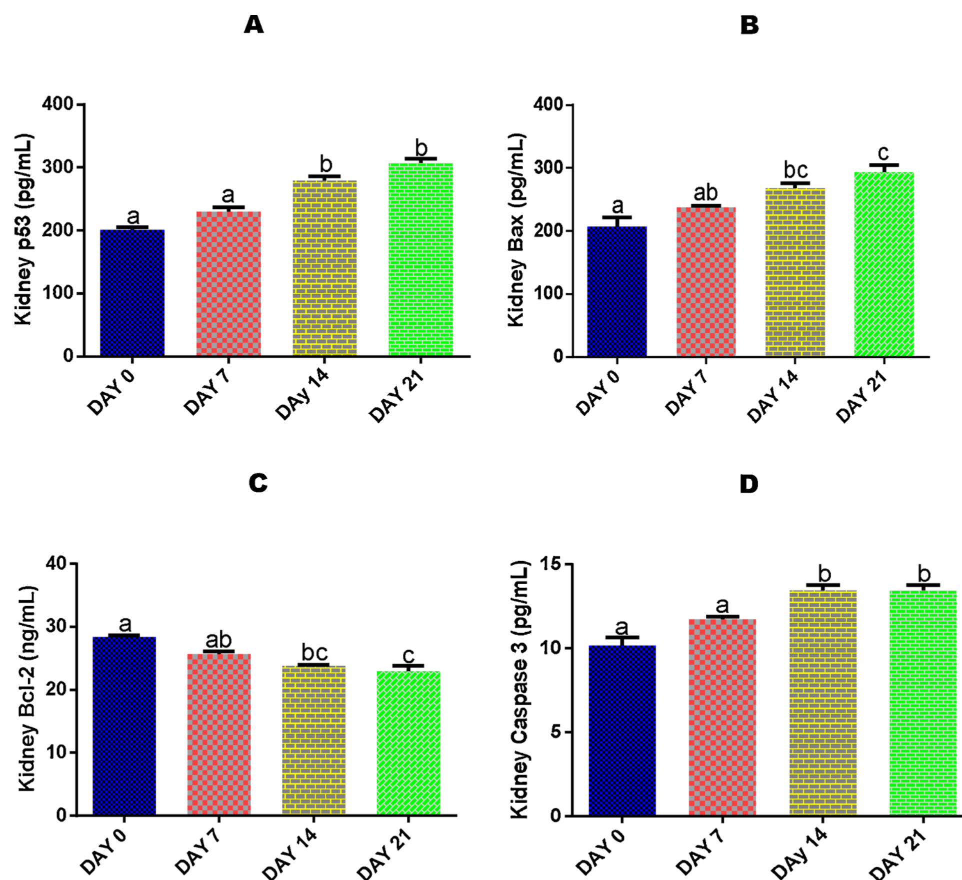

Fig. 5. Time course effect of MC on kidney p53 (5A), Bax (5B), Bcl-2 (5C) and caspase-3 (5D) levels. Values are expressed as mean ± standard error of the mean. Bars labeled with different letters are statistically significant ($p < 0.05$).

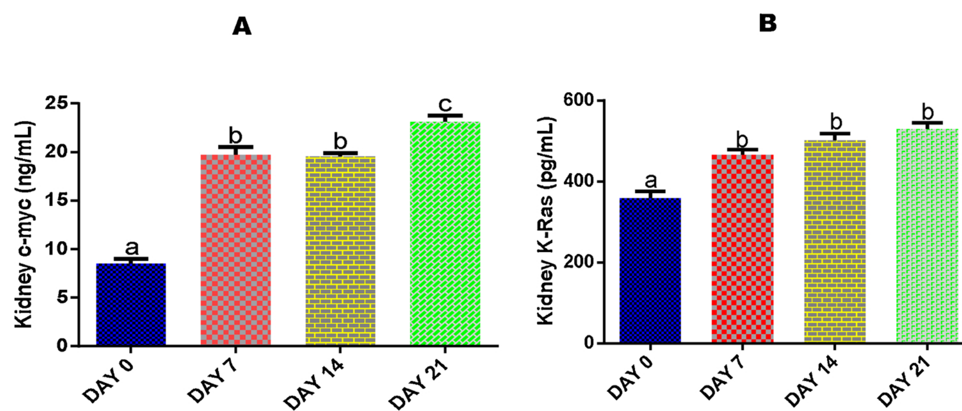

Fig. 6. Time course effect of MC on kidney c-myc (6A) and K-Ras (6B) levels. Values are expressed as mean ± standard error of the mean. Bars labeled with different letters are statistically significant ($p < 0.05$).

overproduction of free radicals that subsequently led to a significant decrease in NO (Fig. 2B). Reactive oxygen species play an important role in NO-based cell signaling [43]. Free radicals can affect NO availability both from production to post-production scavenging and lead to a myriad of vascular disorders due to compromised NO functionality [43].

Both the enzymatic and non-enzymatic antioxidants are the natural defense against free radical-mediated tissue damage in several organs. Antioxidants such as GSH, GPx, GST, SOD, and CAT work synergistically as a team of defense against reactive oxygen species [46]. In this study, MC administrations caused a significant reduction in renal GSH (Fig. 2C) level and SOD (Fig. 3C) activity (after 7 days only), as well as a significant increase in the activities of GPx (after 14 days only) (Fig. 3A) and GST (after 21 days only) (Fig. 3B), suggesting that GSH has played a major role in renal MC detoxification and mopping of generated free radicals (hydrogen peroxide). SOD catalyzes the dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide (H_2O_2) and O_2, while CAT catalyzes the decomposition of H_2O_2 to molecular oxygen and water [47]. GSH serves as a substrate for GPx, and as H_2O_2 is being detoxified, there is concomitant oxidation of GSH to GSSG [48]. GST is one of the phase two drug-metabolizing enzymes and catalyzes the release and transfer of GSH to xenobiotic for their detoxification. Also, the non-significant effect of MC on renal CAT activity recorded in this study (Fig. 3D), suggested that scavenging of H_2O_2 may be through the glutathione family of antioxidants as stated above, and not by CAT. To support our findings, previous studies have

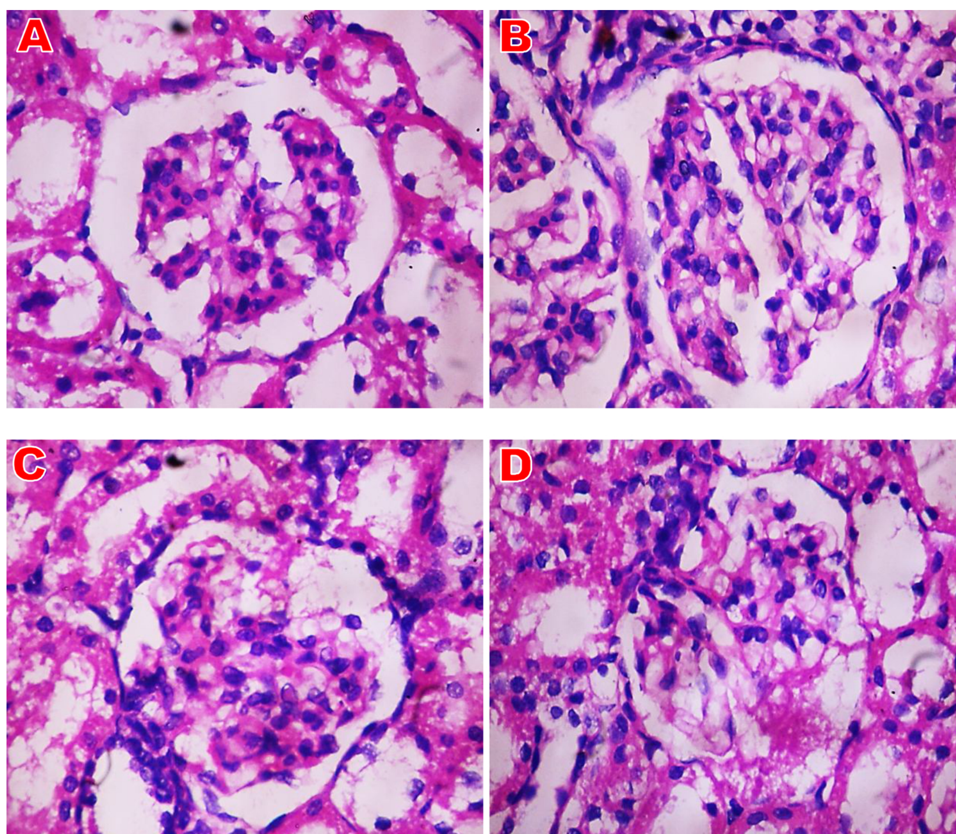

Fig. 7. Kidney microphotographs (x 400) showing (A) normal appearance with intact glomeruli and tubules; (B) disseminated fatty infiltration of glomeruli and tubules with area of tubular degeneration, and focal area of glomerular inflammation (moderate glomerulonephritis); (C) disseminated fatty infiltration of glomeruli and tubules with area of tubular degeneration, glomerular mesangialization, infiltration of bowman capsule with inflammatory cells, as well as glomerular and tubular inflammation (severe glomerulonephritis); and (D) erosion of bowman capsule, disseminated fatty infiltration of glomeruli and tubules with area of tubular degeneration, glomerular mesangialization, infiltration of bowman capsule with inflammatory cells, glomerulosclerosis, as well as glomerular and tubular inflammation (very severe glomerulonephritis). A = Day 0; B = Day 7; C = Day 14; D = Day 21.

reported a significant reduction in the renal activity of endogenous antioxidant enzymes following iron-induced renal toxicity [49], as well as aluminum oxide and/or zinc oxide nanoparticles [50] in rats.

Cytokines act as messengers between tissues and the immune system, enabling them to play a role in many physiological processes *via* either anti-inflammatory or pro-inflammatory properties [51]. Some documented pro-inflammatory cytokines are IL-6, TNF-α, IL-1β, interferon-(IFN-) γ, and IL-17 [52,53], while those involved in anti-inflammation include IL-4 and IL-10 [52,54]. The significant increase in the levels of kidney TNF-α (Fig. 4A), IL-6 (Fig. 4B), and IL-1β (Fig. 4C), as well as decreased level of IL-10 (Fig. 4D) after 7, 14 and 21 days of MC administrations, is an indication of MC-induced renal injury or infection, causing the secretion and recruitment of the proteins, predominantly by the immune cells to the site of injury or infection where they initiate inflammation and trigger pathological pain [55]. Renal tubule cells express specific receptors that mediate the effects of individual cytokines [56–58]. It is believed that the proximal tubule cells [59,60], thick ascending limb [61,62], and collecting ducts [63] act as the immune responder or pro-inflammatory cells responsible for the pathogenesis of renal dysfunction. In a recent study by Somade et al. [64], it was reported that administration of edible camphor also led to the up-regulation of renal TNF-α, IL-1β, and IL-6, while Yousef et al. [50] reported that aluminum oxide, zinc oxide, or their combination resulted in the elevation of both renal TNF-α and IL-6 in rats.

Apoptosis is a strictly regulated process under the control of several signaling pathways, such as the mitochondrial pathway and caspase cascade [65–67]. p53, a well-known tumor suppressor protein can prevent cells from becoming malignant *via* initiation of cell cycle arrest [68,69]. In this study, the significant increase in renal p53 level (Fig. 5A) after 14 and 21 days of administrations is an indication of MC-induced renal damage. MC-induced renal oxidative stress and inflammation recorded in this study may have stimulated the activation of p53, which elicits the induction of cell cycle arrest and activates the apoptotic genes to start apoptosis. p53 is an important regulator of apoptosis and carcinogenesis [70–74]. Many pathways control p53-induced apoptosis, and one of these involves the Bax protein, a p53 target and a pro-apoptotic member of the Bcl-2 family of proteins [75–77]. Bax promotes the cytosolic release of cytochrome *c*, which then activates caspase 3, one of the major executioners of apoptosis [78]. Also, the *Bax* gene is an apoptosis-stimulating member of the *Bcl-2* gene family. The Bcl-2 protein forms heterodimers with the Bax protein *in vivo* and the molar ratio of Bcl-2 to Bax determines whether apoptosis is initiated or inhibited in many tissues [79]. Bax protein coordinates cell death through its involvement in disruption of mitochondria and eventual cytochrome *c* release and therefore considered to be one of the primary p53 targets [80]. In this study, the significant increase in renal Bax (Fig. 5B) and decrease in Bcl-2 (Fig. 5C) levels after 14 and 21 days of MC administrations suggest a p53-induced programmed cell death, since Bax and Bcl-2 are the targets of p53. In response to cellular damage, up-regulated p53 recorded in the study may have stimulated Bax expression and down-regulated Bcl-2 expression. The resulting increase in the level of free Bax may have eventually bound to the mitochondrial membrane, creating pores in it, causing mitochondrial membrane damage and the release of cytochrome c that subsequently initiates cellular apoptosis. Caspase-3 is widely known as one of the key executioners of apoptosis. Caspase-3 activation requires proteolytic cleavage of its inactive form or zymogen into activated p17 and p19 subunits [81]. Bax stimulates the release of cytochrome *c* from the mitochondria and the released cytochrome *c* activates caspase-3 [82]. Cleaved caspase-3 is considered as a primary mechanism of apoptosis. The above may therefore, explain the increased level of kidney caspase-3 (Fig. 5D) after 14 and 21 days of 2-ME administrations. The released cytochrome c following Bax attack on the mitochondrial membrane may have interacted with downstream apoptotic mediators (Apaf-1, caspase-9) to form an apoptosome that cleaved the executioner caspases including caspase-3, that facilitate the programmed cell death.

c-Myc is a proto-oncogene and a transcription factor that is actively involved in the regulation of cell growth, proliferation, differentiation

and apoptosis [83–85]. Deregulation in its expression plays a key role in human diseases, and c-Myc overexpression has been found in most human cancers [86]. *K-Ras* is another proto-oncogene; the most commonly mutated oncogene, and frequently associated with some of the deadliest forms of cancer [87]. It has been reported that Myc is essential for K-Ras-driven cancer [88] and activation of Ras stabilizes Myc [89], enabling myc to render cells vulnerable to DNA damage and apoptosis [90]. Therefore, the significant increase in renal c-Myc (Fig. 6A) and K-Ras (Fig. 6B) levels after 7, 14 and 21 days of MC administrations may be an indication of MC-induced alterations in these oncogenes by amplification or translocation, resulting into their activations and subsequent generation of reactive oxygen species that may have caused DNA damage. Also, activation of these oncogenes may therefore, explains the marked increase in the levels of apoptotic players (p53, Bax, caspase-3) recorded in this study that facilitated apoptosis and boycotted tumor initiation and progression. Recently, it has been generally agreed that Myc plays an important role in the progression of many human cancers, although increased cell growth caused by Myc is halted by greater rates of apoptosis [91].

The toxicity of MC was further confirmed and corroborated by the kidney histopathology (Fig. 7) that revealed various renal pathological disorders such as erosion of bowman capsule, disseminated fatty infiltration of glomeruli and tubules with area of tubular degeneration, infiltration of bowman capsule with inflammatory cells, glomeruloscelorosis, and very severe glomerulonephritis.

In conclusion, exposures to MC in any way should be strictly avoided as it could trigger renal damage through the disorganization of the antioxidant system, up-regulation of inflammatory, apoptotic, and oncogenic markers in rats.

CRediT authorship contribution statement

Oluwatobi T. Somade: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Babajide O. Ajayi:** Methodology, Investigation, Resources, Supervision, Project administration. **Mariana O. Olushola:** Methodology, Investigation, Resources, Project administration. **Esther O. Omoseebi:** Methodology, Investigation, Resources, Project administration.

Declaration of Competing Interest

None to declare.

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