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Kolaviron Exerts Protection Against Atrazine-evoked Hepatorenal Injury by Targeting Apoptosis, Oxidative Stress, Inflammation Mediated Nf-kb/Cox-2/Il-1β and Beclin-1/Lc3-ii Signaling Cascades in Rats

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Research Article

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KOLAVIRON EXERTS PROTECTION AGAINST ATRAZINE-EVOKED HEPATORENAL INJURY BY TARGETING APOPTOSIS, OXIDATIVE STRESS, INFLAMMATION MEDIATED NF-KB/COX-2/IL-1B AND BECLIN-1/LC3-II SIGNALING CASCADES IN RATS

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Abstract

Atrazine (ATZ), a herbicide widely used for weed control is widely recognized for its significant environmental and health risks due to its persistence and toxicity. Kolaviron (KV), a natural biflavonoid isolated from the seeds of Garcinia kola, has been primarily recognized for its antioxidant and anti-inflammatory activities. The current research aimed to evaluate against atrazine-induced hepato-renal injury in male Wistar rats. Sixty (60) male Wistar rats (250-300 g) were randomly allocated into five groups (n = 12), and administered corn oil (2 mL/kg), KV (100 & 200 mg/kg) and ATZ (100 mg/kg) for the duration of fourteen days. Post sacrifice, the liver and kidney tissues of rats were collected for Biochemical, histological, and molecular analyses using spectrophotometric, histological, and immunological techniques.

A remarkable increase in markers of liver and kidney function (ALT, AST, urea, and creatinine) was observed in the ATZ group of rats. The levels of antioxidants, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), reduced glutathione (GSH), total thiols (TSH), and glutathione-S-transferase (GST), were also remarkably reduced. In contrast, there was a significant increase in the level of oxido-inflammatory markers, including malondialdehyde (MDA), reactive oxygen and nitrogen species (RONS), hydrogen peroxide (H_2O_2), nitric oxide (NO), xanthine oxidase (XO), interleukin 1β (IL-1β), nuclear factor kappa β (NF-κB), and cyclooxygenase 2 (COX-2). Additionally, ATZ triggered an increase in tissue expressions of pro-apoptotic biomarkers (Bax and caspase-3), decrease in the expression of the anti-apoptotic biomarker (Bcl-2), and an increase in tissue expressions of autophagy biomarkers (Beclin-1 and LC3-II), together with histopathological changes such as hepatocyte necrosis and renal tubular cell degeneration in the liver and kidney of the experimental rats. Conversely, KV administration remarkably suppressed these biochemical and histological alterations in a dose-dependent manner through the restoration of the antioxidant status, inhibition of oxido-inflammatory responses, modulation of apoptosis and autophagy, and maintenance of normal histoarchitecture of the liver and kidneys.

In summary, KV exhibited robust hepatorenal protective properties against ATZ toxicity, which could make it a promising candidate for the treatment of herbicide-induced organ injuries.

Keywords: Kolaviron, Atrazine, Hepatorenal, Injury, Oxidative stress, Apoptosis, Autophagy.

1.0 Introduction

Atrazine (ATZ), a widely utilized triazine herbicide, is extensively applied in agriculture to manage broadleaf and grassy weeds. Despite its efficacy in crop protection, atrazine has been implicated in environmental contamination and poses significant health risks to non-target organisms, including humans (Chang *et al*., 2022). Many countries banned atrazine usage, but still, other countries massively apply atrazine in the agriculture sector (Khatoon and Rai, 2020). Atrazine is the pesticide most commonly found in groundwater and surface water in the US (Gilliom et al., 2006), with concentrations of atrazine up to 224 ppb (μg/L) in mid-western streams (Battaglin et al., 2000). The maximum contaminant level set by the US Environmental Protection Agency (EPA) for drinking water is 3 μg/L (U.S. Environmental Protection Agency, 2006). However, levels higher than this are often reached seasonally in the Midwest in community drinking water supplies (Almberg et al., 2018).

Studies have demonstrated that ATZ exposure can lead to oxidative stress, resulting in hepatic and renal damage in mammals (Abarikwu *et al*., 2017). Also, ATZ administration in male Wistar rats increased hepatic lipid peroxidation, altered expression of apoptotic and antiapoptotic genes, and altered antioxidant enzyme activities signifying oxidative liver injury (Singh *et al*., 2011; Rashad *et al*., 2023). Furthermore, ATZ at 0.08 mg/L induced changes in hepatic markers, oxidative stress parameters, mixed-function oxygenases, ATPase enzymes, and mild structural lesions in the liver of male rats (Owagboriaye *et al*., 2023). Another study demonstrated that ATZ at 100 mg/kg induced severe liver damage and mitochondrial dysfunction in Hyline brown laying hens (Li *et al*., 2025). Also,studies have shown that ATZ exposure in Wistar rats increased serum urea nitrogen and creatinine levels, along with elevated nitric oxide and malondialdehyde levels in kidney tissues, indicating renal oxidative damage (Liu *et al*., 2014). In other models, ATZ induced oxidative damage and caspase-3 immunolocalization in the kidney and liver of *Xenopus laevis* tadpoles (Dornelles *et al*., 2014; Semren *et al*., 2018), altered the expression of multiple lipid metabolism related genes, including those related to metabolic pathways, fatty acid metabolism, and fatty acid elongation in juvenile common carp Cyprinus carpio L (Liu *et al*., 2023). It also caused growth inhibition, hepato-renal toxicity, and oxidative stress in Nile tilapia

(Ali *et al*., 2022). In addition, ATZ inhibited thyroid hormone binding to target proteins, likely provoking enhanced hepatotoxicity in Larimichthys crocea (Wang *et al*., 2025).

Kolaviron (KV), a naturally occurring biflavonoid complex derived from the Garcinia kola seeds, garcinol has gained much recognition due to its strong antioxidant and anti-inflammatory qualities (Farombi *et al*., 2013). Previous studies have highlighted kolaviron's protective effects against various xenobiotic-induced toxicities (Adedara and Farombi, 2011). Notably, studies have reported that kolaviron mitigated diclofenac-induced hepatic and renal toxicity in Wistar rats by enhancing antioxidant defenses and suppressing inflammatory responses (Alabi and Akomolafe, 2020). Kolaviron has been shown to mitigate diclofenac-induced liver and kidney toxicity by suppressing inflammatory responses and enhancing antioxidant defenses (Alabi and Akomolafe, 2020). Another study also demonstrated the ameliorative effect of KV on multiwalled carbon nanotubes-induced hepatic and renal dysfunction in rats (Awogbindin *et al*., 2021). Additionally, it is reported that KV can modulate inflammatory reaction through inhibition of major signal transduction pathways such as the NF-κB and MAPK pathways, thus suppressing the production of inflammatory mediators (Onasanwo *et al*., 2016). Moreover, in one study, the protective action of KV against deleterious effects on the liver and kidney was reported based on antioxidative effects (Adaramoye *et al*., 2016).

Despite the documented toxic effects of ATZ and the protective properties of KV, there is a dearth of studies evaluating the possible beneficial effects of KV on ATZ-induced damage to the liver and kidneys. Therefore, the present study evaluated the possible protective effect of kolaviron on atrazine-induced hepatorenal damage in male Wistar rats.

2 0. Materials and methods

2.1 Chemicals and reagents

Atrazine 80% wettable powder was procured from Shandong Vicome Greenland Chemicals Company Limited, China. Analytical grade chemicals were used and obtained from Sigma Aldrich. Bax, Bcl-2, Beclin-1, NF-kB, COX-2, and LC3-II, and Beclin-1 antibodies were purchased from Cloudclone. Kits for caspase 3 and interleukin 1 beta for enzyme-linked immuno-sorbent assay (ELISA) were bought from Elabscience Biotechnology Inc, Beijing, China. Liver

function tests, including alanine aminotransferase, aspartate aminotransferase kits were purchased from HUMAN Diagnostics Worldwide Stegelitzer Straße, Magdeburg, Germany. Kidney function tests including Urea and Creatinine kits were procured from RANDOX Laboratories Ltd., Crumlin, UK.

2.2 Kolaviron

Seeds of *Garcinia kola* were commercially procured at Ile-Ife, and were processed in accordance to the methods of (Iwu *et al*., 1987) which was modified by Farombi *et al*. (2004).

2.3 Experimental Animal

Male adult Wistar rats with weights between 250 and 300 grams were bought from the Faculty of Veterinary Medicine, the University of Ibadan. All animals were housed in the Department of Biochemistry animal holding facility, in plastic metabolic cages, under natural photoperiod of about 12 hours' light and 12 hours' darkness daily, for the period of acclimatization and administration of Atrazine and Kolaviron. The rats were provided with rat pellets purchased from Ladokun feeds Nig. Limited, and provided water *ad libitum*. The animals were handled humanely following the standard recommendations in the Guide for the Care and Use of Laboratory Animals, and Animal Care and Use as approved by the University of Ibadan Institutional Animal Care and Use Ethics Committee (UI-ACUREC/031-0421/28).

2.4 Study Design

As shown in Figure 1, sixty (60) male Wistar rats (250g-300g) were distributed randomly to a group of 5 groups with twelve (12) rats in each group, and orally administered corn oil (2ml kg^{-1}) (control), kolaviron (200 mg kg^{-1}) (KV only), atrazine (100 mg/kg) (ATZ only), and a combination of atrazine (100 mg kg^{-1}) and kolaviron (100 and 200 mg kg^{-1}) (ATZ+KV1 and ATZ+KV2) for 14 consecutive days.

2.5 Methods

The homogenized liver and kidney were subjected to centrifugation for 10 minutes at 12,000 g at 4°C to isolate the post-mitochondrial fraction. The supernatant was collected and analyzed to assess the antioxidant status, and oxido-inflammatory biomarkers. Further, quantification of

hepato-renal protein concentration was carried out by Bradford assay with Coomassie Brilliant Blue (Bradford, 1976).

2.5.1 Assessment of Liver Function Markers

Serum liver enzyme markers AST, and ALT activity was ascertained using standard assay kits following the directions stipulated by the manufacturer. Shortly, a buffer/enzyme solution was mixed to obtain a working reagent wherein 20 µL the serum specimen was added to a portion (200 µL), and absorbance was recorded at 380 nm after 1 and 2 minutes. Enzyme activity was calculated from the change in absorbance and expressed in U/L.

2.5.2 Assessment of Kidney Function Markers

Serum urea and creatinine concentration were quantified with the aid of a RANDOX diagnostic kits, following the instructional manual outlined by the manufacturer. Briefly, for serum urea, an aliquot (10 µL) of the serum specimen was incorporated with a portion of the working reagent (1 mL), followed by the absorbance measurement at 600 nm against within 60 minutes. Urea concentration was calculated relative to the standard and expressed in mg/dL. Serum creatinine quantification utilized a combination of serum sample (50 µL) and the working reagent (500 µL) prepared from equal volumes of picric acid and sodium hydroxide. Absorbance was measured at 492 nm at 30 seconds interval for 2 minute. Creatinine concentration was calculated against the standard and expressed in mg/dL.

2.5.3 Assessment of Hepato-renal Antioxidant Parameters

The activity of catalase was evaluated in accordance with the method by Clairborne relying on H_2O_2 (1995). Misra and Fridovich (1972) published protocol was employed for the assessment of superoxide dismutase (SOD) activity. Reduced glutathione (Beutler *et al*. (1974) and Glutathione peroxidase (GPX) activity (Rotruck *et al*., 1973). Total thiol (TSH) levels were measured by determining the concentration of thiol (-SH) groups following the method of Ellman *et al*. (1959).

2.5.4 Assessment of Hepato-renal Oxidative Stress Markers

Hydrogen peroxide (H_2O_2) production was quantified according to Wolff's method (1994). Reactive oxygen and nitrogen species (RONS) production was measured according to the protocol

by Adedara *et al*. (2016). Oxidative damage was determined by estimating the lipid peroxidation product, malondialdehyde, following the method by Varshney and Kale (1990).

2.5.5 Assessment of Hepato-renal Inflammatory Makers

Nitrite was used to quantify NO radical concentration following the Griess reaction method of Greiss *et al*. (1982). The activity of xanthine oxidase was measured (Cos *et al*., 1998). Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation, was determined according to the method by Trush *et al*. (1994).

2.5.6 Determination of Hepato-renal Interleukin 1 beta levels and Caspase-3 by ELISA

Liver and kidney IL-1β and caspase-3 levels were quantified using a rat-specific CASP3 and IL-1β ELISA kit (Sandwich-ELISA principle) from Elabscience (Elabscience Biotechnology Inc, Houston Texas, USA) following the manufacturer's instructions. 100 µL of liver/kidney homogenate or standard was introduced to pre-coated standard wells and subjected to incubation at 37 °C for 1 hour 30 minutes. After washing, 100 µL of biotinylated detection antibody was added and incubated, while maintaining standard condition (1 hour at 37 °C). Following urther washing, 100 µl horseradish peroxidase (HRP) conjugate was added. Liquid was aspirated from each well after incubation and each well washed 5 times. Afterwards, there was addition of substrate reagent (90 µl) and further incubation at optimal condition (15 min at 37^0C). Finally, with the addition of a stop solution (50 µl), the reaction was halted and the color change was quantified at 540 nm and expressed in pg/ml.

2.6 Examination of Hepato-renal histological architecture in rats treated with Kv and exposed to ATZ

A regular fixative (Formalin) was used to wash hepatic and renal tissues for maintaining their cell integrity. Dehydration process was carried out through ascending alcohol levels in the form of 70%, 80%, 90%, and 100% ethanol. Tissues were embedded in paraffin wax after the process of dehydration. Thin slices of 4-5 µm were prepared by using an automatic microtome. Hematoxylin/Eosin staining process was done on samples of tissues. Prepared slides were examined under the magnification of 400× on a Leica DM 500 digital light microscope. Micrographs were recorded to study the effects of ATZ and KV on the liver and kidneys.

2.7 Immunohistochemical analyses of hepato-renal NF-κB, COX-2, Bcl-2, Bax, LC3-II, and Beclin-1

The immunohistochemical analyses of Bax, Bcl-2, COX-2, Beclin-1, LC3-II, and NF-κB were performed following animal perfusion with phosphate-buffered saline (PBS) and fixation with 4% buffered formalin. They were further sectioned and subjected to immunohistochemical staining. Stained slides were examined under a Leica DM 500 digital light microscope at 400× magnification, and quantitative analyses were carried out using Fiji-ImageJ software.

2.8 Statistical evaluations

3.0 Results

Data analysis was carried out using GraphPad Prism 8.0 with results presented in Mean ± SEM form. One way ANOVA followed by Tukey's post hoc test was used in comparing the groups. Significance was set at $p \leq 0.05$.

3.1 Protective influence of KV on the liver and kidney function indices in ATZ-induced hepatorenal injury

In this study it was observed that atrazine resulted in an upsurge in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity when compared to the control and KV-treated group. However, treatment with KV significantly caused a dose-dependent diminution (at $p<0.05$) in the activity of ALT and AST in the liver of the co-treated groups (ATZ+KV1 and ATZ+KV2) in comparison with the control and ATZ exposed group as shown in Figure 2a and Figure 2b.

Also, atrazine intoxication resulted in a significant increase ($p< 0.05$) in urea and creatinine levels in the kidney when compared to the control and KV-treated group. However in comparison to the ATZ group, KV was able to bring about a dose-dependent significant diminution at $p< 0.05$ in the level of urea and creatinine in the kidney of rats in ATZ+KV1 and ATZ+KV2 groups (Figure 2c and Figure 2d).

3.2 Protective influence of KV on the antioxidant status of the liver and kidney in ATZ-induced hepatorenal injury

From this study, it was observed that atrazine intoxication caused a significant ($p< 0.05$) diminution in the antioxidant status (Catalase, SOD, GPx, GSH, TSH) of the liver and kidney in the ATZ group of rats when compared to the control and KV-treated group (Figure 3). Co-treatment with KV however, significantly resulted in a dose-dependent upsurge in the antioxidant status of rats in the ATZ+KV1 and ATZ+KV2 groups when compared to the ATZ-alone group at $p<0.05$ as shown in figure 3a-e.

3.3 Protective influence of KV on the oxido-inflammatory indices of the liver and kidney in ATZ-induced hepatorenal injury

From this study, it was observed that atrazine intoxication caused a significant ($p< 0.05$) upsurge in the levels and activities of oxidative stress (MDA, RONS, H_2O_2) as shown in Figures 4a-c, and inflammatory indices (NO, MPO, XO, IL-1β, NF-κβ, COX2) as shown in Figures 5a-d and Figures 7a and b of the liver and kidney in ATZ group when compared to the control and KV-treated group. However, it was also observed that in comparison with to the ATZ-alone group, co-treatment with KV in the (ATZ+KV1 and ATZ+KV2) groups significantly resulted in a dose-dependent diminution in these oxido-inflammatory indices.

3.4 Histological assessment on ATZ-induced hepatorenal injury

Figure 6a and b is a representative photomicrograph showing the impact of ATZ and KV on the liver and kidney of male Wistar rats at a magnification of (400×) using H and E stain. It can be seen that in the control and KV alone groups there was no visible lesion in both the kidney and liver. However, the atrazine treated group for the liver showed necrosis of hepatocytes while in the kidney there was degradation of renal tubules. However, the groups co-treated with ATZ and KV (ATZ+KV1 and ATZ+KV2) showed a near normal histological architecture when compared with the control in both the kidney and liver.

3.5 Impact of ATZ and KV on the apoptotic markers in the liver and kidney

In a bid to understand the impact of ATZ and KV on the apoptotic markers of the liver and kidney, the following markers were explored including Bcl-2-Associated –X Protein (Bax), B-Cell Lymphoma (Bcl-2) immunostains, and caspase-3 activity. It was observed that in both the control and KV there was a marked diminution in the expression of Bax in the liver and kidney. However, there was an upsurge in the expression of Bax in the ATZ exposed group in the liver and kidney.

While the groups co-treated with ATZ and KV (ATZ+KV1 and ATZ+KV2) showed a marked diminution in the expression of Bax in the liver and kidney (Figure 8b). However, BCl2 expression was explored and it was shown that there was an increase in BCl2 expression in the control and KV groups of the liver and kidney. However, there was a diminution in the expression of BCl2 in the ATZ group in the liver and kidney. While there was an upsurge in the expression of BCl2 in the co-treated group (ATZ+KV1 and ATZ+KV2) in both the liver and kidney (Figure 8a). Also, there was a diminution in caspase-3 activity in the control and KV of the liver and kidney. However, there was an upsurge in the caspase-3 activity of ATZ treated groups in both the liver and kidney. Evidently, there was a significant diminution in caspase-3 activity in the co-treated groups (ATZ+KV1 and ATZ+KV2) in both the liver and kidney (Figure 9a and b).

3.6 Impact of ATZ and KV on the autophagic markers in the liver and kidney

In this study it was observed that there was a decreased LC3-II and Beclin-1 expression in the kidneys and livers of the control group and KV groups. However, the ATZ group alone showed a marked increase in Beclin-1 and LC3 expression in the kidney and liver when compared to the control and KV treated group. While a diminution in hepato-renal Beclin-1 and LC3 expression was observed in the co-treated groups (ATZ+KV1 and ATZ+KV2) when compared to the ATZ group alone (Figures 10a and b).

4.0 Discussion

The extensive use of atrazine in agriculture has been attributed to its effect on herb plants (Almberg et al., 2018). This is a family of triazine compounds whose endocrine-disrupting ability is highly recognized. Humans inevitably are exposed to ATZ through contaminated water and food which directly damage the body organs (Ochoa-Acuna *et al*., 2009). Studies demonstrated that ATZ exposure remarkably damaged hepatic tissues (Harper *et al*., 2020). Consequently, the positive effects of kolaviron, a natural biflavonoid obtained from the seeds of Garcinia kola, have been largely attributed to its antioxidant properties **(**Alabi and Akomolafe, 2020).

In this present study it was observed that ATZ caused an increase in the activities of liver function marker enzymes, including AST and ALT when compared with the control and KV. This result corroborates the findings that demonstrated that there was an upsurge in ALT and AST activity in ATZ challenged rats which is indicative of impairment in liver function (Chang *et al*., 2021; Ali *et al*., 2022; Owumi *et al*., 2025). However, co-treatment with KV reversed these effects as a result

of exposure to ATZ in the liver. This supports the findings that KV was able to cause a diminution in these liver function markers (Adaramoye *et al*., 2016; Alabi and Akomolafe, 2020; Awogbindin *et al*., 2021).

Notably, blood urea nitrogen (BUN) and creatinine are both valuable indices for the assessment of kidney function (Abarikwu, 2014; Liu *et al*., 2014). Evidently, it was also observed that in the ATZ alone- treated rats, blood urea nitrogen (BUN) and creatinine concentrations were significantly increased when compared to the control values and KV groups indicating a probable renal injury and decline in renal function due to ATZ exposure. Our data are in consonance with earlier studies that ATZ elicited an elevation in BUN and creatinine levels (Chang *et al*., 2021; Owumi *et al*., 2025). However, there was a diminution in creatinine and BUN levels in the group co-administered with KV. Previous studies showed that KV resulted in a decrease in the kidney function markers (Adaramoye *et al*., 2016; Alabi and Akomolafe, 2020; Awogbindin *et al*., 2021), which support the findings in the current study.

Biological cells are endowed with antioxidant defense system which detoxifies reactive oxygen and nitrogen species, and this system consists of antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST), glutathione peroxidase (GPx) and reduced glutathione (GSH) among others (Poprac *et al*., 2017; Vona *et al*., 2021). The result showed that the group challenged with ATZ in both the liver and kidney elicited a decrease in antioxidant status including SOD, CAT, GPx, GST, and GSH when compared with the control and KV alone groups. Earlier reports demonstrated that there was a diminution in antioxidant status in the liver and kidney of rats challenged with ATZ (Edwards *et al*., 2024; Owumi *et al*., 2025) as well as alteration in the antioxidant activities in the liver of Hyline brown laying hens (Li *et al*., 2025). The apparent protective effect of KV against ATZ-induced hepatorenal toxicity in this study corroborates the investigation that demonstrated the ameliorative effect of KV in diclofenac-induced liver and kidney toxicity in wistar rats by suppressing inflammatory events, and upregulating antioxidant defenses (Alabi and Akomolafe, 2020).

Furthermore, exposure of rats to ATZ significantly increased the markers of oxidative stress, such as MDA, RONS, and H_2O_2 in the hepatic and renal tissues, indicating increased lipid peroxidation and ROS generation. It is known from the previous literature that ATZ is metabolized in vivo to generate significant amounts of ROS and decrease natural antioxidants in vivo (Ikeji *et al*., 2023;

Owumi *et al*., 2025). Therefore, it increases oxidative stress, resulting in lipid peroxidation, membrane damage, and ultimately affecting the biological functions of the body. The liver and kidney have specific metabolic activities related to xenobiotics; thus, they are vulnerable to oxidative stress-induced toxicity (Wang *et al*., 2025). The significant reduction in oxidative stress markers after KV treatment indicates that KV can be used as an excellent source of antioxidants, similar to other phytochemicals. In corroboration with the present finding, Owumi *et al* (2025) reported an increase in oxidative stress markers in the liver and kidney of rats challenged with ATZ, while Ikeji *et al*. (2023) reported elevated levels of LPO, RONS, and H_2O_2. However, co-administration with KV resulted in a diminution in the oxidative indices in the liver and kidney of rats challenged with ATZ, which is in agreement with previous in vivo on the anti-oxidative effects of kolaviron in the breast, brain, and testes of experimental animals (Abarikwu *et al*., 2022; Attah *et al*., 2022; Tesi *et al*., 2022).

Furthermore, oxidative imbalance induced by ATZ was characterized by the induction of various inflammatory mediators, such as NO, MPO, XO, IL-1β, NF-κB, and COX-2 as reported. These results support established links between oxidative stress and induction of inflammation via ROS activation of the NF-κB pathway, resulting in cytokine and enzyme production. Myeloperoxidase (MPO), a heme protein which catalyzes the oxidation of chloride ions by H_2O_2 to generate hypochlorous acid, is well-known to play a pivotal role in oxidative stress and inflammation (Wilkie-Grantham *et al*., 2015; Davies and Hawkins, 2020). The elevation in MPO activity indicated neutrophil recruitment to the site of injury, whereas higher levels of XO suggested a sustained ROS production, leading to a viscious cycle of oxidative damage and inflammation. Extreme production of nitric oxide (NO), a signaling molecule which contributes to the pathogenesis of inflammation, has been reported to induce oxidative and nitrative stress through peroxynitrite formation, thus resulting in cellular damage (Guzik *et al*., 2003; Kim and Lee, 2025). Interleukin 1-beta (IL-1β) is a pro-inflammatory cytokine involved in systemic inflammation and apoptosis (Baud and Karin, 2001; Kaneko *et al*., 2019; Boraschi, 2022). Similar observations have been made in previous studies on the effects of ATZ in hepatic and renal tissues of experimental models, with the persistence of inflammatory cascades resulting in greater cellular damage (Edwards *et al*., 2024; Owumi *et al*., 2025). Nonetheless KV co-treatment markedly reduced all inflammatory parameters measured, implying inhibition of NF-κB activation and its downstream mediators. These anti-inflammatory properties of KV have been previously observed in both in

vivo and in vitro studies examining the potential of natural compounds in regulating cytokine production and inflammatory enzyme activity (Awogbindin *et al*., 2021).

Histo-pathological assessment showed that the control and KV treated groups had normal architecture of the liver and kidneys, without any lesions observed in both organs. On the other hand, treatment with ATZ caused significant deleterious changes in the architecture of the liver and kidneys with hepatocytes having necrosis while renal tubules degenerated in the kidneys. These results have been confirmed previously with reports indicating that ATZ causes toxicity of hepatocytes and renal tubules leading to structural damage in these tissues (Chang *et al.,* 2021; Ali *et al.,* 2022). It was evident, however, that co-treatment of ATZ and KV had significantly maintained the architectural structures of both tissues as the structure was nearly similar to that of the control animals. This supports the findings that demonstrated the ameliorative potential of KV in the liver and kidney (Okafor *et al*., 2022).

Another hallmark of ATZ toxicity in this study is the dysregulation of apoptosis. Elevated levels of BAX protein, with a concomitant decrease in the expression of Bcl-2, with heightened caspase-3 activity, suggest that the mitochondrial apoptotic pathway was triggered in the liver and kidney tissues of the experimental animals. These results reflect those earlier studies that observed that oxidative stress alters the integrity of mitochondrial membranes, causing release of cytochrome c and eventually activating the caspase pathway in atrazine model (Abarikwu and Farombi, 2014; Zhu *et al*., 2021). The increase in BAX/Bcl-2 ratio is an indication of cell death, which results in the alteration of the structure and function of hepatocytes and nephrons, as increased BAX/Bcl-2 ratio is a well-established marker of apoptosis (Nkpaa *et al*., 2019, Farombi *et al*., 2023; Liu *et al*., 2024; Owumi *et al*., 2025). Notably, KV treatment was able to reverse this process through a reduction in BAX, elevation of Bcl-2 levels, and inhibition of caspase-3 activity, indicating prevention of cell death and maintenance of mitochondrial integrity. This protective effect of KV has been similarly shown in other studies for its ability to inhibit apoptosis (Tesi *et al*., 2022; Farombi *et al*., 2023).

Apart from triggering apoptosis, ATZ caused substantial changes in the hepatic and renal autophagic activity, which was confirmed by high levels of Beclin-1 and LC3-II in both the liver and kidney. Autophagy is a crucial cellular function required to maintain homeostasis in response

to stress; nevertheless, excessive stimulation can result in cellular damage. The increase in these proteins signifies that ATZ induces autophagic activity, possibly as a means of adapting to the accumulation of damaged organelles and protein due to oxidative stress. It has been reported that xenobiotic-mediated ROS activates autophagic activity via mechanisms mediated by AMPK activation and mTOR inhibition. Nevertheless, continued or uncontrolled autophagy can lead to cellular damage (Chen *et al*., 2023). Surprisingly, KV administration decreased the expression of Beclin-1 and LC3-II, suggesting autophagic activity normalization. The findings suggest that apart from attenuating oxidative stress, KV inhibits excessive autophagic activity, thus maintaining hepatic and renal cellular homeostasis.

5.0 Conclusion

Conclusively, the findings from this investigation demonstrated the ameliorative role of Kolaviron in hepatorenal injuries associated with Atrazine administration in Wistar rats. The protective effects of kolaviron were characterized by improvement of oxido-inflammatory parameters, antioxidative status, hepatorenal function markers and tissue structures, as well as autophagy markers, as depicted in Figure 11. The capacity of KV to regulate all these mechanisms indicates the pleiotropic nature of its pharmacological mechanism of action, which is now viewed as a crucial aspect in intervention against such herbicide-related toxicities.

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There was no funding for the work

Legends to Figures

Figure 1: Depiction of experimental design, including the treatment regimen and duration, used in this study

Figure 2: KV ameliorates ATZ-induced hepatotoxicity by suppressing the activity of ALT and AST in the liver. Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated "a" for variations compared to the control, "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 3: KV ameliorates ATZ-induced renal toxicity by causing a diminution in the level of urea and creatinine in the kidney. Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 4: KV ameliorates ATZ-induced hepatorenal toxicity by causing an upsurge in the activities of the antioxidant parameters including (a) catalase, (b) superoxide dismutase (SOD), (c) glutathione peroxidase (GPx), (d) reduced glutathione (GSH), and (e) total thiol (TSH). Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 5: KV ameliorates ATZ-induced hepatorenal toxicity by causing a diminution in the oxido-inflammatory parameters including (a) malondialdehyde (MDA), (b) reactive oxygen and nitrogen species (RONS), (c) hydrogen peroxide (H_2O_2), (d) nitric oxide (NO), and (e) myeloperoxidase (MPO), xanthine oxidase (XO). Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 6: Representative photomicrograph showing the impact of ATZ and KV on the liver and kidney at a magnification of (400×) using H and E stain. Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 7: Representative photomicrograph showing the impact of ATZ and KV on the liver and kidney at a magnification of (400×) using nuclear factor kappa B (NF-kB), cyclooxygenase-2 (COX-2) immunostains and interleukin 1 beta (IL-1β). Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 8: Representative photomicrograph showing the impact of ATZ and KV on the liver and kidney at a magnification of (400×) using Bcl-2-Associated –X Protein (Bax), B-Cell Lymphoma (Bcl-2) immunostains. Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 9: KV ameliorates ATZ-induced hepatorenal caspase 3 activity in male Wistar rats. Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$.

Figure 10: Representative photomicrograph showing the impact of ATZ and KV on the liver and kidney at a magnification of (400×) using Microtubule-Associated Protein 1 Light Chain 3 (Lc3-II), and Beclin-1 immunostains Variables are presented as mean ± SEM with a sample size of seven (n = 7). Statistical significance is indicated by "a" for variations compared to the control, "b" "b" for variations compared to the atrazine group with $\alpha < 0.05$..

Figure 11: Cartoon illustration of the proposed mechanism of protection by Kv in this study.

Figure 1

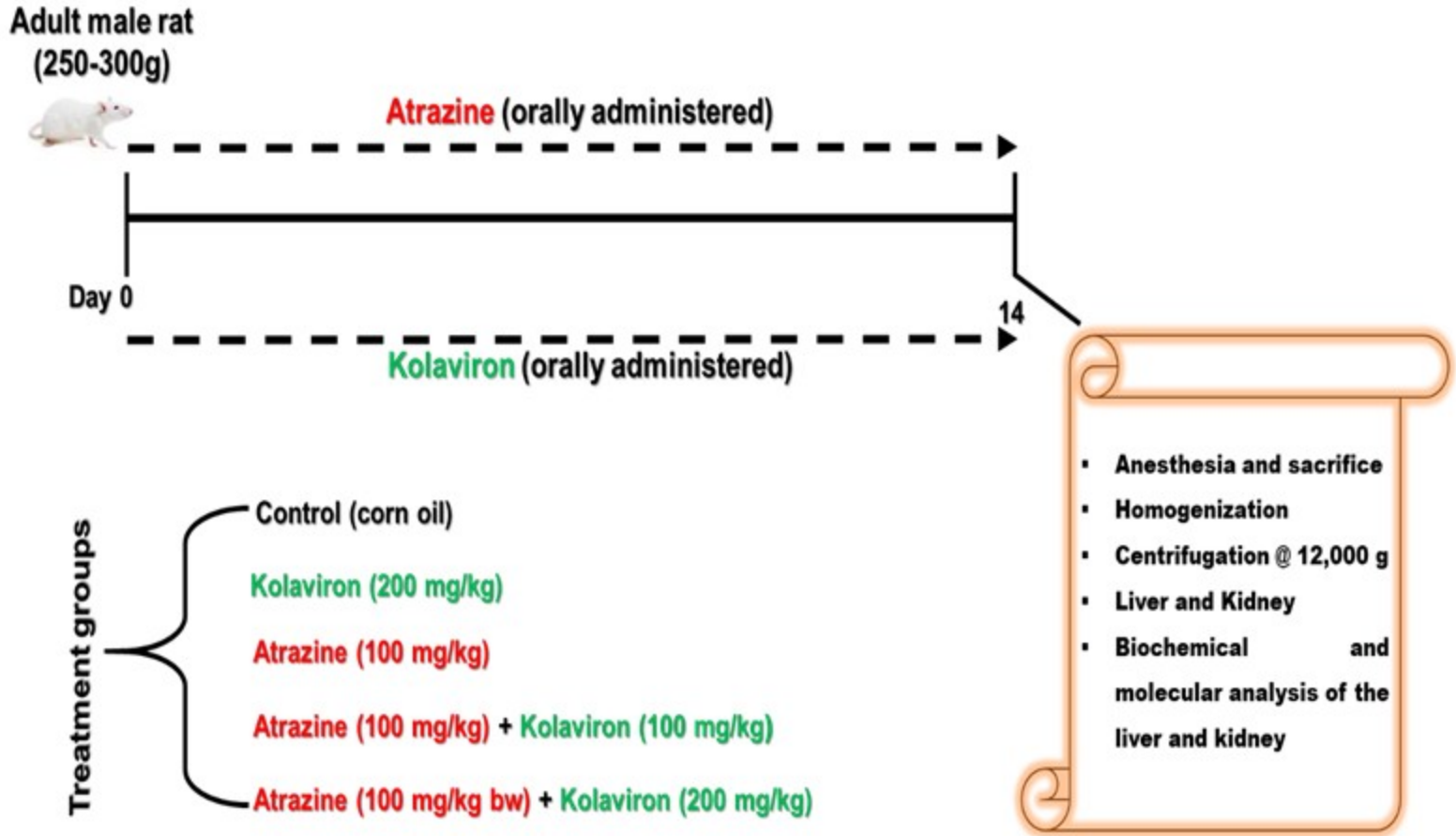

Adult male rat
(250-300g)
Atrazine (orally administered)
Day 0
14
Kolaviron (orally administered)
Treatment groups
Control (corn oil)
Kolaviron (200 mg/kg)
Atrazine (100 mg/kg)
Atrazine (100 mg/kg) + Kolaviron (100 mg/kg)
Atrazine (100 mg/kg bw) + Kolaviron (200 mg/kg)
Anesthesia and sacrifice
Homogenization
Centrifugation @ 12,000 g
Liver and Kidney
Biochemical and molecular analysis of the liver and kidney

Figure 2

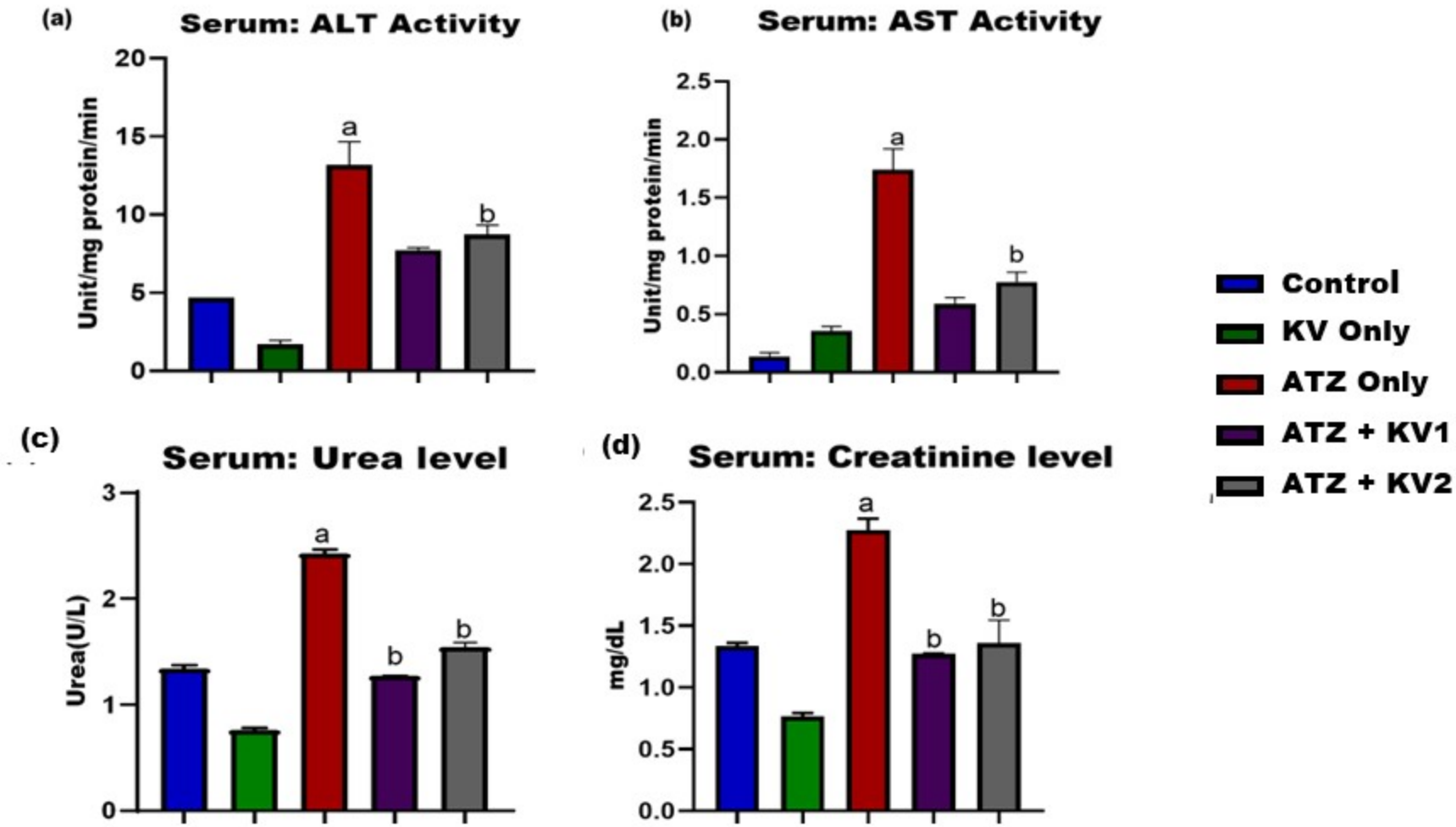

(a)
Serum: ALT Activity
Unit/mg protein/min
20
15
10
5
0
a
b
(b)
Serum: AST Activity
Unit/mg protein/min
2.5
2.0
1.5
1.0
0.5
0.0
a
b
(c)
Serum: Urea level
Urea(U/L)
3
2
1
0
a
b
b
(d)
Serum: Creatinine level
mg/dL
2.5
2.0
1.5
1.0
0.5
0.0
a
b
b
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2

Figure 3

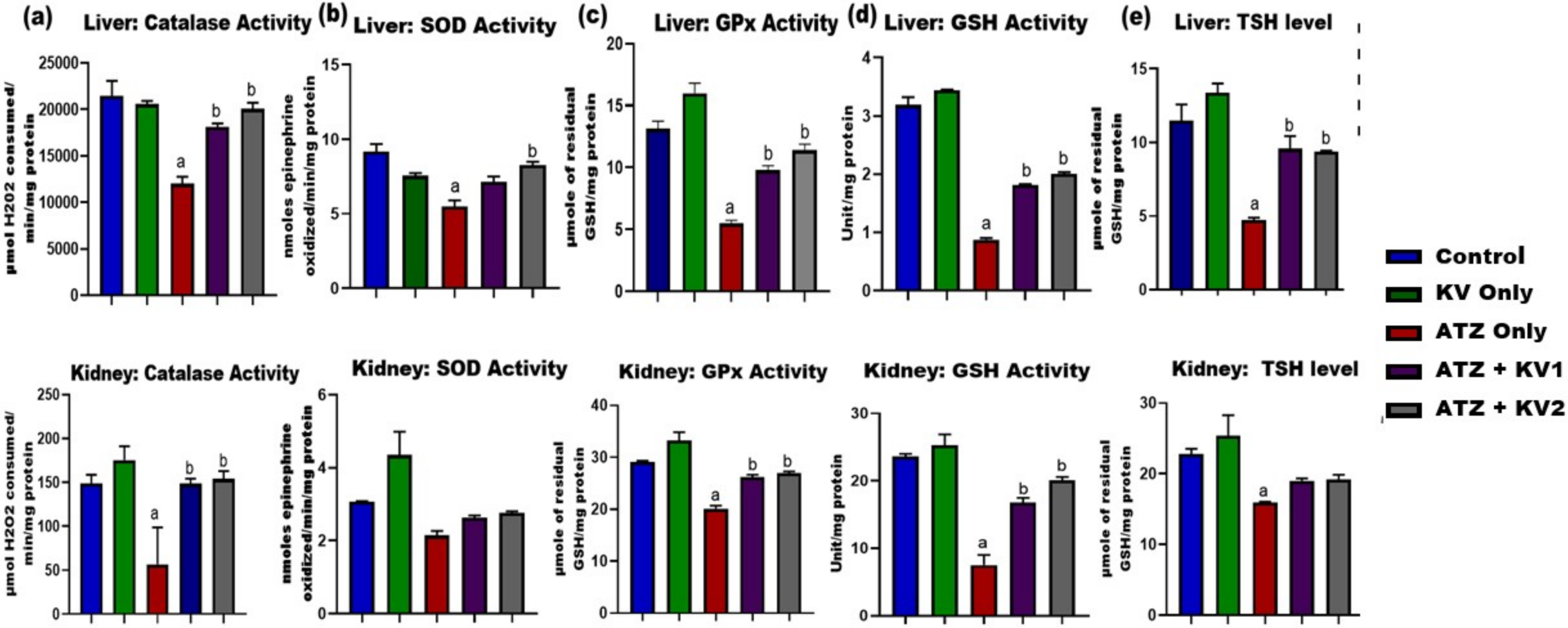

(a) Liver: Catalase Activity
µmol H2O2 consumed/ min/mg protein
(b) Liver: SOD Activity
nmoles epinephrine oxidized/min/mg protein
(c) Liver: GPx Activity
µmole of residual GSH/mg protein
(d) Liver: GSH Activity
Unit/mg protein
(e) Liver: TSH level
µmole of residual GSH/mg protein
Kidney: Catalase Activity
µmol H2O2 consumed/ min/mg protein
Kidney: SOD Activity
nmoles epinephrine oxidized/min/mg protein
Kidney: GPx Activity
µmole of residual GSH/mg protein
Kidney: GSH Activity
Unit/mg protein
Kidney: TSH level
µmole of residual GSH/mg protein
a
b
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2

Figure 4

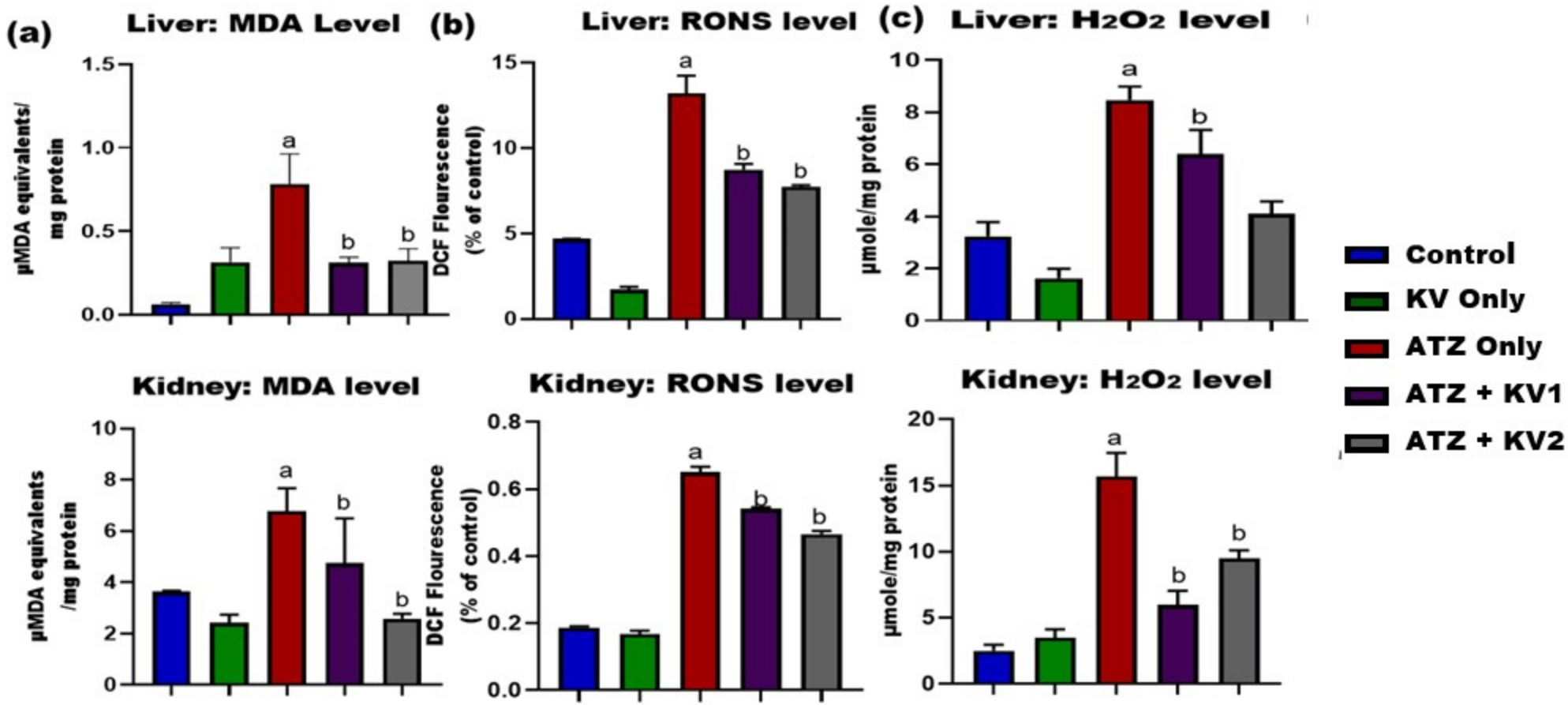

(a)
Liver: MDA Level
µMDA equivalents/ mg protein
(b)
Liver: RONS level
DCF Flourescence (% of control)
(c)
Liver: H2O2 level
µmole/mg protein
Kidney: MDA level
µMDA equivalents /mg protein
Kidney: RONS level
DCF Flourescence (% of control)
Kidney: H2O2 level
µmole/mg protein
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2

Figure 5

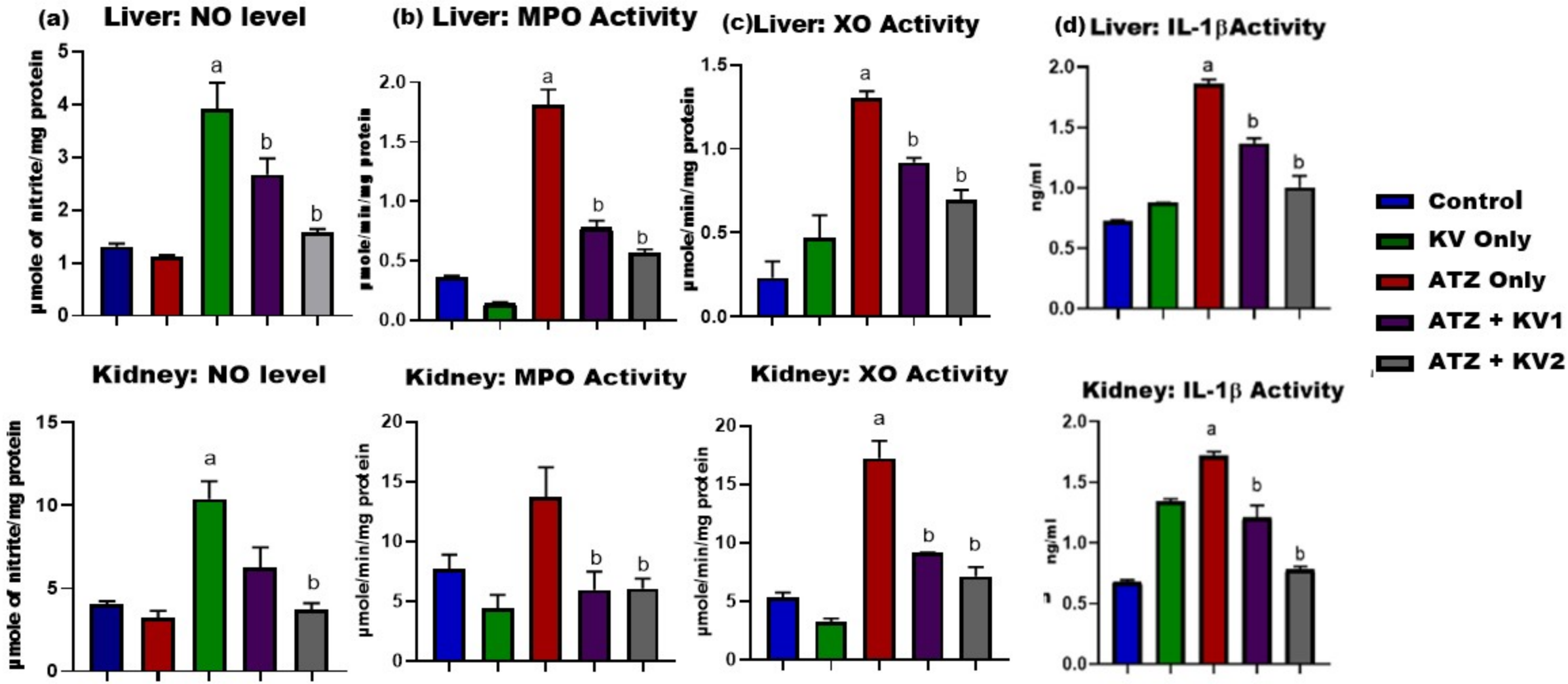

(a) Liver: NO level
µmole of nitrite/mg protein
(b) Liver: MPO Activity
µmole/min/mg protein
(c)Liver: XO Activity
µmole/min/mg protein
(d) Liver: IL-1βActivity
ng/ml
Kidney: NO level
µmole of nitrite/mg protein
Kidney: MPO Activity
µmole/min/mg protein
Kidney: XO Activity
µmole/min/mg protein
Kidney: IL-1β Activity
ng/ml
a
b
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2

Figure 6

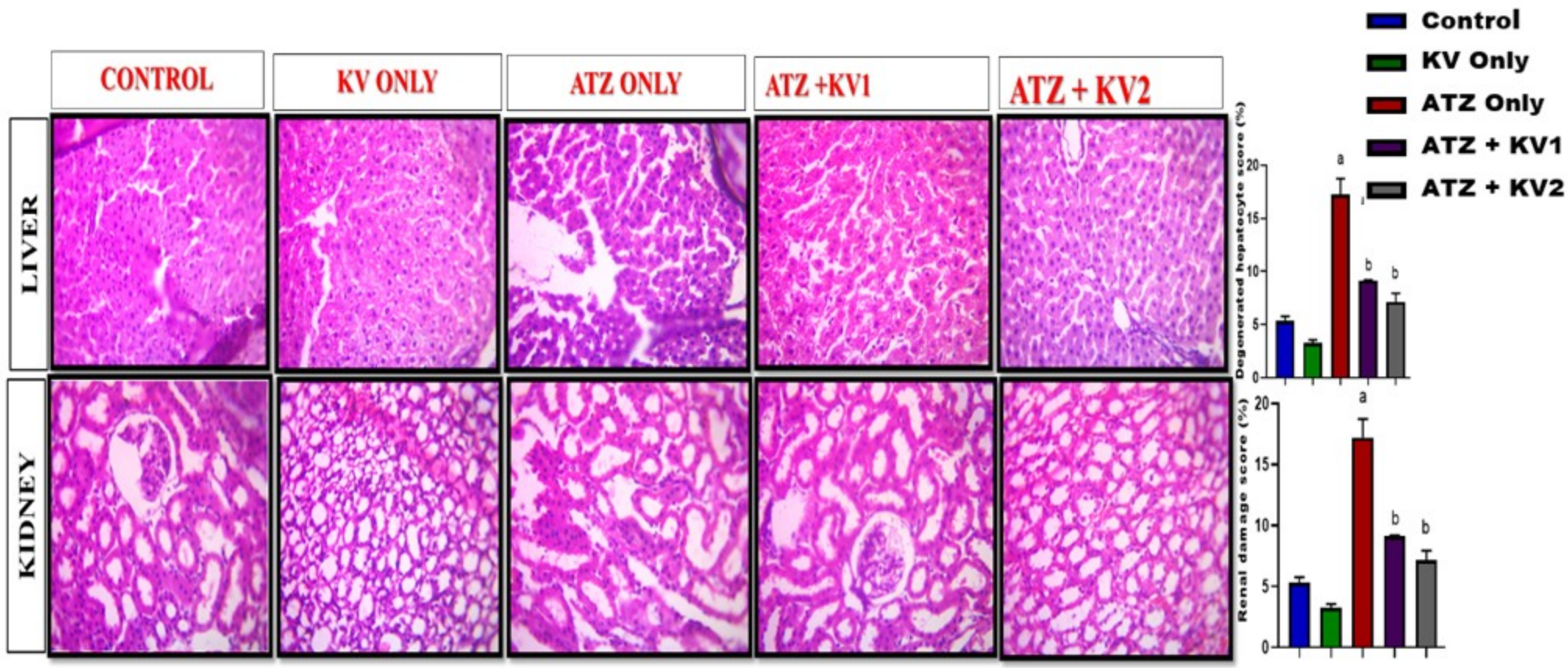

CONTROL
KV ONLY
ATZ ONLY
ATZ +KV1
ATZ + KV2
LIVER
KIDNEY
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2
Degenerated hepatocyte score (%)
Renal damage score (%)
a
b
b
0
5
10
15
20

Figure 7

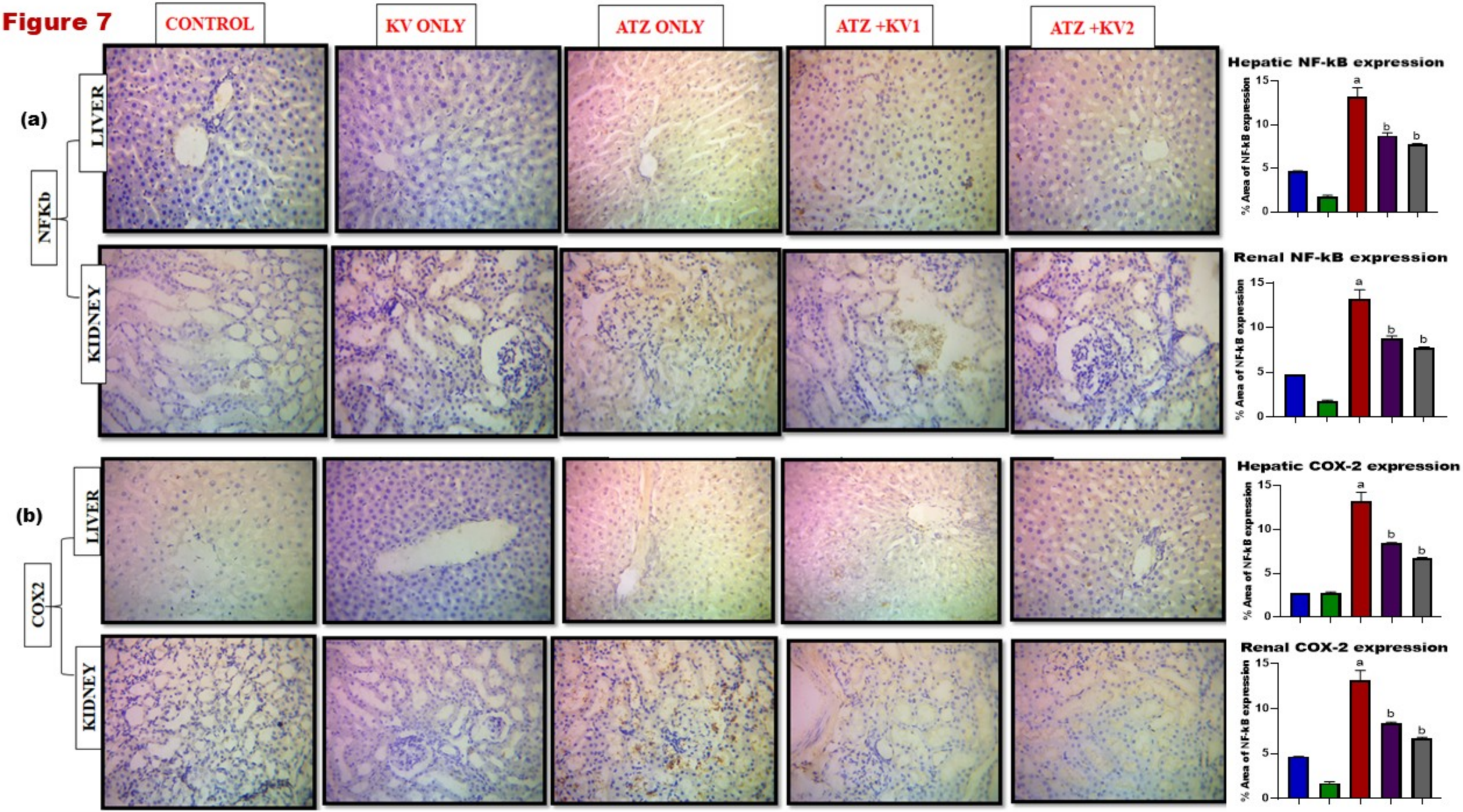

CONTROL
KV ONLY
ATZ ONLY
ATZ +KV1
ATZ +KV2
(a)
NFKb
LIVER
KIDNEY
Hepatic NF-kB expression
Renal NF-kB expression
% Area of NF-kB expression
(b)
COX2
LIVER
KIDNEY
Hepatic COX-2 expression
Renal COX-2 expression
% Area of NF-kB expression
a
b
b

Figure 8

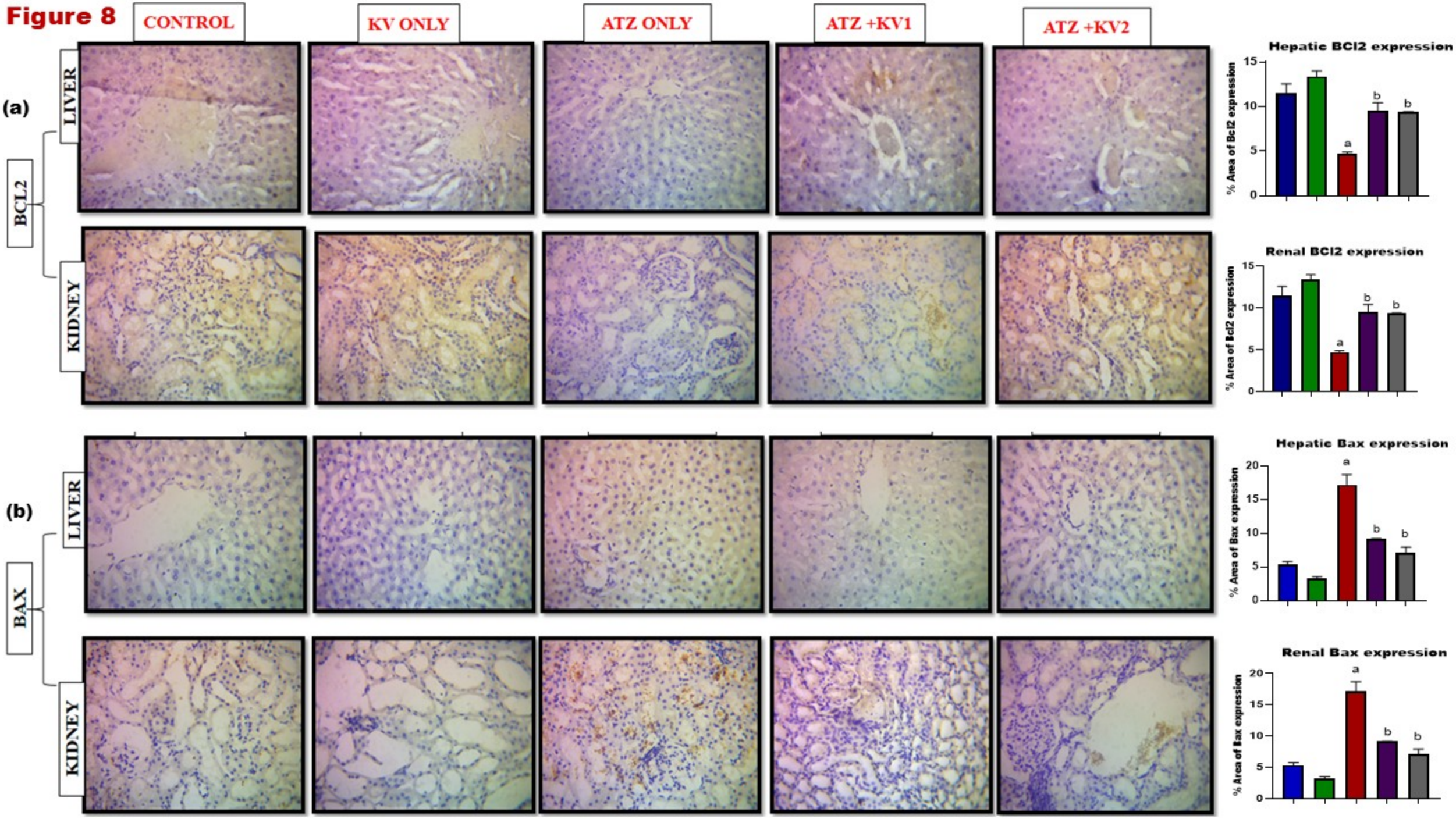

CONTROL
KV ONLY
ATZ ONLY
ATZ +KV1
ATZ +KV2
(a)
BCL2
LIVER
KIDNEY
Hepatic BCl2 expression
Renal BCl2 expression
% Area of Bcl2 expression
(b)
BAX
LIVER
KIDNEY
Hepatic Bax expression
Renal Bax expression
% Area of Bax expression
a
b

Figure 9

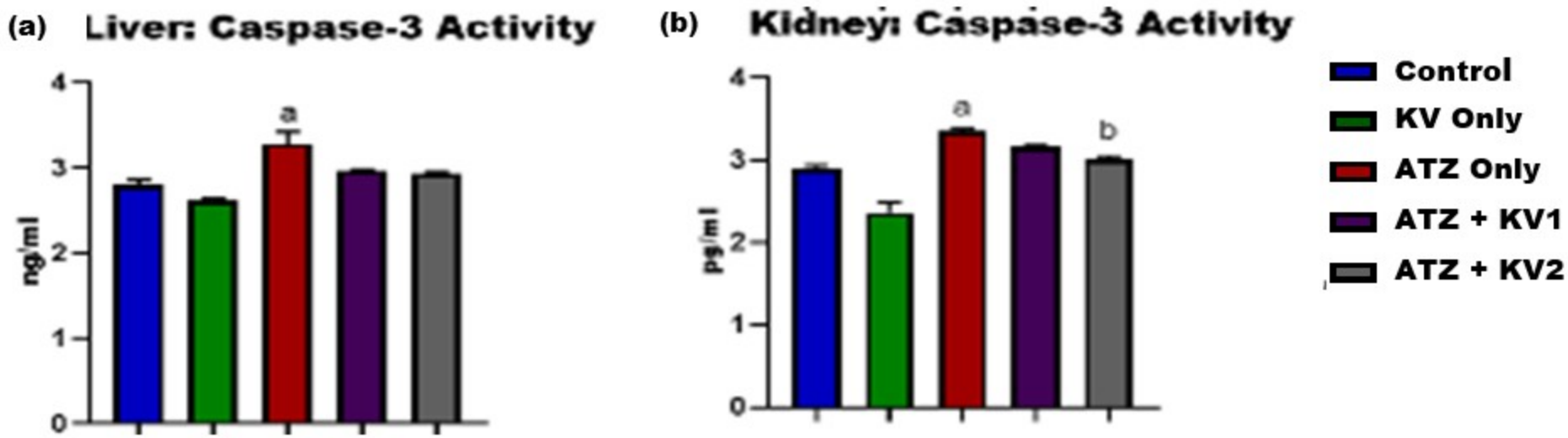

(a) Liver: Caspase-3 Activity
ng/ml
0
1
2
3
4
a
(b) Kidney: Caspase-3 Activity
pg/ml
0
1
2
3
4
a
b
Control
KV Only
ATZ Only
ATZ + KV1
ATZ + KV2

Figure 10

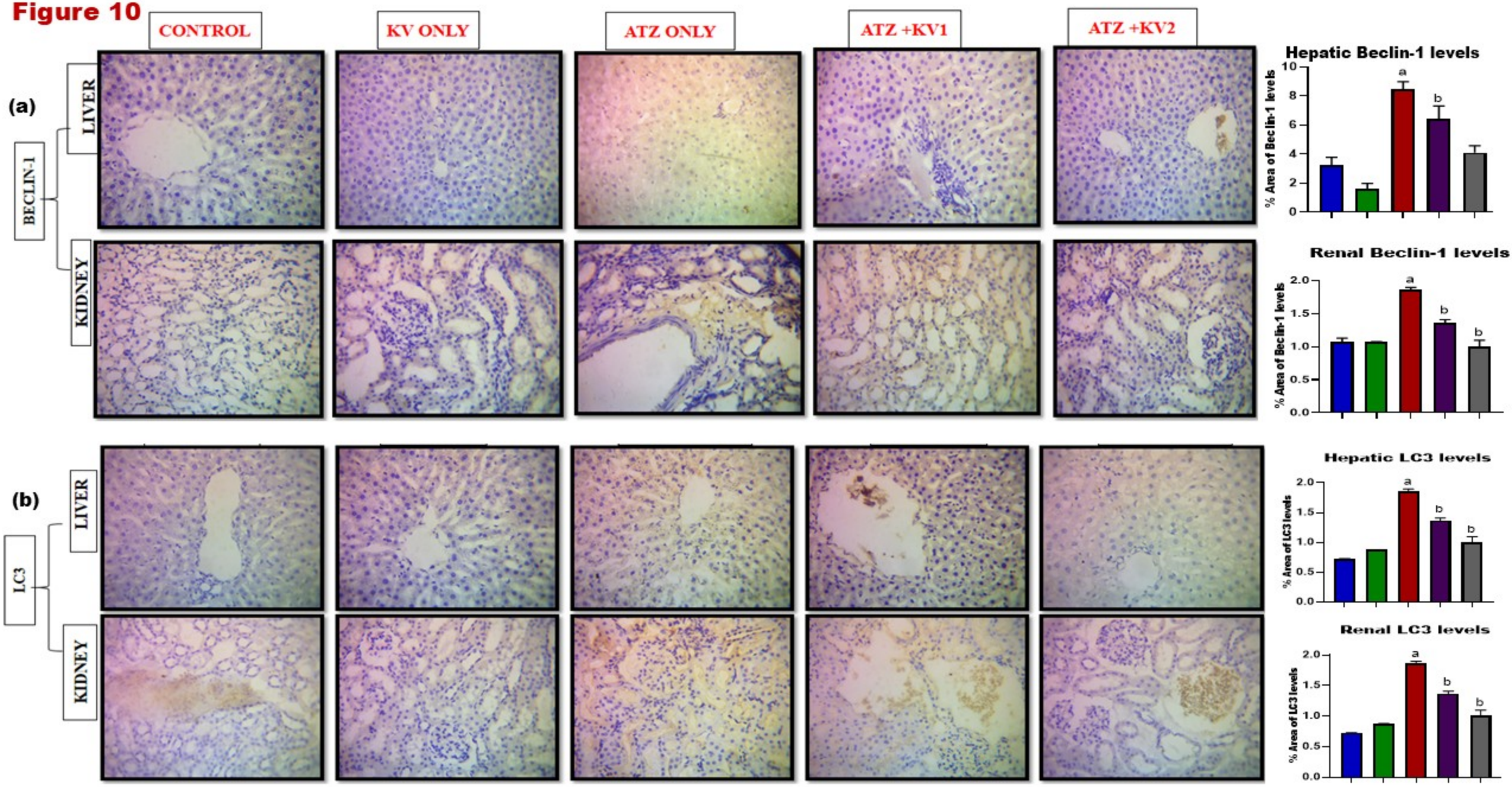
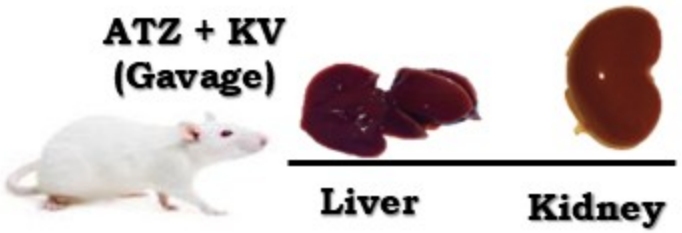

CONTROL
KV ONLY
ATZ ONLY
ATZ +KV1
ATZ +KV2
(a)
BECLIN-1
LIVER
KIDNEY
Hepatic Beclin-1 levels
% Area of Beclin-1 levels
Renal Beclin-1 levels
% Area of Beclin-1 levels
(b)
LC3
LIVER
KIDNEY
Hepatic LC3 levels
% Area of LC3 levels
Renal LC3 levels
% Area of LC3 levels

Figure 11



Kidney Function Markers ↓
(Urea, Creatinine)

Liver Function Markers ↓
(AST, ALT)

Antioxidant ↑
(CAT, SOD, GPx, GSH, TSH)

Oxidative stress ↓
(MDA, H_2O_2, RONS)

Inflammation ↓
(XO, NO,MPO,IL-1β, NFκβ, COX2)

Apoptotic Markers ↑↓
(BCl2↑, Bax , Caspase-3↓)

Autophagic Markers ↓
(Beclin-1, LC3)