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Research Article

Ethanollic extract of *Jatropha gossypifolia* exacerbates Potassium Bromate-induced clastogenicity, hepatotoxicity, and lipid peroxidation in rats

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ABSTRACT: Extracts of *J. gossypifolia* L. have been reported to have several medicinal values, including potential anti-cancer and anti-inflammatory properties. In this study, we investigated the anti-clastogenic and hepatoprotective, effects of the ethanollic leaf extract of *J. gossypifolia* L. in potassium bromate (KBrO₃)-induced toxicity in rats. The general trend of the results indicates significant increases ($p < 0.05$) in mean values when toxicant (KBrO₃) only group is compared with normal control group, except for catalase where a significant decrease ($p < 0.05$) was recorded. Surprisingly, treatment of the toxic effects of KBrO₃ by *J. gossypifolia* did not lower the mean values of any of these parameters investigated. Instead, there were significant increases ($p < 0.05$) in the mean number of bone marrow micronucleated polychromatic erythrocytes (mPCEs), plasma malondialdehyde (MDA) concentration, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase activities, and sodium, while the increases in mean concentrations of creatinine, urea, and potassium were not significant ($p > 0.05$). Also, there was a further reduction in the activity of catalase by *J. gossypifolia* treatment, and was also not significant ($p > 0.05$). We therefore concluded that the ethanollic leaf extract of *J. gossypifolia* may not have a protective role against chromosomal and liver damage in KBrO₃-induced toxicity, but complicating effects.

KEYWORDS: *Jatropha gossypifolia*, KBrO₃, ethanollic extract, toxicity, rats.

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INTRODUCTION

Potassium bromate (KBrO₃) plays an important role as a food additive in bread making, production of fish paste and in fermented beverages. Moreover, it is an important potential contaminant in hypochlorite, and it is also a by-product of ozone used as disinfectant in drinking water, because ozonation of drinking water containing bromide may lead to the formation of bromate (Oduechere and Farombi, 2012). In addition, KBrO₃ is used in cold-wave hair lotion (Moore and Chen, 2006; Nishioka *et al.*, 2006). In Nigeria, and in many parts of the world, use of potassium bromate as bread improver has been banned; however, some bread makers

and bakeries in Nigeria have continued to include potassium bromate in their bread (Ekop *et al.*, 2008). KBrO₃ has been reported to be a potent nephrotoxic agent that can mediate renal oxidative stress, toxicity and tumor response in rats. It also enhances renal lipid peroxidation and hydrogen peroxide formation with reduction in renal antioxidant enzymes (Khan *et al.*, 2004). Also, potassium bromate contributes to the cellular redox status and impairment of membrane protein activities in rats (Farombi *et al.*, 2002).

Proper care must be ensured in the uses and preparations of medicinal plants in order to avoid their indiscriminate use.

One such plant is *J. gossypifolia* Linn (Euphorbiaceae), a bushy gregarious shrub that grows wildly almost throughout India, and South Western part of Nigeria. It has significant anticancer and pesticidal activities (Hartwell, 1969; Chatterjee *et al.*, 1980). Decoction prepared from the leaves of *J. gossypifolia* is used for disinfecting wounds (Labadie *et al.*, 1989). The stem sap stops bleeding and itching of cuts and scratches (Morton, 1968; Morton, 1980). Histamine, apigenin, vitexin, isovitexin and tannins have all been found in *J. gossypifolia* leaves. Jatrophine and jatrodien, an alkaloid and a lignin respectively have been found in the stem of the plant (Matsuse *et al.*, 1999; Omoregbe *et al.*, 1996). A decoction of the bark is used as an emmenagogue, and the leaves have been used for treatment of stomachache, venereal disease and as 'blood purifier' (Kirtikar and Basu, 1996; Banerjee and Das, 1993).

Some studies have been conducted using the aqueous, methanolic and petroleum ether extract of *J. gossypifolia* (Panda *et al.*, 2009; Purohit and Purohit, 2011; Kumari and Roy, 2014). Previous studies on the plant have shown that it can be used as haemostatic agent (Oduola *et al.*, 2005; Oduola *et al.*, 2007), antimicrobial (Seth and Sarin, 2010; Purohit and Purohit, 2011; Kumari and Roy, 2014), anti-inflammatory (Purohit and Purohit, 2011; Nagaharika *et al.*, 2013), antifeedant (Bullangpoti *et al.*, 2012), and antifertility (Jain *et al.*, 2013). Therefore, this present study focused on the use of ethanolic extract of *J. gossypifolia* against KBrO₃-induced clastogenicity, hepatotoxicity, and lipid peroxidation in wistar rats.

MATERIALS AND METHODS

Test materials, kits, and chemicals

KBrO₃ and other chemicals used were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England. Alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine, urea, sodium, and potassium kits were products of Cypress Diagnostics, Langdorp, Belgium.

Plant material

Fresh leaves of *J. gossypifolia* were harvested from a garden not far from the Federal University of Agriculture, Abeokuta. Identification was done at the Biological Science Department of the University. The identified and authenticated leaves were air-dried at room temperature under standard laboratory procedures. The dried sample was pulverized into fine coarse powder with an electric blender to powdery form.

Plant extracts preparation

Three hundred and eighty grams (380 g) of the milled leaves was extracted in 80 % v/v ethanol for 72 hours. The mixture was then filtered and the filtrate was left to evaporate to

dryness under reduced pressure in a rotary evaporator. The dried extract was stored at -20 °C until use. The *J. gossypifolia* ethanolic leaf extract was administered to the rats according to their body weights.

Animals

Twenty (20) Wistar albino rats weighing averagely 200 g were collected from the Department of Veterinary Medicine of our university. The animals were housed in metallic cages in the experimental animal house of the department. They were allowed to acclimatize for two weeks before the commencement of the experiment with 12 h light/dark cycle and temperature of 28 ± 2 °C, and were fed with normal rat chow diet and water *ad libitum*. The permission to use the animals was approved by the Institution's Animal Ethical Committee.

Experimental design

The rats were randomly divided into four groups of five rats each.

Group 1: served as normal control and administered corn oil orally for seven days.

Group 2: received an oral administration of 200 mg/kg KBrO₃ for the first three (3) days orally.

Group 3: received an oral administration of 200 mg/kg KBrO₃ for first 3 days, followed by 300 mg/kg ethanolic extract of *J. gossypifolia* for seven (7) days orally, starting from the first day.

Group 4: received 300 mg/kg ethanolic extract of *J. gossypifolia* for seven (7) days.

All treatments were administered for seven (7) days.

Estimation of biochemical parameters

Twenty four (24) hours after the last administration, animals were anaesthetized using diethyl ether. Blood was collected directly from the abdominal artery with heparinized syringes into clean heparinized tubes.

Preparation of plasma

Plasma was separated by centrifugation at 3000 rpm for 10 minutes, and used for the estimation of various biochemical parameters namely: Alanine Transferase activity (ALT), Aspartate Transferase (AST), Alkaline Phosphatase activity (ALP), Gamma-Glutamyl Transferase activity (GGT), sodium, potassium, creatinine, and urea following the methods described in Cypress Diagnostics Kits, Belgium.

Malondialdehyde (MDA), an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 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 a blank.

MDA was calculated using the molar extinction coefficient for MDA-TBA complex of $1.56 \times 10^5 \text{ M}^{-1}\text{CM}^{-1}$. Catalase was assayed according to the method described by Sinha (1972). The reaction mixture (1.5 mL) contained 0.01 M pH 7.0 phosphate buffer (1.0 mL), tissue homogenate (0.1 mL) and 2 M H₂O₂ (0.4 mL). The reaction was stopped by the addition of 2 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio), followed by heating in boiling water for 10 minutes, then cooling at room temperature, and absorbance was read at 570 nm.

Preparation of bone marrow

The femurs from each of the animals were removed and bone marrow was aspirated with a syringe pin, and microscopic slides were prepared according to Matter and Schmid (1971). The slides were then fixed in absolute methanol (BDH Chemical Ltd, Poole, England), air-dried, pretreated with May-Grunwald solution (Sigma-Aldrich, procedure No GS-10) and air-dried. The dried slides were stained in 5% Giemsa solution, and induced in phosphate buffer 0.01 M (pH 6.8) for 30 seconds. Thereafter, they were rinsed in distilled water, air-dried, and mounted. The slides were scored at x400 magnification under a light microscope for micronucleated polychromatic erythrocytes (mPCEs).

Histological analysis

Liver sections were fixed in an aqueous 10% p-formaldehyde solution and washed in 10 mmol/L phosphate buffer pH 7.4 at 4 °C for 12 hours. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye, and finally observed at x400 magnification under a light microscope.

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.

RESULTS

Micronucleus Assay

The mean number of micronucleated polychromatic erythrocytes (mPCEs) scored in the normal control group was 0.25 ± 0.02 (Figure 1). This value is statistically significant ($p < 0.05$) compared with KBrO₃ only administered group. Treatment with *J. gossypifolia* extract significantly ($p < 0.05$) led to about 44% increase in the mean number of mPCEs. Also, there was a significant increase ($p > 0.05$) when *J. gossypifolia* extract only group is compared with the normal control group.

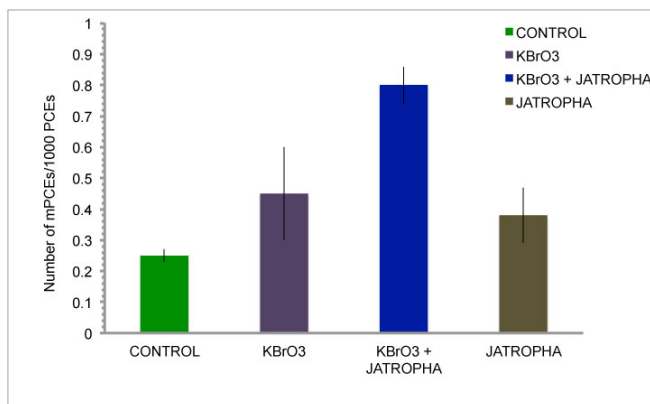


Figure 1. Effects of *J. gossypifolia* treatment on the mean number of micronucleated polychromatic erythrocyte (mPCEs) of KBrO₃ intoxicated rats. Bars having different letters are significantly different ($p < 0.05$); $n = 5$.

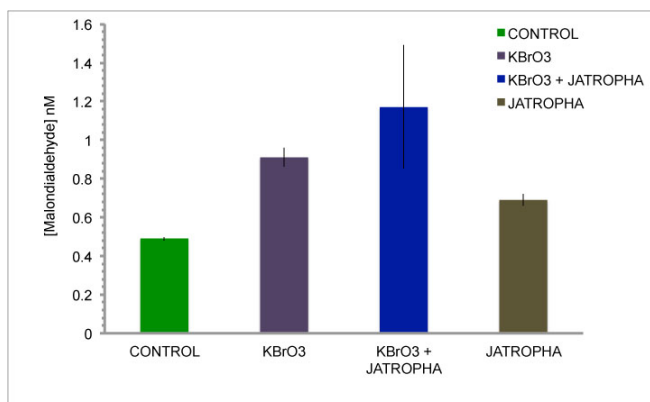


Figure 2. Effects of *J. gossypifolia* treatment on the mean MDA concentrations of KBrO₃ intoxicated rats. Bars having different letters are significantly different ($p < 0.05$); $n = 5$.

Lipid Peroxidation

The result of malondialdehyde (MDA) concentration, an index of lipid peroxidation (Figure 2), showed a significant increase ($p < 0.05$) in the mean value in all the groups compared with the normal control. Treatment with the plant extract did not lower the mean MDA concentration, instead it significantly raised ($p < 0.05$) the mean concentration (group 3) compared with toxicant only group. Also, the mean MDA concentrations obtained in the groups administered *J. gossypifolia* and KBrO₃ were comparable, as there were no significant ($p < 0.05$) differences (Figure 2).

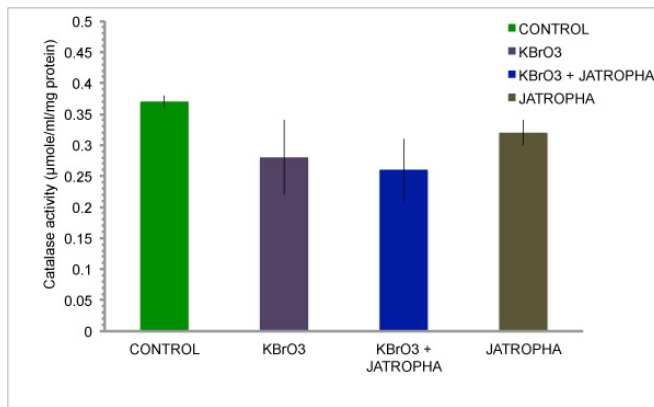


Figure 3. Effects of *J. gossypifolia* treatment on the mean plasma catalase activity of KBrO₃ intoxicated rats. Bars having different letters are significantly different ($p < 0.05$); $n = 5$.

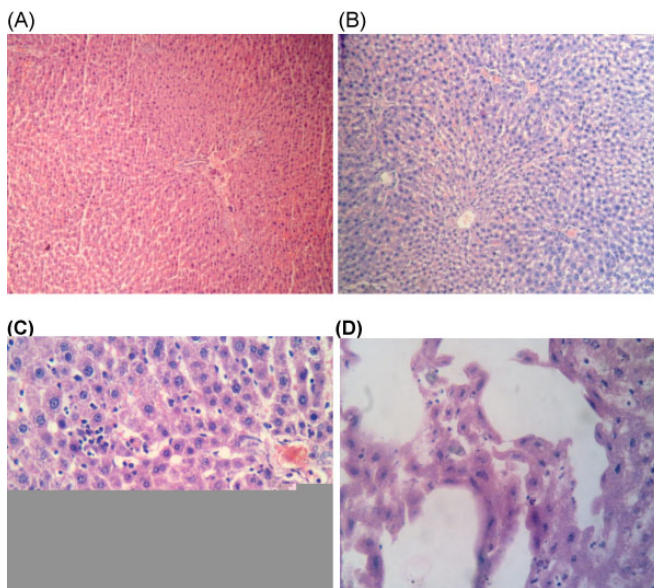


Figure 4. Effects of *J. gossypifolia* treatment on the liver histopathology of KBrO₃ intoxicated rats.

(A) Control group: no visible lesions. Hepatic cells are intact. (B) KBrO₃-intoxicated group: a slight sinusoidal dilation was seen. (C) KBrO₃-intoxicated + *J. gossypifolia* group: mild foci of hepatic necrosis and small aggregates of slight mononuclear cellular infiltration in the sinusoidal and necrotic foci. (D) *J. gossypifolia* only group: multifocal areas of moderate hepatic atrophy and severe sinusoidal dilatation with diffuse hepatic degeneration and necrosis.

Catalase Activity

For the activity of catalase (Figure 3), a significant ($p < 0.05$) reduction in the mean activity of catalase was recorded in the KBrO₃ only, and KBrO₃ plus *J. gossypifolia* groups compared with the normal control group. Although, there was a reduction in the mean catalase activity in the KBrO₃ plus *J. gossypifolia* group (group 3) compared with KBrO₃ only group

(Group 2), this decrease however, is not statistically significant ($p > 0.05$) (Figure 3).

Activities of AST, ALT, ALP, and GGT in the liver

Indices of hepatic function such as AST, ALT, ALP, and GGT were also investigated (Table 1). For AST, there were significant differences ($p < 0.05$) in all the groups. Highest mean activity was seen in the group administered *J. gossypifolia* compared with the normal control rats. *J. gossypifolia* did not lower the mean activity of this enzyme, but rather led to a significant increase ($p < 0.05$) (Table 1).

For ALT, ALP and GGT (Table 1), there were significant differences ($p < 0.05$) in the group administered KBrO₃ only compared with the normal control. Also, *J. gossypifolia* did not protect against hepatic damage, but it significantly increased ($p < 0.05$) the mean activities of the enzymes in group administered KBrO₃ plus *J. gossypifolia*. There was no significant difference ($p > 0.05$) in the *J. gossypifolia* only group and KBrO₃ plus *J. gossypifolia* group for ALT, while there was also no significant difference ($p > 0.05$) between KBrO₃ only and *J. gossypifolia* only groups for ALP and GGT.

Plasma Creatinine, urea, sodium, and potassium concentrations

For creatinine and urea mean concentrations (Table 2), there were significant increases ($p < 0.05$) in KBrO₃ only and KBrO₃ plus *J. gossypifolia* groups compared with the normal control group. Although, there was an increase in the mean concentrations of creatinine and urea in KBrO₃ plus *J. gossypifolia* group compared with KBrO₃ only, this was not statistically significant ($p > 0.05$). There were also significant differences ($p < 0.05$) between the normal control and the *J. gossypifolia* group.

Similar trend was seen in the mean concentrations of sodium and potassium (Table 2). The only difference here is that, for mean sodium concentration, there was a significant difference ($p < 0.05$) between KBrO₃ only group and KBrO₃ plus *J. gossypifolia* group. For potassium, no significant difference ($p > 0.05$) was seen among the toxicant and extract treated groups.

Histopathology

Our liver histopathological results (Figure 4) revealed that there were no visible lesions in the normal control rats. Administration of KBrO₃ caused a slight sinusoidal dilation. Ethanolic extract of *J. gossypifolia* treatment in the liver of KBrO₃-intoxicated rats did not show any corrective effects, but revealed a mild foci of hepatic necrosis and small aggregates of slight mononuclear cellular infiltration in the sinusoidal and necrotic foci, while the liver histopathology of rats administered *J. gossypifolia* only revealed multifocal areas of moderate hepatic atrophy and severe sinusoidal dilatation with diffuse hepatic degeneration and necrosis, suggesting the probable toxicity of the ethanolic extract.

Table 1. Effects of *J. gossypifolia* treatment on liver function indices of KBrO₃-intoxicated rats.

Group	Treatment Regimen	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
I	Control (Vehicle)	5.80 ± 0.02 ^a	3.38 ± 0.13 ^a	1.30 ± 0.03 ^a	1.46 ± 0.08 ^a
II	KBrO ₃ (200 mg/kg)	16.91 ± 0.79 ^b	4.67 ± 0.22 ^b	3.30 ± 0.15 ^b	2.88 ± 0.15 ^b
III	KBrO ₃ (200mg/kg) + JG (300mg/kg)	52.27 ± 3.61 ^c	8.53 ± 0.94 ^c	4.18 ± 0.49 ^c	6.42 ± 0.06 ^c
IV	JG (300mg/kg)	75.37 ± 4.16 ^d	7.00 ± 0.82 ^c	3.74 ± 0.60 ^c	2.27 ± 0.08 ^b

The values are total activities of the indicated enzymes and are expressed as mean ± standard error of mean of five rats each. Values having different superscript are significantly different ($p < 0.05$); JG represents *Jatropha gossypifolia*.

Table 2. Effects of *J. gossypifolia* treatment on renal function parameters of KBrO₃-intoxicated rats.

Group	Treatment Regimen	Creatinine (mg/dl)	Urea (mg/dl)	Sodium (mg/dl)	Potassium (mg/dl)
I	Control (Vehicle)	1.08 ± 0.08 ^a	46.70 ± 0.57 ^a	145.59 ± 8.53 ^a	3.98 ± 0.64 ^a
II	KBrO ₃ (200 mg/kg)	1.77 ± 0.09 ^b	84.40 ± 1.59 ^b	181.35 ± 4.09 ^b	7.84 ± 1.34 ^b
III	KBrO ₃ (200mg/kg) + JG (300mg/kg)	1.83 ± 0.04 ^b	92.33 ± 1.43 ^b	195.16 ± 16.79 ^c	9.32 ± 0.80 ^b
IV	JG (300mg/kg)	1.42 ± 0.03 ^c	76.33 ± 0.97 ^c	158.11 ± 11.38 ^a	6.55 ± 0.52 ^b

The values are plasma concentrations of the indicated parameters and are expressed as mean ± standard error of mean of five rats each. Values having different superscript are significantly different ($p < 0.05$); JG represents *Jatropha gossypifolia*.

DISCUSSION

Plant extracts are used to treat numerous human diseases (Khan *et al.*, 2006) and have prominent effect on the animal system, important therapeutic properties and antimicrobial activities against various pathogens (Moshi *et al.*, 2006; Ahmed *et al.*, 2006; Oladunmoye, 2006). It is therefore advised to take necessary precautionary measures when engaging in the use of medicinal plants, as they may also pose a health risk in humans.

The micronuclei assay is developed for detection of *in vivo* chromosomal breakage more conveniently than the traditional cytogenetic methods (Heddle, 1973). The majority of the micronuclei are found in the polychromatic erythrocyte (PCE) cells and these offer an advantage for the use of the micronucleus assay for screening mutagens (Von Le debut and Schmidt, 1973). It has been used to detect *in vivo*

genetic activity in bone marrow cells (Sai *et al.*, 1992). Our results on micronucleus assay revealed that administration of ethanolic leaf extract of *J. gossypifolia* did not ameliorate KBrO₃-induced clastogenicity, but has contributed to the increased number of mPCEs scored (Figure 1).

Antioxidant delays or inhibits oxidative damage to target molecules (Halliwell, 1996). Measurement of thiobarbituric acid (TBARS) is mostly used to monitor lipid peroxidation and indirectly, oxidative stress *in vitro* and *in vivo* (Beltowski *et al.*, 2000). The lipid oxidation causes disruption of the bilayer and cell integrity accompanied by leakage of cellular content from the damaged organ into the blood stream (Ologundudu *et al.*, 2010). Our results showed that the ethanolic leaf extract of *J. gossypifolia* may not exert any antioxidant effects. Treatment of KBrO₃-induced lipid peroxidation by *J. gossypifolia* was further complicated (Figure 2).

Reactive oxygen species are continuously formed in the body, which cause cell damage. It is therefore necessary for tissues to be protected against this oxidative injury through intracellular and extracellular antioxidants (Halliwell and Gutteridge, 1999). Under normal conditions, antioxidant enzymes such as superoxide dismutase catalyze the conversion of superoxide radicals (O₂⁻) into hydrogen peroxide (H₂O₂) and O₂ (Oyedemi *et al.*, 2010) and catalase further detoxifies H₂O₂ into H₂O and O₂ (Fridovich, 1986). From this study, treatment with ethanolic extract of *J. gossypifolia* leaves did not help in conserving the activity of catalase enzyme of the animals. It further overwhelmed the activity of the enzyme, though not significant ($p > 0.05$) when compared with KBrO₃ only group (Figure 3).

The activities of enzymes such as AST, ALT, ALP and GGT are used in the diagnosis of hepatic injuries and diseases. The activities of these enzymes are known to increase in the blood as a result of hepatic damage or injury (Lum and Gambino, 1972). Treatment of bromate-induced hepatotoxicity with ethanolic leaf extract of *J. gossypifolia* only further exposed the liver of the animals to injury. These were observed by the significant increase ($p < 0.05$) in the activities of the enzymes when compared with bromate only group (Table 1).

The kidney is an excretory organ. In checking its sufficiency, we also assessed the levels of plasma creatinine, urea, sodium, and potassium. Treatment of KBrO₃-induced increase in concentrations of these parameters with *J. gossypifolia* proved abortive. There were increases in the concentrations of the renal function parameters, though not significant ($p > 0.05$) except for sodium, when compared with KBrO₃ only group (Table 2).

Histopathological investigations further corroborated all the obtained results in this study (Figure 4). Treatment of KBrO₃-induced toxicity with *J. gossypifolia* revealed in the liver; mild foci of hepatic necrosis and small aggregates of slight mononuclear cellular infiltration in the sinusoidal and necrotic foci, while multifocal areas of moderate hepatic atrophy and

severe sinusoidal dilatation with diffuse hepatic degeneration and necrosis were seen in the *J. gossypifolia* only group.

Conclusion

We therefore concluded that the exacerbation of KBrO₃-induced clastogenicity, hepatotoxicity, and lipid peroxidation by *J. gossypifolia* ethanolic extract may be due to the presence of toxic phytochemicals acting as agonist by promoting KBrO₃- toxicity. Proper care needs to be ensured in the consumption or use of the plant *in vivo*.

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