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Evaluation of the effect of *Jathropha interrigima* (Euphorbiaceae) on Hepatic Function of Male Albino Wistar Rats Exposed to Lead

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Authors' contributions

This work was carried out in collaboration among all authors. Author CCO designed the study, authors EON and ESB wrote the protocol, and wrote the first draft of the manuscript. Author MJA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to evaluate the effect of *Jathropha interrigima* on hepatic function of male Albino Wistar rats exposed to lead.
Study Design: The study is an experimental study.
Place and Duration of Study: Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria, between March 2020 and December 2020.
Methodology: A total of 30 male Albino Wistar rats that weighed between 140-210g were used for this study. This study was done in three phases: Acute, Sub-chronic and Chronic phases and *Jathropha interrigima* extract was given to the rats as a prophylaxis (PRE) within the three phases and as a therapeutic (POST) substance within the three phases. Blood samples were collected at the end of each phase for both PRE and POST by cardiac puncture and separated into microvials tubes for the spectrophotometric evaluation of total protein, albumin, Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline phosphatase (ALP) using a Selectra Pro-S

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automated chemistry analyzer. Liver tissues were also harvested for histological studies using haematoxilin stain. Data was analysed using SPSS version 22.0 and p values less than 0.05 were considered statistically significant.

Results: The results showed that for the Acute Phase, the rats exposed to Lead (Treatment Group) given the Jathropha extract prophylactically(PRE) had mean Total protein level(40 ± 4.00g/l) which was significantly lower than the positive control (64.0 ± 0.01g/l) and the Negative control(66.0 ± 2.80g/l); Mean AST level(109.7±13.80iu/l) was significantly lower than the Positive control (124.0±2.80iu/l) and the Negative control(154.0 ±2.80iu/l) whereas for those given the Jathropha extract therapeutically (POST) had mean Albumin level (33.3 ± 1.53g/l) which was significantly lower than the negative control (37.0 ± 6.83). In the Sub-chronic phase, Rats in the PRE had Total Protein in TG (49.7 ± 1.53) was significantly lower than the NC and PC; AST(82.7±4.04iu/l) and ALT(49.0 ± 3.61) in the TG were significantly higher than in the PC and NC whereas those in POST had mean Total protein level(66.0 ± 1.73)and Mean Albumin level(34.0± 1.00)which were significantly higher than PC and NC; mean AST (54.0±2.00), Mean ALT(34.3±2.08) and Mean ALP(33.0±1.73) which significantly lower than PC and NC. A similar result occurred at the chronic phases for both PRE and POST. Histological examination also showed inflammatory infiltrates in the PRE and mitotic activity in the POS.

Conclusion: The results of this study indicate that exposure to Lead caused a hepatic injury and *Jathropha interrigima* may have the potential to heal or reverse the injurious effect if given as a therapy.

Keywords: Jathropha interrigima; hepatic function; male albino rats; lead.

1. INTRODUCTION

Metals are ubiquitous in the environment. They are present in varying amounts in the various compartments of the ecosystem including the biotic and abiotic factors. Some of these metals, even in trace amounts, are deleterious to the ecosystem, as a result of their non-bio-degradability, they easily enter the food chain where they are eventually consumed by humans. In humans, most of these metals, which are not easily excreted may bio-accumulate in the system and may become injurious to the Liver which is the organ to detoxify most metals thereby resulting in impaired hepatic function due to injury to the liver [1]. The incidence of liver diseases is increasing over the years and ranks fifth cause of death worldwide. It is the second cause of death from digestive diseases in the United States [2]. A study carried out in 2009 by Mathew Cave suggested that at least 1 in 3 adult Americans had unexplained Liver disease and at least 60% was attributed to environmental pollution (Science Daily 2009). In 2017, there were 48,789 deaths from liver disease in Nigeria forming about 2.4% of total death in Nigeria [3]. These death tolls may be attributable to the traditional factors (viruses, heavy alcohol use, infections, certain medications and toxins) and environmental pollution [3].

Management or treatment of Liver diseases which may ultimately involve a liver transplant is very expensive and may not be readily affordable by the general population. The hub of pharmaceutical research is on the discovery of medicinal plants which may be an alternative to orthodox therapy, one of such plant is the Jathropha family. The Jathropha plants are readily available in the tropical rainforest of the South -South region of Nigeria. Studies involving *Jathropha gossypiifolia* has been shown to have potential hepato-protective action. This study was based on the extracts of this plant in carbon tetrachloride induced liver damage in rats. The petrol, ether methanol and water extracts from the aerial parts of *Jathropha gossypiifolia* presented significant protective action on the liver, bringing the liver enzymes back to almost normal after inducement [4]. However, information about *Jathropha interrigima* is scarce. This study therefore evaluated the effect of *Jathropha interrigima* on hepatic function of male albino rats exposed to heavy metals using Lead as a case study.

Lead is a highly toxic metal whether inhaled, ingested or absorbed through the skin as it affects virtually every organ in the body. There is no safe level of Lead exposure. Most ingested Lead is absorbed into the blood stream the primary cause of its toxicity is its interference with proper functioning of enzymes, this it does by binding to sulfhydryl groups found on enzymes [5] mimicking and displacing other metals which act as cofactors in many enzymatic

processes [6]. Lead easily interacts with calcium, iron and zinc, although high levels of calcium, iron and zinc could provide protection against lead poisoning [6]. In animals, lead exhibits toxicity in many organs, damaging the nervous, renal, reproductive, hematopoietic, and cardiovascular systems after ingestion, inhalation, or skin absorption. A study conducted by Nabil et al. [7] on the effect of Lead acetate on male albino rats showed that Lead acetate was hepatotoxic giving rise to marked increase in the Liver enzyme. Its prevalence in the body in very minute amounts could lead to poor mental outcomes. Lead can cause severe damage to the brain and kidneys and, ultimately, death. By mimicking calcium, lead readily crosses the blood-barrier. It degrades the myelin sheaths of neurons, reduces their numbers, interferes with neuro-transmission routes, and decreases neuronal growth [5]. In the human body, lead inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrinIX, the final step in hemesynthesis. This causes ineffective heme synthesis and microcytic anaemia [8].

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 30 male albino wistar rats that weighed between 140-210g were used for this study. The rats were purchased from the Department of Animal and Environmental Biology of Rivers State University, Port Harcourt. The rats were kept in well ventilated cages at room temperature; and were allowed to acclimatize for 2 weeks and were fed with standard feeds (Top feeds finisher mash, Sapele, Warri) and were given clean double distilled water. The animals were well treated according to the guidelines for the care and use of Laboratory animals [9].

2.2 Plant Material

Jathropha interrigima plant was obtained from a garden in Ibeto road, Port Harcourt town, Rivers State and was identified by Prof. Ben Ekeke of the Department of Forestry, Rivers State University, Port Harcourt, Nigeria.

2.3 Study Design

This study is an experimental study and was carried out in three phases: Acute phase (15 days), Sub- Chronic Phase (30 days) and Chronic Phase (60 Days). A total of 30 male albino rats were used for the study. The rats were divided into two sets: the Prophylaxis set (PRE) where *Jathropha interrigima* leaf extracted and given by oral gavage according to body weight for 15 days before exposure to a calculated dose of the Lead according to body weight. In the therapeutic Set (POST), the rats were exposed on first day to a calculated dose of Lead then from the second day given the diluted Jathropha leaf extract for next fifteen days by oral gavage. In the Sub chronic phase, the PRE rats were given Jathropha extract for 30 days and on the 31st day they were given a calculated dose of the metal, whereas, the POST rats were exposed to the metal first then given Jathropha afterwards for 30 days before sample collection. Same methodology was used for the chronic phase as the study was stretched to 60 days before sample collection. There were also negative controls (NC) which were rats given only food and water, and positive controls (PC) which were rats exposed to the metal only.

2.5 Sample Collection

Blood samples were collected from the rats by cardiac puncture at the end of each phase into heparinized vacutainer bottles for analysis. Each blood sample was centrifuged at 3000 rpm for 5 minutes and serum aliquots were put into of micro- vial tubes for the measurement of hepatic parameters such as Total Protein (TP), Albumin (ALB), AST, ALT and Alkaline Phosphatase (ALP) spectrophotometrically using Selectra Pro-S automated analyser. Total protein was analysed using the Biuret method, Albumin by Bromocresol Green method, AST, ALT by enzymatic- colorimetric method.

Harvested liver tissues were fixed in 10% Formol saline for a minimum of 48hrs and representative tissue blocks of 3mm were taken for standard processing into paraffin embedded blocks. The blocks were sectioned in a rotary microtome at 3µm and tissue slides stained with the standard Haematoxylin and Eosin staining technique. Photomicrographs of the liver tissues were taken as shown in the result section.

2.6 Laboratory Analysis

2.6.1 Analysis of Alanine Aminotransferase (ALT) [10]

The measurement of Alanine aminotransferase was carried out using Frankel and Reithman

automated method. An automated chemistry analyzer (Selectra Pro-S) was used for the end-point enzymatic measurement and results were read against known standard calibration. Trans-amination is the process in which an amino group is transferred from an amino to an α-ketone acid. The enzymes responsible for transamination are called transaminases. The substrate in the reaction is α-ketoglutamic acid (α-KG) plus L-alanine for ALT. The products formed by enzyme action are glutamates and PYRUVATE. Addition of 2,4-dinitrophenylhydrazine result in the formation of hydrazone complex with the keto acids. A reddish-brown color was produced on the addition of sodium hydroxide the intensity of color is related to enzymatic activity.

2.6.2 Analysis of Aspartame Aminotransferase (AST) [10]

AST was estimated quantitatively using the Reithman & Frankel method as described by Randox laboratories limited (United Kingdom). Selectra Pro-S automated chemistry analyzer was used for its measurement. The substrate in the reaction is α-ketoglutaric acid (α-KG) plus L-aspartate for AST. The products formed by enzyme action are glutamates and oxaloacetate. Addition of 2,4- dinitrophenylhydrazine result in the formation of hydrazone complex with the keto acids. A red colour is produce on the addition of sodium hydroxide the intensity of color is related to enzymatic activity.

2.6.3 Analysis of total protein

The determination of Total Protein was done using an automated chemistry analyzer (Selectra Pro-S). It is an end point colorimetric method. Cupric ions in copper sulphate reagent join with the peptide bonds of the protein molecules in an alkaline solution to form a blue-violet colored complex which is read spectrophotometrically at 540nm wavelength.

2.6.4 Analysis of albumin

Albumin was determined quantitatively using the colorimetric Bromocresol Green method. It was analyzed using an automated chemistry analyzer (Selectra Pro-S). Bromocresol Green (BCG) is an indicator which is yellow between pH 3.5-4.2. When it binds to albumin, the color of the indicator changes from yellow to blue green, the absorbance of the color is measured in a spectrophotometer at 600nm wavelength.

2.6.5 Analysis of alkaline phosphatase (ALP)

ALP was measured quantitatively using an end point colorimetric method [9] and modified by Teco Diagnostics, Canada. It was analyzed using Selectra Pro-S automated analyzer. The ALP acts upon the AMP- buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue color which is measured with a spectrophotometer at 580nm wavelength.

2.6.6 Histological analysis

The liver of the rats in the acute phase study were harvested on the 17th day after the blood sample collection; those of the Sub-chronic phase were harvested on the 31st day and the chronic phase on the 61st day concurrently for histological examination. The tissues were preserved in 10% formol saline solution for a minimum of 48 hours. Each tissue was labeled according to the labels on the rats. The tissues were sent to the Rivers State University Teaching Hospital Histology Laboratory for any possible signs of toxicology of pathological changes.

The tissues were dissected and representative blocks of 3mm were processed with their labels in a tissue cassette. The fixed tissue blocks were fixed in ascending grades of alcohol, xylene, infiltrated and embedded in molten paraffin wax. The embedded tissues were sectioned on a microtome. Deparaffinized sections were stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on slide were examined and photomicrographs captured with x 400 objective lenses.

The paraffin wax was removed by dipping into xylene 1 & 2 for a minute each. The slide was immersed in absolute alcohol for 30 seconds and hydrated in descending grades of alcohol for 30 seconds. It was rinsed in tap water for a minute, stained in Erlich'shaematoxylin for 30 minutes and rinsed in running water until color turns blue. 1% aqueous eosin was used for a counterstain for 5 minutes and rinsed for 30 seconds. The slide was dehydrated again by passing it through ascending grades of alcohol for 30 seconds and was cleared in xylene for a minute.

2.7 Statistical Analysis

Statistical analysis was done with Statistical Package for Social Sciences (SPSS) of Windows

Stat Pack (version 22.0). Data generated were recorded as mean and standard deviations (Mean ± S. D), ANOVA (including Tukey's Multiple Comparative Test) and p values less than 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Acute Phase Mean levels of the parameters under study are presented in Table 1 and Fig. 1 & 2. Total protein level in the Treatment Group when *Jathropha interrigima* extract was given as prophylaxis (40 ± 4.00g/l) was significantly lower than the positive control (64.0 ± 0.01g/l) and the Negative control (66.0 ± 2.80g/l); Mean AST level(109.7±13.80iu/l) was significantly lower in the treatment group than the Positive control (124.0±2.80iu/l) and the Negative control(154.0 ±2.80iu/l) whereas for those given the Jathropha extract therapeutically (POST) had mean Albumin level (33.3 ± 1.53g/l) which was significantly lower than the negative control (37.0 ± 6.83 g/l). This may indicate than the exposure to Lead may have caused a hepatic injury or dysfunction leading to a reduction in the level of total protein which the Jathropha extract when given as prophylaxis could not protect, this is at variance with the study conducted by Bhagat and colleagues [11] which stated that *Jathropha gossypiifolia* had hepato-protective properties. In the Sub-chronic phase, Rats in the PRE had Total Protein in TG (49.7 ± 1.53) which was significantly lower than the NC and PC; AST (82.7±4.04iu/l) and ALT (49.0 ± 3.61 iu/l) in the TG were significantly higher than in the PC and NC.

The significant increase in AST/ALT levels may actually indicate a hepatic cells injury which the Jathropha extract could not mitigate against, however, those in POST had mean Total protein level (66.0 ± 1.73) and Mean Albumin level (34.0± 1.00) which were significantly higher than PC and NC; mean AST (54.0±2.00), Mean ALT (34.3±2.08) and Mean ALP (33.0±1.73) which significantly lower than PC and NC. The significant increase in the Total protein and albumin levels at this phase may indicate a restorative process which is in tandem with the studies carried out by [4], which states that some Jathropha species may have anti-inflammatory properties. A similar result occurred at the chronic phases for both PRE and POST. Histological examination also showed inflammatory infiltrates in the PRE and mitotic activity in the POST. The results of this study indicate that exposure to Lead caused a hepatic injury and *Jathropha interrigima* may have the potential to heal or reverse the injurious effect if given as a therapy over a long duration of time maybe between 30 -60 days.

One of the major functions of the liver is in the production of protein and albumin. The liver of rats exposed to Lead were seen to lose this function gradually even when Jathropha was given as a prophylaxis. This is shown in Table 1, but as the study progressed, those that were given the Jathropha as therapy after exposure showed an improvement in the Total protein level as well as in their Albumin portion. Also the liver enzyme AST which easily gets elevated in inflammation of Hepatic cells as seen in the PRE was lowered as the *Jathropha* was given after exposure and for a longer period of time as seen in Table 3. This could further be buttressed in Fig 1 and 2.The histological report of the PRE Lead liver tissue shows an infiltration of inflammatory cells due to the Lead toxicity but in Fig. 2, which is the POST Lead, there is a mitotic activity because *Jathropha Interrigima* has a healing effect and the Liver has the potency to regenerate itself. This is in agreement with [4].

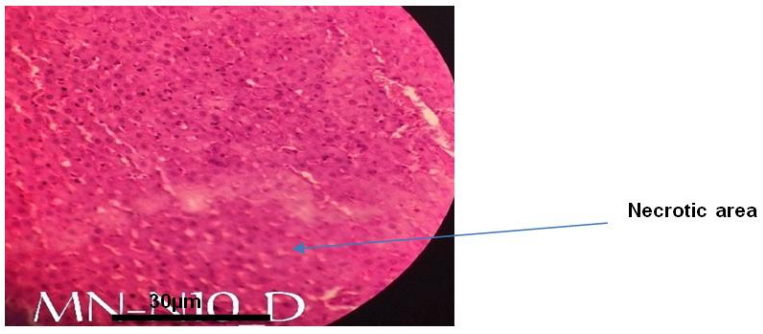

Fig. 1. Lead exposed Liver tissue treated with *Jathropha interrigima* at Acute phase of (PRE)

Table 1. Effect of *Jathropha interrigima* (PRE and POST) on Hepatic Function of Male Albino Rats Exposed to Lead in Acute Phase

Phases	Groups	Parameters Mean ± SD									
		Pre					Post				
		TP (g/l)	ALB (g/l)	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/l)	ALB (g/l)	AST (U/L)	ALT (U/L)	ALP (U/L)
15 Days	NC	66.0 ± 2.8	37.0±2.8	154.5±10.6	55.5±4.9	128.0± 1.2	66.0±2.8	37.0±6.8	154.5±10.6	55.5±4.9	128±21.2
	PC	64.0 ± 0.0	33.5±2.1	124.0±2.8	53.0±1.4	62.5±2.12	60.0±0.0	29.0±0.0[1]	157.0±0.0	59.0±0.0	98.0±0.0
	TG	49.0 ± 4.0[*#]	33.0±1.0	109.7±13.[*]	52.7±2.3	83.7±22.5	69.3±5.1	33.3±1.53	154.0±2.0	64.3±2.0	126.3±5.1
	p-value	*0.007*	*0.168*	*0.029*	*0.607*	*0.060*	*0.134*	*0.027*	*0.833*	*0.064*	*0.087*
	F-value	*22.143*	*2.887*	*9.700*	*0.568*	*6.162*	*3.454*	*10.135*	*0.191*	*5.925*	*4.793*
	Remark	*S*	*NS*	*S*	*NS*	*NS*	*NS*	*S*	*NS*	*NS*	*NS*

S– Significant at p<0.05 (ANOVA)
NS – Non-significant at p<0.05 (ANOVA)
1 – significant at p<0.05, PC compared with NC (Tukey's post hoc)
*# and * - significant, at p<0.05, TG compared with PC and NC respectively (Tukey's post hoc)*

Table 2. Effect of *Jathropha interrigima* (PRE and POST) on Hepatic Function of Male Albino Rats Exposed to Lead in Sub-Chronic Phase

Phases	Groups	Parameters Mean ± SD									
		PRE					POST				
		TP (g/l)	ALB (g/l)	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/l)	ALB (g/l)	AST (U/L)	ALT (U/L)	ALP (U/L)
30 Days	NC	59.0±1.4	34.0±1.4	61.0±1.4	39.0±1.4	56.0±1.4	56.0±2.8	29.5 ±0.71	50.5±2.12	36.0 ±1.4	43.0±0.0
	PC	64.0±1.4	33.5±0.7	66.0±1.4	46.5±2.1	62.5±2.1	60.0±0.0	29.0±0.00	157±0.1[1]	59.0±0.0[1]	98.0±0.0[1]
	TG	49.7±1.5[*#]	30.3±1.5	82.7±4.0[*#]	49±3.6[*]	63.0±4.3	66±1.73[*]	34.0±1.0[*#]	54.0±2.00[#]	34.3±2.0[#]	33.0±1.7[*#]
	p-value	*0.001*	*0.068*	*0.003*	*0.044*	*0.161*	*0.010*	*0.004*	*0.000*	*0.000*	*0.000*
	F-value	*61.165*	*5.65*	*35.7*	*7.58*	*2.982*	*17.95*	*31.143*	*2493.6*	*154.9*	*1811.9*
	Remark	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*

S– Significant at p<0.05 (ANOVA)
NS – Non-significant at p<0.05 (ANOVA)
1 – significant at p<0.05, PC compared with NC (Turkey's post hoc)
*# and * - significant, at p<0.05, TG compared with PC and NC respectively (Turkey's post hoc)*

Table 3. Effect of *Jathropha interrigima* (PRE and POST) on Hepatic Function of Male Albino Rats Exposed to Lead in Chronic Phase

Phases	Groups	Parameters Mean ± SD									
		PRE					POST				
		TP (g/l)	ALB (g/l)	AST (U/L)	ALT(U/L)	ALP (U/L)	TP (g/l)	ALB (g/l)	AST (U/L)	ALT (U/L)	ALP (U/L)
60 Days	NC	59.0±1.4	34.0±1.41	61.0±1.4	39.0±1.41	56.0±1.4	56.0±2.8	29.5±0.7	50.5±2.1	36.0±1.4	43.0±0.0
	PC	64.0±1.4	33.5±0.7	66.0±1.4	46.5±2.1	62.5±2.1	60.0±0.0	29.0±0.0	157±0.1[1]	59.0±0.0[1]	98.0±0.0[1]
	TG	49.7±1.5[*#]	30.3±1.5	82.7±4.0[*#]	49.0±3.6[*]	63.0±4.3	66.0±1.7	34.0±1.0[*#]	54.0±2.0[#]	34.3±2.0[#]	33.0±1.7[*#]
	p-value	*0.001*	*0.068*	*0.003*	*0.044*	*.161*	*0.010*	*0.004*	*0.00*	*0.000*	*0.000*
	F-value	*61.165*	*5.654*	*35.714*	*7.582*	*2.982*	*17.95*	*3.143*	*2493.634*	*154.9*	*1811.9*
	Remark	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*

S– Significant at p<0.05 (ANOVA)
NS – Non-significant at p<0.05 (ANOVA)
1 – significant at p<0.05, PC compared with NC (Tukey's post hoc)
*# and * - significant, at p<0.05, TG compared with PC and NC respectively (Turkey's post hoc)*

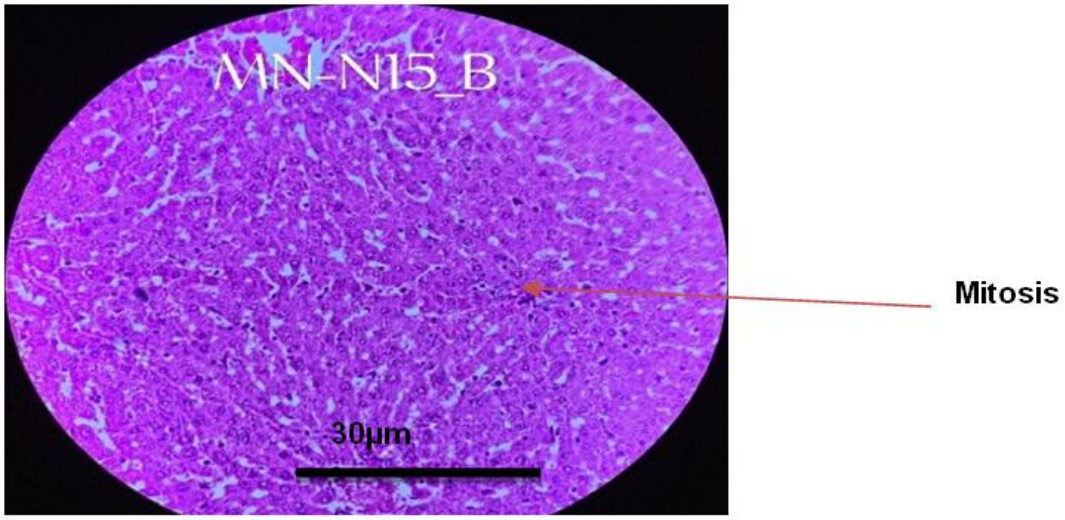

Fig. 2. Lead exposed Liver tissue treated with Jathropha interrigima at acute phase of (POST)

4. CONCLUSION

In this study, *Jathropha interrigima* extract exhibited anti-inflammatory properties, not at acute phase but when duration was increased to 30 days. It also had restorative potentials especially as regards hepatic injury. It may be better to give this herb as a therapeutic agent than prophylactic.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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