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Post-treatment with *Hibiscus Sabdariffa* Linn calyx extract enhances liver function following chronic paracetamol exposure in mice.

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ABSTRACT

This study evaluated the impact of administration of aqueous extract of *Hibiscus sabdariffa* Linn calyx on liver function following chronic exposure to paracetamol. Four (4) groups were involved: control, extract only, paracetamol only and post-treatment (drug at zero time and extract 8h later). There were 5 mice in each group. Paracetamol and *Hibiscus sabdariffa* Linn calyx extract (HSCE) were administered orally 500 mg/kg body weight and 250 mg/kg body weight respectively, on a daily basis for 16 weeks. At the end of the experiment, all the animals were sacrificed. Activities of liver marker enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and levels of bilirubin, albumin, total protein and total cholesterol were assessed in serum. Malondialdehyde (MDA) and antioxidant status (superoxide dismutase and catalase activities, and reduced glutathione level) were estimated in liver homogenate. Histopathological examinations of liver sections were also carried out on experimental mice. Paracetamol toxicity was evidenced by a significant increase ($P \leq 0.05$) in biochemical indices of liver function with decreased protein content. Post-treatment with the extract was seen to mitigate the changes prompted by exposure to paracetamol; this was also confirmed by histopathological studies. The observed amelioration by the extract may not be unrelated to the presence of phenolic compounds, anthocyanins, flavonols and protocatechuic acid (PCA), which has been widely reported.

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KEYWORDS: Antioxidant status, *Hibiscus sabdariffa* Linn calyx extract (HSCE), liver function, paracetamol, post-treatment.

INTRODUCTION

Hibiscus sabdariffa Linn is an annual shrub that is cultivated between April and November in the tropics and it belongs to the Malvaceae family [1].

It is thought to be native to Africa and Asia [1]. In some areas around the world, it is cultivated as a home garden crop, cultivation can be achieved either through the seeds, rooting or shoot cuttings on a well-drained soil with an annual rainfall of 130-260 mm in the first quarter of growth. Dry weather is favorable during the later months of growth. Proper monitoring of the water content and careful avoidance of mixed cultivation with crops susceptible to root and stem rot will prevent root and stem rot in *Hibiscus sabdariffa* Linn [2].

Parmar *et al*. [3] reported that it is very difficult to find any drug capable of protecting the liver from damage or help in hepatic cell regeneration despite the success in modern medicine recorded. However, Ukegbu *et al*. [4] reported the frequency in which many active plant extracts are utilized to treat a wide variety of clinical diseases including liver disease. *Hibiscus sabdariffa* Linn has been reported to show the presence of phenolic compounds, anthocyanins, flavonols, protocatechuic acid (PCA), etc [5]. Herrera-Arellano *et al*. [6] described the positive effect of this plant extract in reducing blood pressure in experimental animals and humans. The hypo-cholesterolemic activity of the plant was confirmed when they observed lowering effect in the different lipid fraction of hypercholesterolemic rat [7]. The ability of the extract to protect the liver from radiation and over-dosage effect of acetaminophen were also reported [8, 9].

The liver is the most significant organ of the body for detoxification; disorders to this organ remain some of the most serious health problems. The organ is very important in regulation of physiological processes involved in several vital functions like storage, secretion and metabolism. It is also heavily involved in the detoxification of a variety of drugs and xenobiotics and plays central role in the transformation and clearing of chemicals [10]. Hepatotoxicity has been known to cause severe metabolic disorders and sometimes eventually death. Hepatic damage occurs mostly because the liver is involved in different functions, chief of which is detoxification of xenobiotics, ultimately leading to oxidative stress which when in excess distorts all of its functions [11].

Acetaminophen (paracetamol or *N*-acetyl-*p*-aminophenol (APAP)) is a derivative of para-aminophenol and is used as an analgesic and antipyretic drug. It belongs to the para-aminophenol class of the nonsteroidal anti-inflammatory drugs [12]. At therapeutic doses, paracetamol is not harmful or toxic, but at higher doses, it could lead to acute liver damage. The hepatotoxicity of APAP has been linked to the formation of toxic highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) which causes oxidative stress and glutathione depletion

[13]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at larger doses [14].

The unending interests in this plant up to now have led to numerous scientific discoveries that exceed the traditional medicinal belief. However, the present study examined the hepato-regenerative potential of *H. sabdariffa* on paracetamol induced liver damage in mice using the liver function tests, total cholesterol, lipid peroxidation and antioxidants as biomarkers.

MATERIALS AND METHODS

Plant material

The calyces of *Hibiscus sabdariffa* Linn were purchased from a well-known market in Abuja, Nigeria's capital city. The plant was identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Subsequently a voucher specimen of the plant (voucher number UBHm 0261) was deposited at the Herbarium, University of Benin, Benin City. The extract was prepared as earlier described [15].

Experimental Animals

Twenty (20) mice weighing between 27 to 32 g were used for the study. They were obtained from a breeder in Benin City and housed in wooden cages in controlled optimum conditions in the animal house of the Department of Biochemistry, University of Benin. The mice were given free access to food and tap water for two weeks to allow for acclimatization before commencement of treatment. The study was conducted according to the conventional procedures accepted by National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Ethic Committee of the Faculty of Pharmacy, University of Benin, Benin city, Nigeria.

Paracetamol preparation

Paracetamol powder was first dissolved in dimethyl sulfoxide (DMSO) (2.5% aqueous solution of DMSO), then mixed with distilled water to make up the required quantity.

Experimental design and treatment schedule

Four (4) groups were involved: control, extract only, paracetamol only and post-treatment (drug at zero time and extract 8h later). There were 5 mice in each group. Paracetamol (500 mg/kg body weight) and *Hibiscus sabdariffa* Linn calyx extract (HSCE) (250 mg/kg) were administered orally. The experiment lasted for 16 weeks after which all the mice were sacrificed.

Biochemical analyses

The mice were sedated using chloroform, after which blood was taken by cardiac puncture and placed in plain sample bottles. The liver was also harvested for biochemical analyses and histopathological investigations. Analyses carried out on serum include alanine aminotransferase (ALT) [16], aspartate aminotransferase (AST) [16], alkaline phosphatase (ALP) [17], gamma glutamyl transferase (GGT) [18], total cholesterol [19], albumin [20], total protein [21] and bilirubin (total and direct) [22]. Reduced glutathione (GSH) [23], malondialdehyde (MDA) [24], superoxide dismutase (SOD) [25] and catalase [26] were carried out on liver homogenate.

Statistical analysis

Data obtained were presented as mean ± S.E.M. Analysis for significance was done by one way ANOVA and mean values that differed significantly were identified using the Duncan's multiple range test. $P \leq 0.05$ was considered significant.

Results

Effects of post-treatment of HSCE on chronic paracetamol exposure

Liver function parameters

Table 1 shows results for liver function tests carried out in serum. After 16 weeks, it was observed that post-treatment with extract brought about a significant reduction close to the control for ALT, AST, total cholesterol, ALP, GGT, Total bilirubin and Direct Bilirubin levels while for Total Protein and Albumin levels there was a significant increase with values returned close to the control, compared to paracetamol only.

Table 1:

Antioxidants and lipid peroxidation in the liver

Table 2 shows results for antioxidants and lipid peroxidation tests carried out in liver homogenate. After 16 weeks, it was observed that post-treatment with extract led to a significant reduction very close to the control for MDA levels while for the antioxidants there was a significant increase and returned close to the control, compared to paracetamol only.

Table 2:

Histopathological findings

Liver ultrastructure of mice exposed to paracetamol before extract

The control mouse showed normal architechure of the liver, composed of portal vein (A) and hepatocytes (B), separated by sinusoids (C) (plate 1). The mouse that received extract only showed moderate Kupffer cell activation (A) (plate 2). The mouse that received paracetamol only showed portal congestion (A) and moderate infiltrates of inflammatory cells (B) (periportal

hepatitis) (plate 3), while the mouse that received paracetamol at zero time and extract 8h later showed moderate periportal lymphocytosis (A) and vascular congestion (B) (plate 4).

Figure 1:

DISCUSSION

This study has shown that the ALT, AST and ALP levels which were significantly increased in the paracetamol only group relative to control, significantly reduced when the HSC extract was added 8 hours later. This is consistent with the work of [27] who showed a similar trend when water extract of HSC was added following oxidation induced by paracetamol. Similarly, according to the work of [28] a polyphenol extract of Hibiscus Sabdariffa ameliorated acetaminophen induced hepatic steatosis. A possible mechanism could be due to a strong antioxidant activity, which ensures reduction of cellular damage by reducing oxidative stress and by stopping mitochondrial dysfunction through decreasing Bax and tBid expression in the liver [28].

For total protein and albumin, paracetamol only group showed significant decreased total protein and albumin content when compared with the control and there was a significant rise in the TP and Alb level after the HSCE was added 8 hours later. This agrees with the work of [29] who also reported a significant decrease in total protein and albumin levels when paracetamol toxicity was induced. Similar report was made by [30] who worked on the amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements. It is known that hepatic cytolysis and loss of biochemical functions have the ability to cause inhibition of albumin production, ultimately causing a decrease in albumin. Albumin ensures oncotic pressure is maintained and various substances such as iron, fatty acids, calcium, hormones, and bilirubin are transported.

With respect to total and direct bilirubin, it was observed that there was a significant increase in the paracetamol administered group, compared to the control; while their levels significantly decreased in the group that received the extract 8 hours later, relative to paracetamol only group. The increase in value of T-BIL and D-BIL are also associated to DNPH induced liver toxicity [31]. The hepatic cytolysis is followed by the alteration of the cell membrane with the loss of functions thereof. These results are in line with those obtained by [32]. These results enable to assert once again the hepatotoxicity of paracetamol exposure and highlight the important role of the liver in detoxification of toxic substances.

In the lipid peroxidation and anti-oxidant analysis, it was observed that malondialdehyde (MDA) was increased significantly in the paracetamol administered group and when the

extract was added 8 hours later, there was a significant decrease when compared with the paracetamol administered group, while for the anti-oxidant analysis, all three anti-oxidants namely catalase, superoxide dismutase and reduced glutathione showed significant decrease in the group administered paracetamol only when compared to the control but a marked increase very close to the control when the extract was added 8 hours later. The results of lipid peroxidation test are in tandem with those obtained by some authors [33]. The high value of paracetamol significantly different ($P<0.05$) from that of the control group, indicates lipid peroxidation of polyunsaturated membrane leading to cell necrosis with accumulation of MDA in the serum of rats [33]. The production of MDA in biological tissues mainly results from free radical attacks during oxidative stress. The cell membrane is altered ultimately leading to the loss of biochemical and physiological functions of the cell that occurs in cell necrosis. These results would mean that the exposure to paracetamol causes an oxidative stress with an excessive production of free radicals which tends to tilt the antioxidants/pro-oxidants balance in favor of the latter. Excess paracetamol exposure could also lead to a failure of the antioxidant defense system through the inactivation of enzymes, biochemical substrates, and trace elements. The superoxide formation encourages peroxynitrite generation and protein nitration that may further result into oxidative damage to proteins, DNA and lipids [34]. Furthermore, the mitochondria can interact with both paracetamol and NAPQI inducing depletion of mitochondrial glutathione content, decline in ATP content, and uncoupling of the mitochondrial respiratory chain combined with electron leakage [35]. Glutathione is a ubiquitous tripeptide present in all cell types in millimolar concentrations. Glutathione plays major role in the maintainance of the intracellular redox balance and elimination of xenobiotics and ROS [36]. According to the decline in hepatic glutathione content, it was evident that paracetamol-induced toxicity led to a change in cellular redox status toward a state of oxidative stress. A wide variety of oxidizing molecules such as ROS and/or depleting agents can affect glutathione redox state, which normally is maintained by the activity of glutathione-depleting (GPX, GST) and glutathione-replenishing enzymes. Therefore, it can be assumed that the decrease in glutathione concentration might cause the effectiveness of GST and GPX activity to be restricted, as evident by the intensification of lipid peroxidation. The decrease in catalase activity from our result also indicates a decrease in the antioxidant capacity. The decreased activity of SODs may be attributed to the consumption of these enzymes in ROS detoxification. Halliwell and Gutteridge, [37] reported that antioxidant enzymes can be inactivated by lipid peroxides and ROS. SOD is inhibited by hydrogen

peroxide, while GSH and catalase are inhibited by an excess of superoxide radical on the same grounds as indicated in our study [38]. Lipid peroxidation according to [39] is expected to cause the destruction and damage to cell membranes, lead to changes in membrane permeability and fluidity and enhance the protein degradation in mice.

Total cholesterol levels were significantly increased in the paracetamol administered group only as compared to the control but was significantly reduced after 8 hours when HSCE was added. This shares so much similarity with the work of [40] who also reported elevated levels with paracetamol induced toxicity and reduced levels when the extract of *Hibiscus sabdariffa* was added, albeit it was in rats unlike this study that is on mice. The result shows that the extract has the ability to reduce total cholesterol levels by improving HDL production from the liver. Improving the synthesis of HDL from the liver could be as a result of regeneration of liver cells or mopping up of free radicals from the system.

The histopathological findings confirmed the results of all biochemical assays undertaken. Histopathological examination of the liver of the animals treated with paracetamol showed damage to hepatic structural integrity by revealing severe destruction of hepatic architecture [15], necrosis, and infiltration of inflammatory cells. Similar observations have been previously reported [41]. Administration of HSC at 250 mg/kg significantly attenuated hepatocyte necrosis and inflammatory cell infiltration into paracetamol-injured liver.

In conclusion, hepatic injury is more common than we give credit to, and from the above findings, it has been revealed that taking acetaminophen at larger doses than recommended ultimately leads to hepatotoxicity. *Hibiscus sabdariffa* Linn calyx extract has been shown to attenuate liver toxicity and therefore more experimentation is required to properly ascertain the particular active ingredient that ensures the attenuation of hepatic injury.

REFERENCES

1. Gaet, N. (1999). *Hibiscus sabdariffa* Linn. ed. by Ivan A. New Jersey: Humana Press; 1999. pp. 165-170.
2. Tabe, F. N., Yanou, N. N., Kamdje, A. H. N. and Ntso, A. A. (2015). Calyx Juice of *Hibiscus sabdariffa* Linn (Malvaceae): A Possible Therapeutic Supplement to Antiretroviral Drugs. Journal of Diseases and Medicinal Plants, 1(4): 59-67.
3. Parmar, S. R., Patel, H. V. and Kiran, K. (2010). Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. J. Herb. Med. Toxicol. 4: 101-106.

4. Ukegbu, C. Y., Arome, O., Affiong, E., Ogechukwu, A. and Ike C. (2016). Anti-diabetic effect of the methanolic leaf extract of *Axonopus compressus* (*P. beauv*) in alloxan induced diabetic rats. Int. J. Biochem. Res. Rev. 12: 1-5.
5. Fakeye, T. O., Pal, A., Bawankule, D. U. and Khanuja, S. P. S. (2008). Immunomodulatory effect of extracts of *Hibiscus sabdariffa* L. (Family Malvaceae) in a mouse model. Phytother. Res. 22: 664-668.
6. Herrera-Arellano, A., Flores-Romero, S., Chavez-Soto, M. A. and Tortorie-Ilo, J. (2004). Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. Phytomedicine, 5: 375-382.
7. El-Saadany, S. S., Sitohy, M. Z., Labib, S. M. and El-Massry, R. A. (1991). Biochemical dynamics and hypocholesterolemic action of *Hibiscus sabdariffa* (Karkade). Mol. Nutr. Food Res. 35: 567-576.
8. Adaramoye, O., Ogungbenro, B., Anyaegbu, O. and Fafunso, M. (2008). Protective effects of extracts of *Vernonia amygdalina*, *Hibiscus sabdariffa* and vitamin C against radiation-induced liver damage in rats. J. Radiat. Res. 49: 123-131.
9. Olaleye, M. T. and Rocha, B. T. J. (2008). Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. Exp. Toxicol. Pathol. 59: 319-327.
10. Pal, R. K. and Manoj, J. (2011). Hepatoprotective activity of alcoholic and aqueous extracts of fruits of *Luffa cylindrical* Linn in rats. Ann Biol Res, 2 (1): 132-141.
11. Wolf, P. L. (1999). Biochemical diagnosis of liver diseases. Indian J Clin Biochem,14 (1): 59-90.
12. Aghababian, R. V. (2010). Essentials of emergency medicine. Burlington: Jones & Bartlett Publishers, pp. 814.
13. Shah, V. N. and Deval, K. (2011). Hepatoprotective activity of leaves of *Parkinsonia aculeata* Linn against paracetamol induced hepatotoxicity in rats. Int J Pharm, 1 (2): 59-66.
14. Hurkadale, P. J., Shelar, P. A., Palled, S. G., Mandavkar, Y. D. and Khedkar, A. S. (2012). Hepatoprotective activity of *Amorphophallus paeoniifolius* tubers against paracetamol-induced liver damage in rats. Asian Pac J Trop Biomed, (1): S238-S242.
15. Orji, B. O. and Obi, F. O. (2017). Effects of concurrent administration of Paracetamol and aqueous extract of *Hibiscus Sabdariffa* Linn calyx on

Paracetamol Hepatotoxicity in Mice. Journal of Pharmaceutical, Chemical and Biological Sciences, 5(2):108-117.

16. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28:56-63.
17. Tietz, N. W. (1976). Fundamentals of Clinical Chemistry. 2nd edition. Philadelphia: W. B. Saunders Company, pp. 897.
18. Szasz, G. (1969). A kinetic photometric method for serum gamma glutamyl transpeptidase. Clinical Chemistry, 22:124-136.
19. Trinder, P. (1969). Enzymatic end point method. Annals of Clinical Biochemistry, 6: 24.
20. Grant, G. H. (1987). Amino acids and proteins. In: Fundamentals of Clinical Chemistry. Tietz NW. editor, WB Saunders Company Philadelphia, USA, pp. 328-329.
21. Tietz, N. W. (1995). Clinical Guide to Laboratory Tests. 3rd edition, WB Saunders Company. Philadelphia, pp. 518-519.
22. Jendrassik, L. and Groff, P. (1938). Determination of direct and total bilirubin in serum. Biochemistry, 297:81-89.
23. Tietz, F. (1969). Enzymic method for quantitative determination of nanogram amount of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical Biochemistry, 27:502-22.
24. Buege, J. A. and Aust, S. D. (1978). Microsomal lipid peroxidation. Methods in Enzymology, 52: 302-310.
25. Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase. Journal of Biological Chemistry, 247: 3170-3175.
26. Cohen, G., Dembiee, D. and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. Analytical Biochemistry, 34: 30-38.
27. Ali, B. H., Mousa, H. M. and El-Mougy, S. (2003). The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* L. on paracetamol-induced hepatoxicity in rats. Phytotherapy Research, 17(1): 56–59.
28. Lee, C. H., Kuo, C. Y., Wang, C. J., Wang, C. P., Lee, Y. R., Hung, C. N., *et al.* (2012). A polyphenol extract of

Hibiscus sabdariffa L. ameliorates acetaminophen-induced hepatic steatosis by attenuating the mitochondrial dysfunction *in vivo* and *in vitro*. Bioscience, Biotechnology, and Biochemistry, 76(4): 646–651.

29. Kane, A. E., Mitchelld, S. J., Mach, J., Huizer-Pajkos, A., McKenzie, C., Jones, B., *et al.* (2016). Acetaminophen hepatotoxicity in mice: Effect of age, frailty and exposure type. Exp Gerontol, 73: 95–106

30. Hamza, R. Z. and Al-Harbi, M. S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian Pacific Journal of Tropical Biomedicine, 5(7): 521-531.

31. Singh, A., Bhat, T. K. and Sharma, O. P. (2011). Clinical Biochemistry of hepatotoxicity. J Clin Toxicol, S4:001.

32. Douhri, B., Idaomar, M., Senhaji, N. S., Ennabili, A. and Abrini, J. (2014). Hepatoprotective effect of *Origanum* elongatum against carbon tetrachloride (CCl4) induced toxicity in rats. Eur J Med Plants, 4:14-28.

33. Ahmad-Raus, R., Jamal, P. and Mohd-Isa, E. S. (2012). *Hibiscus sabdariffa* aqueous extracts prevents progression of acute liver injury induced by acetaminophen. Pertanika J Trop Agric Sci, 35:511-20.

34. Abdel-Zaher, A. O., Abdel-Hady, R. H., Mahmoud, M. M. and Farrag, M. M. (2008). The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. Toxicology, 243: 261-270.

35. Donnelly, P. J., Walker, R. M. and Racz, W. J. (1994). Inhibition of mitochondrial respiration *in vivo* is an early event in acetaminophen-induced hepatotoxicity. Arch Toxicol, 68: 110-118.

36. Myhrstad, M. C., Carlsen, H., Nordström, O., Blomhoff, R. and Moskaug, J. O. (2002). Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. Free Radic Biol Med, 32: 386-393.

37. Halliwell, B. and Gutteridge, J. M. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J, 219: 1-14.

38. Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., *et al*. (1990). Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Dev, 51: 283-297.

39. El-Megharbel, S. M., Hamza, R. Z., Refat, M. S. (2014). Preparation, spectroscopic, thermal, antihepatotoxicity, hematological parameters and liver antioxidant capacity characterizations of Cd(II), Hg(II), and Pb(II) mononuclear complexes of paracetamol anti-inflammatory drug. Spectrochim Acta A Mol Biomol Spectrosc, 131: 534-544.

40. Joshua, P. E., Ukegbu, C. Y., Eze, C., Nkwocha, C. C., Okafor, J. O., Amaechi, O., *et al*. (2017). Biochemical Effects of the Aqueous Extract of *Hibiscus sabdariffa* on Liver Marker Enzymes and Lipid Profiles in Acetaminophen-challenged Rats. Research Journal of Medicinal Plants, 11: 48-54.

41. Hasanein, P. and Sharifi, M. (2017). Effects of rosmarinic acid on acetaminophen-induced hepatotoxicity in male wistar rats. Pharmaceutical Biology, 55(1): 1809–1816.

Table 1: Effects of post-treatment with aqueous HSCE on liver function parameters in serum of mice on chronic paracetamol exposure:

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Paracetamol (zero time), then extract (8 h later)
ALT (U/I)	8.64±0.39d	8.96±0.39d	17.28±0.78a	10.88±0.32c
AST (U/I)	53.90±0.86e	53.20±0.70e	91.70±0.70a	62.30±0.70c
TP (g/dL)	6.88±0.08b	7.28±0.08a	5.24±0.10e	5.92±0.15d
ALBUMIN(g/dL	3.26±0.03b	3.65±0.04a	2.34±0.05f	2.86±0.03d
TOTAL CHOLESTEROL (mmol/L)	8.38±0.12c	8.29±0.10c	12.62±0.10a	10.98±0.10b
ALP (IU/L)	41.59±0.72d	42.18±0.59d	77.59±0.36a	56.34±0.29b
GGT (U/I)	23.16±0.00d	20.84±2.32d	53.27±2.84a	37.06±2.32c
TOTAL BILIRUBIN (mg/dL)	0.34±0.02d	0.32±0.00d	0.65±0.00a	0.41±0.02b
DIRECT BILIRUBIN (mg/dL)	0.17±0.03b	0.17±0.03b	0.32±0.03a	0.26±0.03b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Table 2: Effects of post-treatment with aqueous HSCE on antioxidants and lipid peroxidation in the liver of mice on chronic paracetamol exposure:

Biochemical parameter (liver)	Control	Extract only	Paracetamol only	Paracetamol (zero time), then extract (8 h later)
MDA (mol/g tissue)	0.08±0.00d	0.08±0.00d	0.13±0.00a	0.11±0.00b
SOD (Units/mg tissue)	0.06±0.00a	0.06±0.00a	0.04±0.00c	0.05±0.00b
CATALASE (Units/g tissue)	7.95±0.24a	8.44±0.30a	5.08±0.14d	6.16±0.08b
REDUCED GLUTATHIONE (mmol/L)	0.08±0.00a	0.08±0.00a	0.05±0.00b	0.06±0.00b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

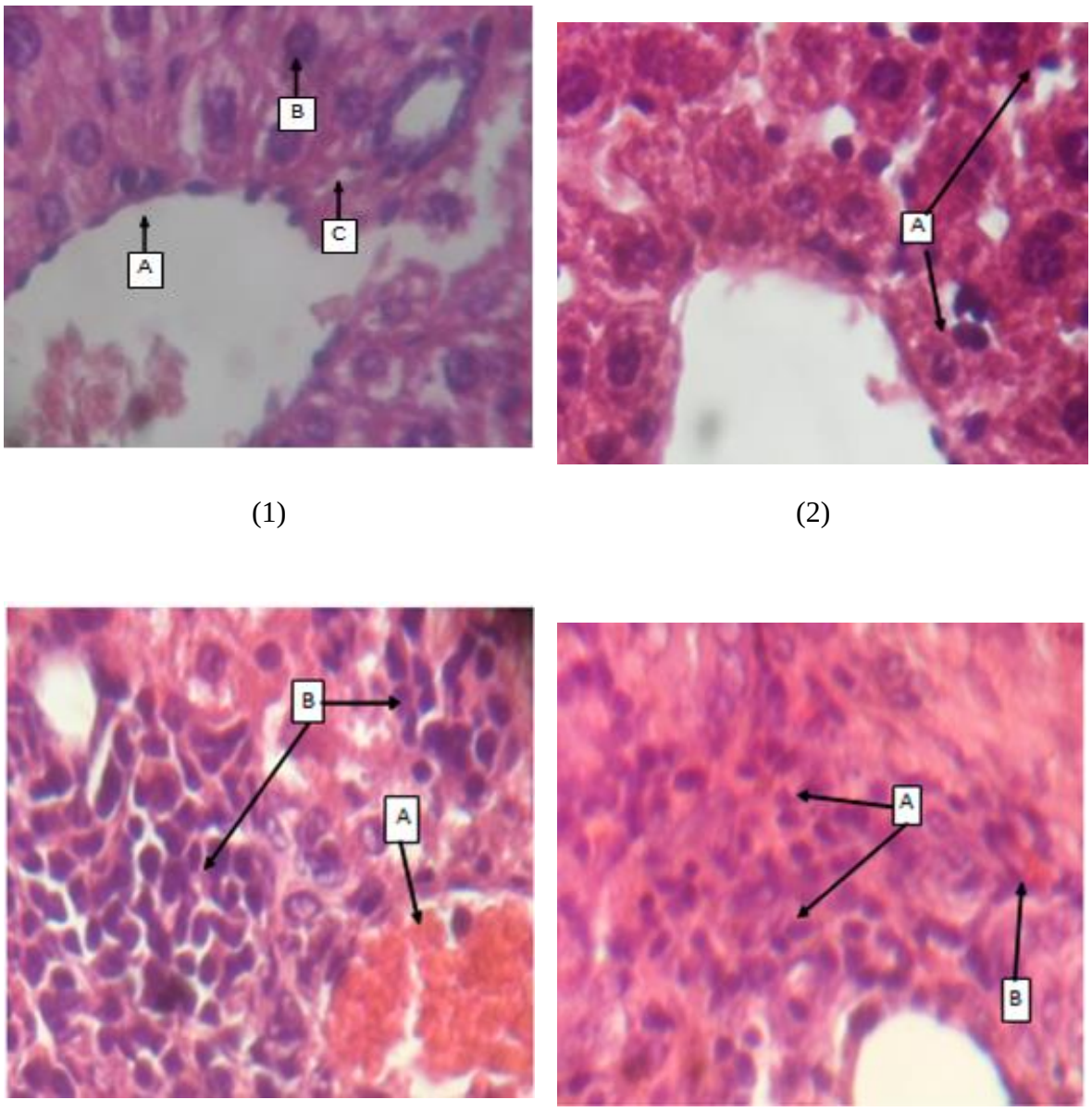

Figure 1: Photomicrograph of liver tissues from mice (H & E, x400). (1) Control (2) HSCE-only (3) Paracetamol-only (4) Paracetamol at zero time and HSCE 8 h later.