JMBR: A Peer-review Journal of Biomedical Sciences
December 2015, Vol. 14 No. 2 pp 118-128

PROTECTIVE PROPERTIES OF YOYO CLEANSER BITTERS AGAINST MERCURY II CHLORIDE (HGCL2)-INDUCED KIDNEY DAMAGE IN ADULT WISTAR RATS

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ABSTRACT

This study was aimed at investigating the effects of oral administration of Yoyo Cleanser Bitters on the mercuric chloride-induced kidney damage in adult Wistar rats. Thirty adult Wistar rats weighing between 180 and 210 g were grouped into six groups of five rats each. Group A animals served as control that were neither administered with $HgCl_2$ nor Yoyo Cleanser Bitters. Groups B, E and F animals were treated with oral administration of 5 mg/kg body weight $HgCl_2$. In addition, groups E and F were treated daily with 0.429 ml/kg body weight and 0.857 ml/kg body weight of Yoyo Cleanser Bitters respectively, for 28 days. Groups C and D animals were treated with oral administration of 0.429 ml/kg body weight and 0.857 ml/kg body weight of Yoyo Cleanser Bitters only respectively, for 28 days. After 28 days, the animals were anesthetized via chloroform inhalation and the kidneys were harvested for routine histological and oxidative stress analysis. Significant ($P < 0.05$) increase in MDA level and significant ($P < 0.05$) decrease in SOD level were observed in the group treated with mercuric-chloride only when compared with the control group. There was no significant differences ($P>0.05$) in MDA and SOD levels when the groups treated with Yoyo Cleanser Bitters only were compared with the control group. More so, there was no significant differences ($P>0.05$) in MDA and SOD levels when the groups treated with mercuric chloride followed by Yoyo Cleanser Bitters were compared with the control group. It could, thus, be inferred from this that Yoyo Cleanser Bitters modulated the changes in MDA and SOD caused by mercuric-chloride. The histological results also showed that the kidneys from the groups treated daily with oral administration of 5 mg/kg body weight of HgCl2 followed 0.429 ml/kg body weight and 0.857 ml/kg body weight of Yoyo Cleanser Bitters for 28 days respectively were essentially normal similar to the control group. From this study, it can be concluded that Yoyo Cleanser Bitters has a protective ability on mercuric chloride-induced kidney toxicity.

INTRODUCTION

Mercuric chloride ($HgCl_2$) is a white crystalline substance that is currently used as a catalyst or reagent in various chemical reactions, and to a lesser extent as a disinfectant or pesticide.[1] The acute lethal dose for most inorganic mercury compounds including $HgCl_2$ for an adult is 1 to 4 grams or 14 to 57 milligrams per kilogram body weight for a 70 kg person.1,2 Acute poisoning with typically $HgCl_2$ generally targets the gastrointestinal tract and the kidneys. Extensive precipitation of enterocyte proteins occurs, with abdominal pain, vomiting, and bloody diarrhoea with potential necrosis of the gut mucosa. This may produce death either from peritonitis or from septic or hypovolemic shock. Surviving patients commonly develop renal tubular necrosis with anuria.[3,4] Chronic poisoning with mercury salts is rare, usually also involving concomitant occupational exposure to mercury vapour. Kidney toxicity involves either renal tubular necrosis or autoimmune glomerulonephritis, or

KEYWORDS: *Protective, Yoyo Cleanser Bitters, Mercuric Chloride, kidney damage, Wistar rats*

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both.[3,4]

Findings from several studies suggest that one of the mechanisms involved in renal cellular injury induced by either in vivo or in vitro exposure to mercury involves the induction of oxidative stress.[5] The high affinity between mercuric ions and thiols suggests that the depletion of intracellular thiols either directly causes, or predisposes, proximal tubular cells to oxidative stress.[5] More so, depletion of cellular antioxidants like ascorbic acid and vitamin E had been reported in the kidneys of rats treated with mercuric chloride.[5,6] There also appears to be depletion of several antioxidant enzymes after in vivo exposure of rats to mercuric chloride. For instance, decreases in the activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione disulphide reductase in the renal cortex had been reported in male Sprague-Dawley rats after mercuric chloride administration.[5,7]

The discovery that compounds derived from plants could act as potential therapeutic weapons against various human, animal and even plant diseases, in addition to their food and nutritionalvalues, has made plants invaluable and indispensable to human and animal lives.[8] The inability to afford modern medical healthcare in developing countries have forced patients to seek traditional medical attentions and as a result, herbal medicine is renowned as the most common form of alternative medicine.[9] This form of medicine has however become an essential solution to health problems regardless of gender, marital status, education, socio-economic status, place of residence and religious affinity.[10,11] In this regard, the World Health Organization (WHO) has estimated that about eighty percent of the world populations rely chiefly on traditional medicines.[12]

Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases.[13,14] Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. The rationale for their utilization has rested largely on long-term clinical experience.[14] Now, with the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants had helped and continues to help in validating their folkloric usage.[13]

In Nigeria several plants have been claimed, traditionally, to have medicinal potentials for the treatment of various ailments in both man and animals.[15,16] However, their efficacy and safety remain doubtful as only a few of these have been properly identified and documented.[17,18]

Herbal bitters are most often polyherbal liquid formulations prepared from mixtures of many plant parts obtained from various plant species and families. Yoyo Cleanser Bitters is a mixture in the class of the internationally recognized bitters, manufactured by Abllat Nigeria Limited, a Nigerian health care product provider. Certified by National Agency for Food, Drugs and Control (NAFDAC) in 2003 as real bitters without alcohol, coloring or artificial preservatives,this organic drug has received wide acceptance and usage by the general populace since its introduction into the Nigerian drug market.[19] It is a powerful blend of some premium quality herbs well formulated to reduce free radical damage and removal of harmful toxins in the body, thereby supporting the immune system and the

body's ability to resist disease.[19]

The ingredients used for the production of Yoyo Cleanser Bitters include Aloe vera, *Acinos avensis, Chenopodium murale,Citrus aurantifolia and Cinnamomum aromaticum.*[19] These various constituents have been known for their various antioxidative properties.[20,21,22,23] This drug was thus, formulated in such a way that ingredients have a synergistic effect on the management of digestive system, circulatory system, nervous system, urinary and excretory, ulceration and hardening of tissues.[19]

Whereas the medicinal values of Yoyo Cleanser Bitters are well-documented, no study has been carried out on the effect(s) of this substance against a known agent with renal toxicity. This study was therefore aimed at investigating the effects of oral administration of Yoyo Cleanser Bitters (which has strong antioxidant properties) on the mercuric chloride-induced kidney damage in adult Wistar rats, bearing in mind that mercuric chloride induces oxidative stress to the kidney.

MATERIALS AND METHOD

EXPERIMENTAL DESIGN

The experimental animals used for this study comprised of thirty (30) adult male Wistar rats, weighing between 180 g and 210 g. The animals were procured from the Animal Holdings of Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo state, Nigeria. The animals were allowed to acclimatize for 2 weeks. All the animals were allowed free access to food (rat chow) and water during acclimatization and throughout the duration of the experiment. The experimental protocol was approved by the Research Ethics Committee (REC), College of Medical Sciences, University of Benin, Benin, Benin City, Edo State, Nigeria.

The animals were grouped into six groups of five rats each.

Group A animals served as control that were neither administered with $HgCl_2$ nor Yoyo Cleanser Bitters.

Group B animals were treated daily with oral administration of 5 mg/kg body weight $HgCl_2$ only.

Group C animals were treated with oral administration of 0.429 ml/kg body weight of Yoyo Cleanser Bitters only, for 28 days.

Group D animals were treated with oral administration of 0.857 ml/kg body weight of Yoyo Cleanser Bitters only, for 28 days.

Group E animals were treated daily with oral administration of 5 mg/kg body weight of $HgCl_2$ followed by 0.429 ml/kg body weight of Yoyo Cleanser Bitters, for 28 days.

Group F animals were treated daily with oral administration of 5 mg/kg body weight of $HgCl_2$ followed by 0.857 ml/kg body weight of Yoyo Cleanser Bitters, for 28 days.

The drug was administered to groups C and E at the manufacturer's recommended dose for adult human which is 30 ml for 70 kg adult[19] which amounted to 0.429 ml/kg body weight while groups D and F were given double dose of 0.857 ml/kg body weight. Administration of the herbal bitters was performed orally, once daily using metal cannula attached to a 1.0 ml syringe.

METHOD OF SACRIFICE AND TISSUE COLLECTION

After 28 days, the animals were anesthetized via chloroform inhalation, the anterior abdominal wall of the rats were exposed by midline incision and the kidneys were harvested and quickly fixed in 10 % formol-saline for 24 hour before the routine histological analysis.

HISTOLOGICAL TECHNIQUE

Paraffin Tissue Processing: Following the fixation of the kidney tissue in 10 % formol-saline, the tissues were dehydrated in ascending grades of alcohol, cleared in xylene, infiltrated in molten paraffin wax in an oven, embedded with embedding mold in molten paraffin wax and sectioned using a rotary microtome (thickness of 5μ) prior to routine haematoxylin and eosin staining.

Haematoxylin and Eosin Staining Method: Good tissue sections which came out as ribbons were placed in 20 % alcohol for spreading of the tissue, which was then floated in a water bath at temperature of 30°C. The sectioned tissues were picked with slides and allowed to dry.

The tissues were placed in xylene to remove excess paraffin wax, rehydrated by passing them through descending grades of alcohol and water, for about 2 minutes each. The tissues were stained in haematoxylin for about 10-15 minutes and rinsed in water. Excess stains were removed by washing under tap water for 2-3 minutes, followed by differentiation of tissues in one percent acid alcohol for a minute. The tissues were then blued in running tap water, counter- stained with 1 % eosin for 3-5 minutes, rinsed in water and dehydrated rapidly by passing through ascending grades of alcohol, cleared in xylene and finally mounted in DPX (Distrene Plasticizer and Xylene) covered with a cover slip, for photomicroscopic studies.

Photomicrography: The tissue sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at various magnifications (X100 and X400).

ANTIOXIDANT ENZYME ESTIMATION

Preparation of Sample: Known weights of different samples of the kidneys from the experimental animals were dissected out, homogenized in a mortar and pestle with a pinch of acid washed sand and a total of 5 ml normal saline (0.95 %) added sequentially during the homogenization process. The homogenates were centrifuged at 3500 rpm for 5 minutes. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated till needed for the assays.

Superoxide Dismutase (SOD) assay: The SOD activities in these tissues were determined by the method of Misra and Fridovich.[24] The supernatant (0.4 ml) was added to 5vml of 0.05 M carbonate buffer (pH 10.2) equilibrated in a spectrophotometer for 2-3 minutes. The reaction was then initiated by the addition of 0.6 ml of freshly prepared 0.3 Mm adrenaline as substrate to the buffered supernatant mixture which was quickly mixed by inversion and the absorbance taken. The increase in absorbance of 420 nm due to the adenochrome formed was monitored

every second for 120 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50 % inhibition of the auto-oxidation of adrenaline to adenochrome during 120 seconds.

Malondialdehyde (MDA) Assay: Serum MDA levels were estimated by the method of Beuge and Aust using Thiobarbituric Acid (TBA).[25] The acid reacts with MDA to form a stable pink color with maximum absorption at 535 nm. According to this method, 375 mg of TBA was dissolved in 2 mL of 0.25 N (HCl), followed by 15 g of Trichloroacetic Acid (TCA) for a total volume of 100 mL. The solution was heated in a water bath at 508^{o}C to dissolve TBA properly.Then, 1 ml of serum was combined with 2 ml of TCA-TBA-HCl and mixed thoroughly. The solution was heated for15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation. Sample absorbance was then determined at 535 nm against a blank that contained all reagents except the serum sample. Serum MDA concentration was expressed as nmol/ml.

STATISTICAL ANALYSIS

The data generated were analyzed using descriptive and inferential statistics. All the values were presented as mean $\pm$ Standard Error of Means (S.E.M). All statistical analysis was carried out using Statistical Package for Social Sciences (SPSS, version 16, Chicago, Il). The significance of difference in the means of all parameters was determined using One Way Analysis of Variance (ANOVA; 95% confidence interval). Post hoc test was carried out for all groups and compared with control.

RESULTS

Oxidative Stress Profile (See the Table 1) Significant ($P < 0.05$) increase in MDA level and significant ($P < 0.05$) decrease in SOD level were observed in Group B (treated with mercuric-chloride only) when compared with Group A (control group). There was no significant difference ($P>0.05$) in MDA and SOD levels when Groups C, D, E and F were respectively compared with Group A. However, significant ($P < 0.05$) decrease in MDA level and a significant ($P < 0.05$) increase in SOD level was observed in Groups E and F (treated with mercuric chloride followed by Yoyo Cleanser Bitters) when compared with group B (treated with mercuric-chloride only). It could, thus, be inferred fromthis that Yoyo Cleanser Bitters modulated the changes in MDA and SOD caused by mercuric-chloride.

TABLE 1: Comparison of some oxidative stress parameters between all the experimental groups

	Group A	Group B	Group C	Group D	Group E	Group F	ANOVA (P)
MDA	7.89 ± 0.80^{a}	17.72 ± 1.49^{b}	10.54 ± 1.06^{a}	9.85 ± 0.63^{a}	9.85 ± 1.55^{a}	8.60 ± 0.70^{a}	**<0.05***
SOD	4.60 ± 0.21^{a}	2.66 ± 0.19^{b}	4.51 ± 0.21^{a}	4.88 ± 0.13^{a}	4.21 ± 0.29^{a}	4.17 ± 0.50^{a}	**<0.05***

* Statistically significant (ANOVA)

- Post-hoc: Like superscripts =no statistically significant difference (P>0.05)
- Post-hoc: Unlike superscripts = statistically significant difference (P<0.05)

HISTOLOGICAL OBSERVATION

Plate 1 shows the histology of the kidney from the control group with normal glomeruli, Bowman's spaces, tubules and interstitial spaces. Plate 2 shows the histology of the kidney from the group treated daily with oral administration of 5 mg/kg body weight $HgCl_2$ only showing distorted glomerulus, increased Bowman's space, focal tubular swelling, luminal obstruction and renal interstitial hemorrhage. Plates 3 and 4 show the histology of the kidneys from the groups treated with oral administration of 0.429 ml/kg body weight and 0.857 ml/kg body weight of Yoyo Cleanser Bitters only respectively, for 28 days showing normal histology similar to the control group. Plates 5 and 6 show the histology of the kidneys from the groups treated daily with oral administration of 5 mg/kg body weight of $HgCl_2$ followed 0.429 ml/kg body weight and 0.857 ml/kg body weight of Yoyo Cleanser Bitters for 28 days respectively. The result is essentially that of normal histology of the kidney similar to the control group.

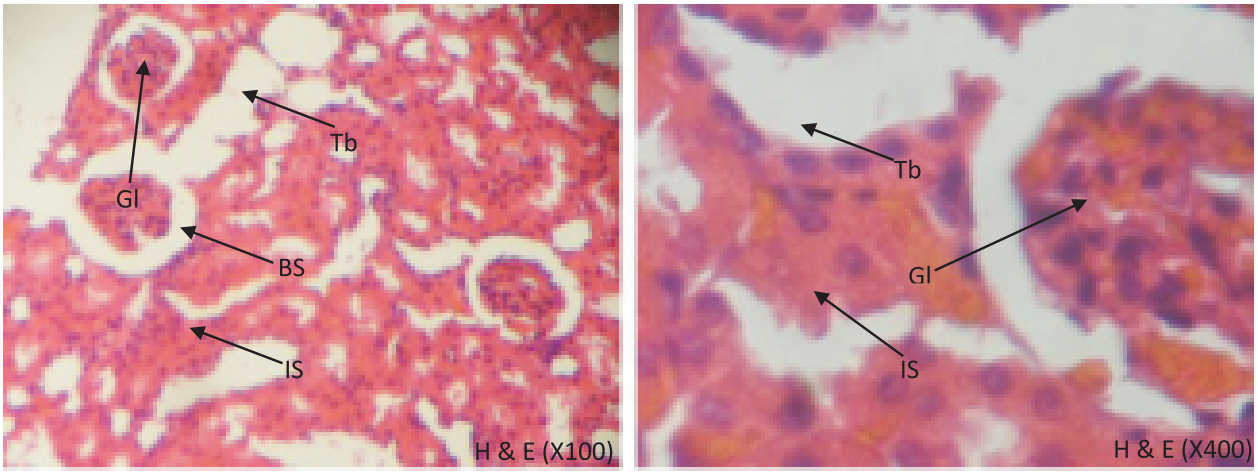

PLATE 1: Photomicrographs of the kidneys of Group A (Control group) showing normal glomeruli '**Gl**', Bowman's spaces **'BS',** tubules '**Tb**' and interstitial space '**IS**'

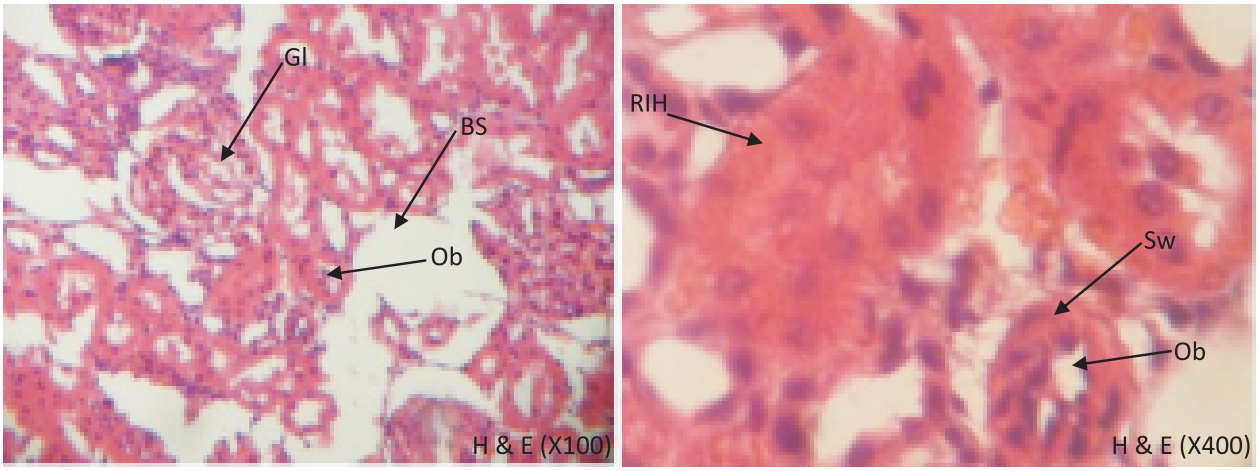

PLATE 2: Photomicrographs of the kidneys of Group B (treated daily with oral administration of 5 mg/kg body weight $HgCl_2$ only for 28 days) showing, increased Bowman's space **'BS'**, focal tubular swelling **'Sw',** luminal obstruction **'Ob'** and renal interstitial hemorrhage **'RIH'**

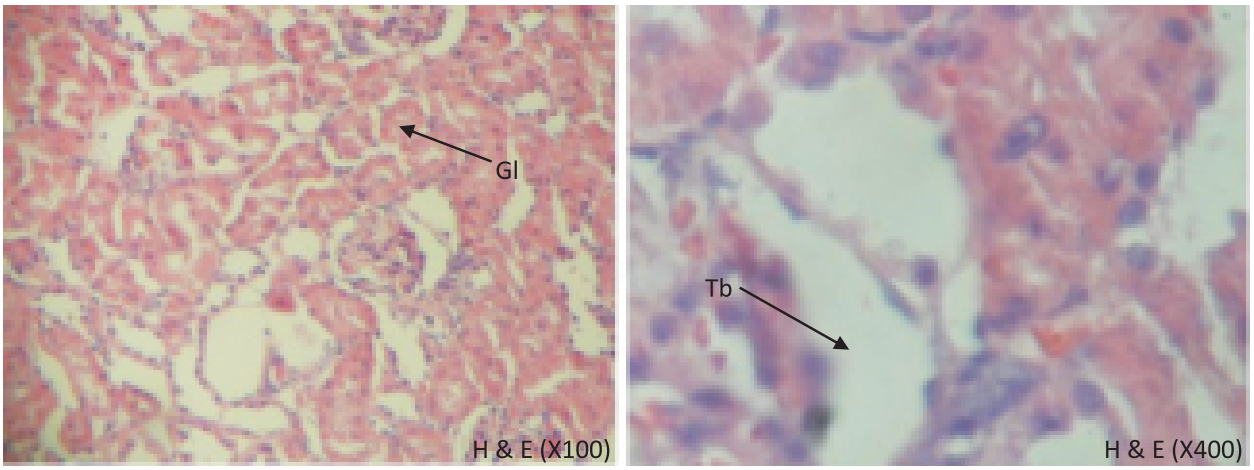

PLATE 3: Photomicrographs of the kidneys of Group **C** (with oral administration of 0.429 ml/kg body weight of Yoyo Cleanser Bitters only, for 28 days) showing normal histology of the kidney similar to the control group with glomerulus **'Gl' and** tubules **'Tb'**.

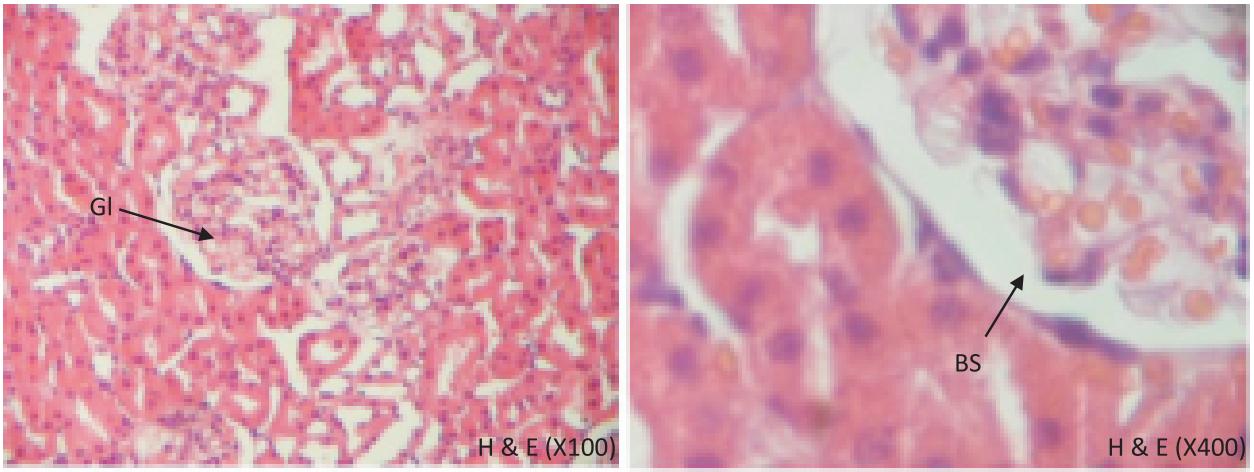

PLATE 4: Photomicrographs of the kidneys of Group E (treated with oral administration of 0.857 ml/kg body weight of Yoyo Cleanser Bitters only, for 28 days) showing normal histology of the kidney similar to the control group with glomerulus **'Gl' and** Bowman's space **'BS'**.

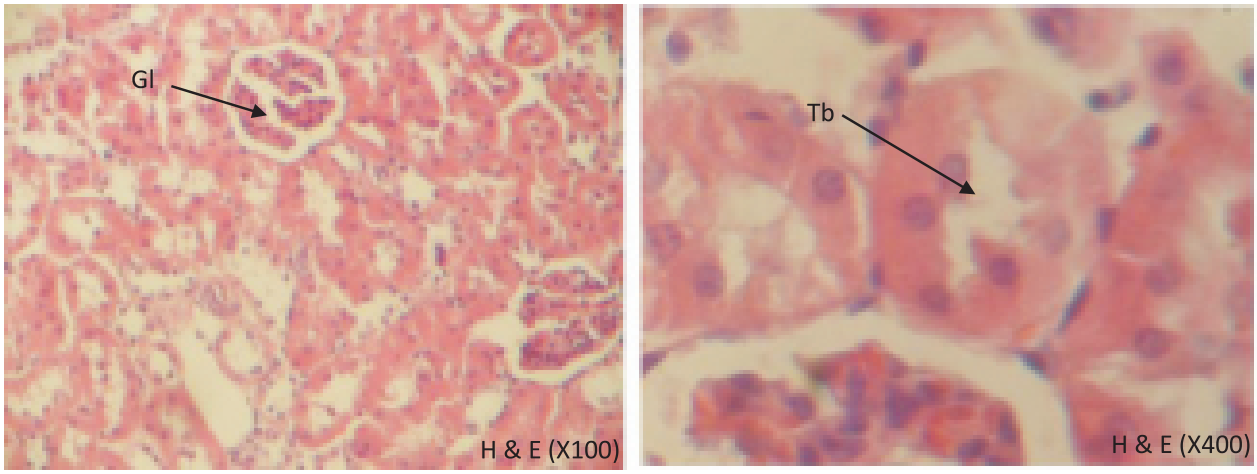

PLATE 5: Photomicrographs of the kidneys of Group D (treated daily with oral administration of 5 mg/kg body weight of $HgCl_2$ and 0.429 ml/kg body weight of Yoyo Cleanser Bitters, for 28 days) showing normal histology of the kidney similar to the control group with glomerulus **'Gl' and** tubules **'Tb'**.

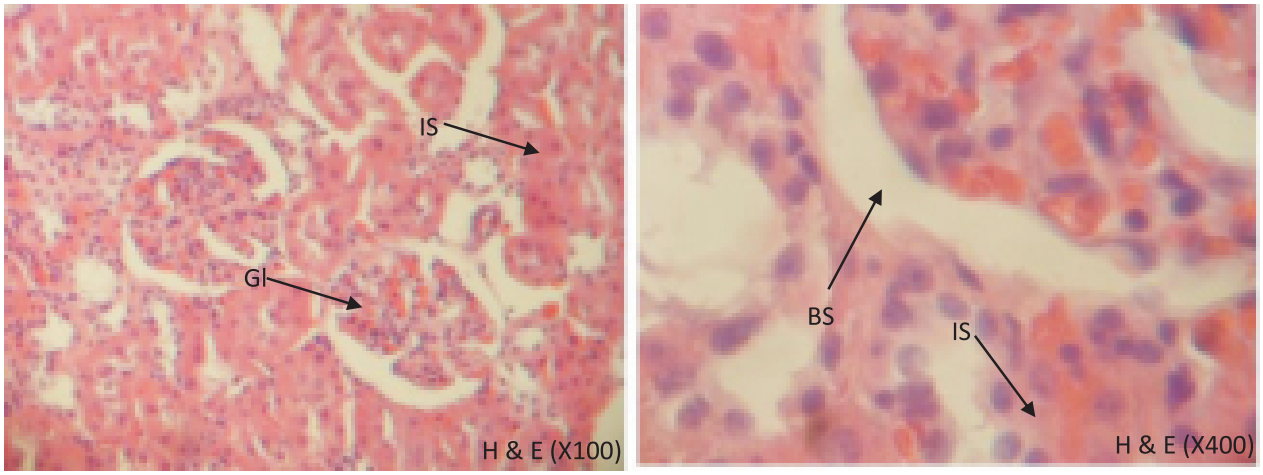

PLATE 6: Photomicrographs of the kidneys of Group F (treated daily with oral administration of 5 mg/kg body weight of $HgCl_2$ and 0.857 ml/kg body weight of Yoyo Cleanser Bitters, for 28 days) showing normal histology of the kidney similar to the control group with glomerulus **'Gl',** Bowman's space '**BS', and** interstitial space **'IS'**.

DISCUSSION AND CONCLUSION

Oxidative stress had been well established as one of the mechanisms of actions in mercury- induced toxicity.[5-7] This results in the excessive release of reactive oxygen species and increased lipid peroxidation in the cells.[26] Free radicals and intermediate products of peroxidation have theability to destroy the integrity and altering the function of biomembranes, which can result in many pathological processes.[27] Various specific enzymes that limit free-radical formation, such as superoxide dismutase (SOD), play important role in the protection of cell membranes against oxidative damage.[28]

This study showed a significant decrease in kidney superoxide dismutase in rats treated with mercuric chloride. This is in agreement with an earlier study.[29] The resultant increase in superoxide dismutase (SOD) levels in the kidneys of rats initially treated with mercuric chloride and followed by the administration of various doses of Yoyo Cleanser Bitters could be as a result of the possible antioxidant properties of Yoyo Cleanser Bitters. An earlier study had discovered the presence of superoxide dismutase in Aloe Vera which is an important constituent of Yoyo Cleanser Bitters.[30]

Malondialdehyde is one of the major oxidation products of peroxidized polyunsaturated fatty acids. Increased malondialdehyde content is an important indicator of lipid peroxidation.[31] This study showed significant increase in malondialdehyde levels in the kidney of rats treated with mercuric chloride when compared with control. This result agrees with previous studies that mercuric chloride increase MDA level in tissues.[32,33] It was observed from this study that there was a significant decrease in MDA levels in all the groups treated with Yoyo Cleanser Bitters following mercuric chloride intoxication.

Induction of renal cortical proximal toxicity by mercuric chloride and renal proximal tubules as the target had been well established experimentally.[34-37] In this study, the observed histological changes following administration of mercuric chloride showed that mercuric chloride induced distortion of the glomerulus, renal interstitial hemorrhage, luminal obstruction and swelling oftubular epithelium. This study also reveals the potency of Yoyo Cleanser Bitters in protecting the kidney against these damages.

Conclusively from this findings, Yoyo Cleanser Bitters is observed to have protective effect on the oxidative stress injury induced by mercuric chloride on the kidney. The beneficial effects of this medication is therefore supported by this study, hence the product is recommended for use.

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