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Oral and Ocular Safety Profile of Whole Plant Aqueous Extract of *Heliotropium indicum* L. in Rodents

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ABSTRACT

Heliotropium indicum is used in folk medicine in Ghana and elsewhere in Africa to manage systemic and ocular disorders (some of which are chronic) amidst inadequate safety data. The aim of this study, therefore, was to assess the toxic effect of medium term (sub-chronic) oral and ocular uses of the rodents to provide an initial predictive safety consequence for human usage. Subchronic toxicity (30-300 mg kg^{-1}) and ocular toxicity studies of whole plant aqueous (0.5-2% w/v) extract of *H. indicum* were conducted in rats and rabbits, respectively. Blood samples were assessed for biochemical and hematological parameters. Organ, such as, the liver, kidney, lungs and spleen were assessed for histopathological signs of toxicity. For the ocular toxicity study, rabbits were assessed for conjunctival, corneal and iris defect by the use of a slit lamp biomicroscope. Blood biochemistry revealed significant ($p \leq 0.05$-0.01) elevations in direct bilirubin levels in all treatment groups but significant ($p \leq 0.01$-0.001) reductions in alkaline phosphatase (ALP) levels at all dose levels. Rats treated with 100 mg kg^{-1} further showed a significant reduction ($p \leq 0.01$) in albumin levels but triglycerides were significantly ($p \leq 0.05$) elevated. Only rats treated with 300 mg kg^{-1} HIE extract showed significant ($p \leq 0.01$) reduction in blood urea nitrogen levels. Repeated exposure of rats to different doses of extract over the 30 day period produced subtle patho-morphological changes in the kidney, liver, spleen except the lungs. The extracts caused no significant damage to the cornea, iris and conjunctival tissues at various time points ($p > 0.05$). Per the findings, the extract used was safe upon topical ocular application however, medium term oral use induced subtle kidney, liver and spleen toxicities.

Key words: Draize test, ocular toxicity, conjunctiva, blood biochemistry, herbal

INTRODUCTION

The use of herbal medicine in traditional medical practices are embedded in the cultural patrimony of many Africans, who practice self medication with familiar herbal products or consult with local herbalists. While their use is evidently motivated by the notion that natural is safe and effective, there are reports of grave adverse effects associated with indiscriminate and excessive use of some herbal products (Bent, 2008; George, 2011). These grave adverse effects have been found to limit the use of the otherwise efficacious preparations, when used appropriately.

One such plant used among some Ghanaian folks and elsewhere in Africa as an antidote for common eye disorders notably conjunctivitis, cataract and high intra-ocular pressure (ocular hypertension) is *Heliotropium indicum*. The major routes of application are oral or topical, either, as a dietary component for postpartum women, poultice to sores or squeezed directly into the eye (PRSPI., 1992; Burkhil, 1985; Kerharo and Bouquet, 1950). Most of these disorders are chronic and require medium to long term use of the plant preparation to achieve curative results. However, there is no available data on the safety profile of therapeutic doses of *H. indicum* upon medium term oral usage (subchronic toxicity) and topical ocular application (ocular toxicity).

This study sought to evaluate the safety of the traditional use of *H. indicum* using rodent models as an initial step in predicting its safety for human use.

MATERIALS AND METHODS

Plant collection: *Heliotropium indicum* (botanical name) commonly called Heliotrope or Indian heliotrope (English name) and locally called Akomfetiko (Twi) was collected in November, 2012, from the University of Cape Coast botanical gardens (5.1036°N, 1.2825°W), Cape coast, Ghana. The plant name was checked with www.the plantlist.org on 13th October, 2012. It was further identified and authenticated by a botanist at the School of Biological Sciences, College Agricultural and Natural Sciences, University of Cape Coast, Cape Coast, Ghana, where a voucher specimen (Specimen number: 4873) has been deposited.

Preparation of the *H. indicum* aqueous extract (HIE): Whole plants of *H. indicum* were washed thoroughly with tap water and shade-dried. The dry plants were milled into coarse powder by a hammer mill (Schutte Buffalo, New York, NY). One kilogram of the plant powder was mixed with one liter of water. The mixture was soxhlet extracted at 80°C, for 24 h. The aqueous extract was freeze-dried (Hull freeze-dryer/lyophilizer 140 SQ, Warminster, PA). The powder obtained (yield 12.2%), was labeled HIE and stored at a temperature of 4°C. This was reconstituted in normal saline to the desired concentration for dosing in this study.

Experimental Animals and Husbandry: Sprague Dawley rats of either sex (weight: 115±15) and New Zealand White rabbits (weight: 1±0.2 kg) were kept in the Animal House of the School of Biological Sciences, University of Cape Coast, Ghana, for use in a subchronic and ocular toxicity study, respectively. The rats were housed in groups of five in an aluminum cages (34×47×18 cm) with soft wood shavings as bedding, while the rabbits were housed in individual cages. The animals were kept under ambient laboratory conditions (temperature 28±2°C, relative humidity 60-70% and a normal light-dark cycle), fed on a normal commercial pellet diet (Agricare Ltd, Kumasi, Ghana) and had access to water *ad libitum*.

Ethical and biosafety considerations: The study protocols were approved by the Institutional Review Board on Animal Experimentation, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (Ethical clearance number: FPPS/PCOL/0030/2013). All activities performed during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC) and Association for Research in Vision and Ophthalmology Statement for use of Animals in Ophthalmic and Vision research. Biosafety guidelines for protection of personnel in the laboratory were observed.

Preliminary phytochemical screening: Screening was performed on HIE to ascertain the presence of phytochemicals using standard procedures described by Harborne (1998) and Kujur *et al*. (2010).

Drugs and chemicals used: The AST, ALT, ALP, urea, triglycerides, cholesterol and creatinine assay kits (Fortress Diagnostic, UK) were used to assess blood biochemistry. All other chemicals were of high purity grade.

Sub-chronic toxicity study: Four groups of seven Sprague-Dawley rats were dosed daily, for thirty days, with either 30, 100 and 300 mg kg^{-1}, *per os*, HIE, or vehicle (dose). The animals were observed individually after dosing during the first 30 min and periodically, during the first 24 h, with special attention given during the first four hours and daily thereafter, for a total of 30 days. Rats were observed for changes in skin and fur, eyes and mucous membranes , respiratory and behaviour pattern. Special attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Animals in each group were weighed before drug administration and every other week during the study period.

Blood samples from animals in each group (via the jugular vein) were collected on 31st day (after the experiment had ended) into ethylenediamine tetra-acetic acid (EDTA) tubes for hematological and biochemical analysis at the Cape Coast Teaching Hospital laboratory using, Sysmex haematology autoanalyzer (Model: KX-21N, Kobe, Japan). The lung, liver, kidney and heart were collected, weighed and preserved in 10% phosphate buffered formalin for histopathological studies at the Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana by an experimental pathologist. Observation for clinical and behavioral signs of toxicity (changes in the skin and fur, eyes, mucous membranes convulsions, salivation, diarrhea, lethargy, sleep, coma, laboured respiration, death, staggering, wobbly gait, hind limbs exaggeration, over compensating and or making splayed movements, feet (primarily hind feet) point outward from body, forelimbs dragging and or showing abnormal positioning, or walking on toes), organ-to-body weight ratios determination, hematological, biochemical analyses and histopathological studies were basis for determining safety in sub-chronic studies.

Ocular toxicity studies: An ocular safety assessment was performed using Draize test with slight modification. New Zealand white rabbits were put in three groups of five, with animals into each group having only their right eye instilled with 0.1 mL of 0.5, 1.0 and 2.0% w/v solution of HIE in normal saline (pH = 7.2). The animals were examined for conjunctival chemosis, redness, discharge, corneal abrasions, iris defects and lens opacities and pupillary reflexes using, a penlight and slit lamp and were scored using, Draize's criteria for determining ocular toxicity after the pH (a principal pre-requite for Draize ocular toxicity testing) of the test substance (HIE) had been established. The examination was done at 1, 24, 48 and 72 h after HIE instillation.

Statistical analysis: GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses to establish the safety of HIE. Data are presented as Mean±SEM or SD and analyzed by One-way ANOVA followed by Dunnett's multiple comparison (*post-hoc*) test or T test used to compare effects. $p \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Preliminary phytochemistry showed that flavonoids, saponins, cyanogenic glycosides, sterol, tannins and alkaloids were present in HIE (Table 1).

Sub-chronic toxicity study

Observation for signs of toxicity: There were no observable abnormal changes in the skin and fur, eyes, mucous membranes convulsions, salivation, diarrhea, lethargy, sleep, coma, respiration and no death was recorded. Further observations of behavioral pattern of gait did not show uncoordinated, staggering, wobbly gait, hind limbs exaggerated, over compensating and or making splayed movements, feet (primarily hind feet) point outward from body, forelimbs dragging and or showing abnormal positioning, nor walking on toes (the heels of the hind feet are perpendicular to the surface).

Hematological and biochemical assessment: Hematological assessments revealed no significant ($p>0.05$) effect of HIE treatment in all treatment group on hematological parameters compared to the control by the 30th day period (Table 2 and 3). However, blood biochemistry

Table 1: Results obtained after preliminary phytochemical screening of HIE

Phytochemical tested for	Results obtained
Anthraquinones	-
Tannins	+
Flavonoids	+
Alkaloids	+
Sterols	+
Glycosides	+
Saponins	+
Triterpenoids	-

+: Present, -: Absent

Table 2: Hematological assessment after treatment of Sprague-Dawley rats with 30, 100 and 300 (mg kg^{-1}) of HIE in a sub-chronic toxicity study

		Concentration of HIE (mg kg^{-1})		
Parameters	Control	30	100	300
WBC ($\times10^9$ L^{-1})	9.69±0.73	7.01±0.66^{ns}	7.59±1.16^{ns}	11.68±1.10^{ns}
RBC ($\times10^6$ μL^{-1})	9.93±0.21	9.76±1.27^{ns}	10.24±1.30^{ns}	9.66±0.24^{ns}
HB (g dL^{-1})	16.82±0.34	17.34±2.26^{ns}	17.90±2.58^{ns}	16.48±0.38^{ns}
HCT (%)	54.90±0.99	52.46±6.11^{ns}	55.34±5.70^{ns}	52.56±1.12^{ns}
MCV (f L)	55.34±1.13	54.08±0.83^{ns}	54.60±1.32^{ns}	54.46±0.84^{ns}
MCH (pg)	16.96±0.17	17.80±0.42^{ns}	17.40±0.37^{ns}	17.08±0.34^{ns}
MCHC (g d^{-1})	30.64±0.61	32.90±0.65^{ns}	31.94±1.29^{ns}	31.38±0.39^{ns}
PLT ($\times10^3$ μL^{-1})	660.00±165.50	609.00±89.78^{ns}	891.20±236.90^{ns}	736.80±88.65^{ns}
PDW	11.40±0.27	10.68±0.50^{ns}	10.06±0.39^{ns}	10.80±0.52^{ns}
MPV (f L)	8.74±0.23	7.84±0.33^{ns}	8.46±0.172^{ns}	8.66±0.36^{ns}
NEUT ($\times10^3$ μL^{-1})	0.29±0.03	0.50±0.18^{ns}	0.81±0.23^{ns}	0.41±0.12^{ns}
LYMP ($\times10^3$ μL^{-1})	0.284±0.03	0.25±0.09^{ns}	0.40±0.07^{ns}	0.44±0.05^{ns}
MONO ($\times10^3$ μL^{-1})	0.31±0.06	0.274±0.0315^{ns}	0.45±0.09^{ns}	0.48±0.09^{ns}
EOS ($\times10^3$ μL^{-1})	0.31±0.06	0.27±0.035^{ns}	0.39±0.05^{ns}	0.49±0.10^{ns}
BASO ($\times10^3$ μL^{-1})	0.00±0.00	0.03±0.01^{ns}	0.02±0.01^{ns}	0.01±0.00^{ns}

WBC: White Blood Cell Count, HB: Hemoglobin, RBC: Red Blood Cell Count, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, LYM: Lymphocytes, RDW-CV and RWD-SD: Red Blood Cell Distribution Width, PLT: Platelet Count, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, P-LCR: Platelet Larger Cell Ratio. Values obtained for the various parameters after treatment. $^{ns}p>0.05$ are the level of significance of hematological profile compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*, NEUT: Neutrophil, LYMP: Lymphocytes, MONO: Monocytes, EOS: Eosinophil, BASO: Basophils

Table 3: Profile of blood biochemistry after treatment of Sprague-Dawley rats with 30, 100 and 300 mg kg^{-1} HIE in sub-chronic toxicity study

		Concentration of HIE (mg kg^{-1})		
Parameters	Control	30	100	300
Creatinine (mg dL^{-1})	0.62±0.33	0.85±0.138^{ns}	0.99±0.16^{ns}	0.98±0.16^{ns}
BUN (mg dL^{-1})	135.60±5.97	147.40±2.94^{ns}	130.40±5.45^{ns}	107.00±3.07**
Cholesterol (mg dL^{-1})	102.00±9.56	118.40±3.94^{ns}	85.40±2.25^{ns}	109.80±4.07^{ns}
Triglycerides (mg dL^{-1})	97.60±15.57	113.00±5.86^{ns}	140.80±5.88*	128.60±9.56^{ns}
Albumin (g dL^{-1})	4.46±0.11	4.02±0.18^{ns}	3.86±0.10**	4.02±0.09^{ns}
Protein (g gL^{-1})	8.78±0.20	8.48±0.26^{ns}	9.08±0.36^{ns}	9.60±0.40^{ns}
Direct bilirubin (mg dL^{-1})	1.74±0.28	4.02±0.58*	3.90±0.07*	4.98±0.71**
Total bilirubin (mg dL^{-1})	2.30±0.11	2.30±0.11^{ns}	2.44±0.12^{ns}	2.64±0.09^{ns}
ALP	585.40±30.74	699.20±27.88*	731.60±24.98**	766.40±20.19***
AST	226.00±5.45	218.20±6.30^{ns}	217.60±6.24^{ns}	250.40±17.06^{ns}
ALT	143.60±2.11	145.20±3.31^{ns}	144.00±3.73^{ns}	147.00±3.78^{ns}

ALT: Alanine transaminase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen.Values recorded are means and standard error of mean . Values obtained for the various parameters after treatment.*$p \leq 0.05$, **$p \leq 0.01$ are the level of significance of biochemical parameters compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*

Table 4: Relative organ weight of Sprague-Dawley rats treated with 30, 100 and 300 mg kg^{-1} HIE in sub-chronic toxicity studies

		Concentration of HIE (mg kg^{-1})		
Organ	Control	30	100	300
Liver	3.67±0.42	3.13±0.09^{ns}	3.23±0.25^{ns}	2.98±0.10^{ns}
Lungs	0.73±0.09	0.85±0.09^{ns}	0.80±0.05^{ns}	0.86±0.11^{ns}
Spleen	0.24±0.04	0.23±0.01^{ns}	0.31±0.05^{ns}	0.27±0.01^{ns}
Kidney	0.81±0.24	0.63±0.00^{ns}	0.82±0.057^{ns}	0.62±0.03^{ns}

Values recorded are Means±SEM. *$p \leq 0.05$, **$p \leq 0.01$ are the level of significance of blood parameters compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*

revealed significant ($p \leq 0.05$-0.01) elevations in direct bilirubin levels in all treatment groups, with significant ($p \leq 0.01$-0.001) reductions in ALP levels at all dose levels. Rats treated with 100 mg kg^{-1} further showed a significant reduction ($p \leq 0.01$) in albumin levels, while the triglycerides were significantly ($p \leq 0.05$) elevated. Only rats treated with 300 mg kg^{-1} showed significant ($p \leq 0.01$) reduction in Blood Urea Nitrogen (BUN) levels.

Histopathological assessment: The relative organ weight of the extract treated groups was not significantly different ($p > 0.05$) from the control group (Table 4). Repeated exposure of the rats to different doses of HIE over the 30 day period produced subtle patho-morphological changes in some of the organs assessed. These changes included edema with loss of glomerulus in some of the corpuscles of the kidney, edematous appearance with some activated Kupffer cells, infiltration of leucocytes within sinusoids of the liver, loss of the marginal zone architecture of the spleen, while the alveolar duct and respiratory bronchiole of the lungs remained normal in all treatment groups (Fig. 1-4).

Ocular toxicity studies: A comparison of the effect of HIE on the right eye to the left eye (control) using, paired T-test, topical administration of 0.1 mL of 0.5, 1.0 and 2.0% w/v indicated that HIE caused no significant damage ($p > 0.05$) to the cornea, iris and the conjunctiva over the entire experimental period (Table 5). The pHs of the 0.5, 1.0 and 2.0% w/v of HIE were recorded as (Mean±SD), 7.24±0.08, 7.18±0.19 and 7.08±0.07, respectively.

DISCUSSION

The presence of bioactive compounds as found in this study (Table1) indicated to be responsible for the biological activity of *H. indicum* have been widely reported (Boye *et al*., 2012; Dash and Murthy, 2011). The available studies that reported the safety of oral use of extracts of *H. indicum* adopted doses far higher than the therapeutic doses for either single dosing acute or repeated

Table 5: Ocular toxicological profile of topical administration of HIE on conjunctival, cornea and iris tissues of rabbit right eyes compared to the left eye under

	Control			HIE 0.5%w/v		
Time (h)	Conjunctiva	Cornea	Iris	Conjunctiva	Cornea	Iris
1	0.00±0.00	0.00±0.00	0.00±0.00	0.400±0.40	0.02±0.02	0.11±0.09
24	0.00±0.00	0.00±0.00	0.00±0.00	0.014±0.02	0.01±0.00	0.00±0.00
48	0.00±0.00	0.00±0.00	0.00±0.00	0.000±0.00	0.01±0.00	0.01±0.00
72	0.00±0.00	0.00±0.00	0.00±0.00	0.000±0.00	0.00±0.00	0.00±0.00
	HIE 1% w/v			HIE 2% w/v		
Time (h)	Conjunctiva	Cornea	Iris	Conjunctiva	Cornea	Iris
1	0.40±0.40	0.10±0.10	0.20±0.02	0.040±0.02	0.02±0.00	0.04±0.02
24	0.03±0.02	0.01±0.00	0.01±0.01	0.00±0.00	0.00±0.01	0.01±0.01
48	0.00±0.00	0.01±0.01	0.01±0.01	0.00±0.00	0.01±0.00	0.00±0.00
72	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values recorded are Means±SEM (n = 5). There was no significant damage (p>0.05) to the conjunctiva, cornea and iris of the right eye instilled with the test drug compared to the left eye as control

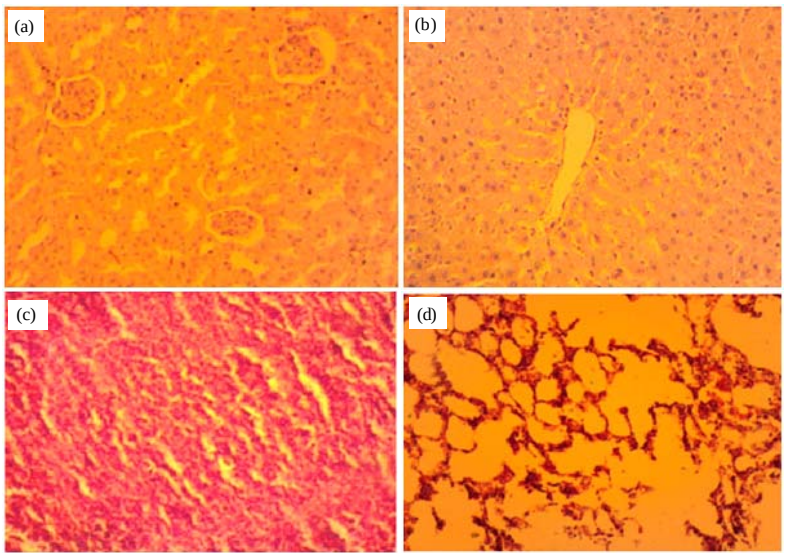

Fig. 1(a-d): Photomicrograph of the kidney, liver, spleen and lung of experimental rats (control) (H and E×100), (a) Normal histology of tubules, renal corpuscles and a well defined capsular space, (b) Liver section reveals normal histological structure of hepatocytes; hepatocytes polygonal in shape, tightly parked, containing basophilic central rounded nuclei separated by the hepatic sinusoids radiating from the central vein and with the presence of non-activated spindle shaped Kupffer cells within the sinusoids, (c) Section showing white pulp and the marginal zone of normal spleen and (d) Histological section of the lung of experimental rats showing normal alveolar duct and respiratory bronchiole

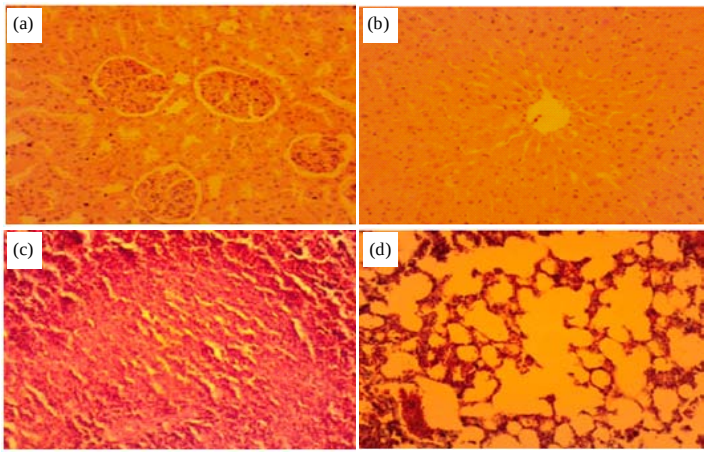

Fig. 2(a-d): Photomicrograph of the kidney, liver, spleen and lung of experimental rats (30 mg kg^{-1} treated) (H and E×100), (a) Normal histology of tubules, renal corpuscles and a well defined capsular space, (b) liver section reveals normal histological structure of hepatocytes; hepatocytes polygonal in shape, tightly parked, containing basophilic central rounded nuclei separated by the hepatic sinusoids radiating from the central vein and with the presence of non-activated spindle shaped Kupffer cells within the sinusoids, (c) Section showing white pulp and the marginal zone of normal spleen and (d) Histological section of the lung of experimental rats showing normal alveolar duct and respiratory bronchiole

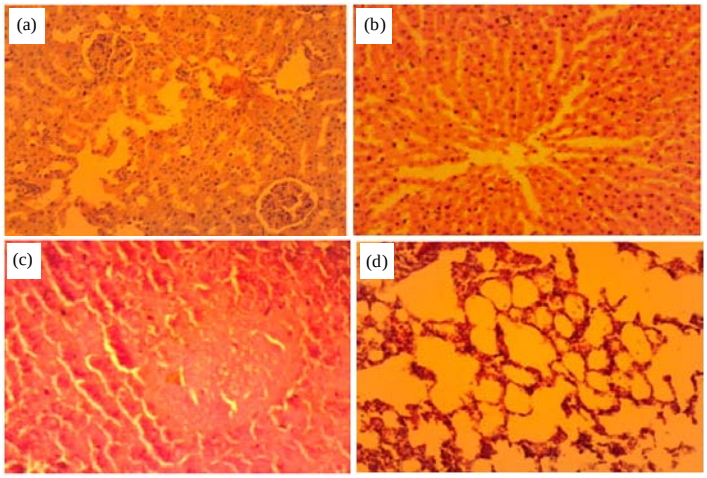

Fig. 3(a-d): Photomicrograph of the kidney, liver, spleen and lung of experimental rats (100 mg kg^{-1} treated) (H and E×100), (a) Histological sections appear edematous with loss of glomerulus in some of the corpuscles, with urinary space intact, (b) Tissue sections appear edematous with some Kupffer cells appearing activated and presence of infiltrated leucocytes within sinusoids, with dilatation of the CV, (c) Section showing altered white pulp and the marginal zone of the spleen showing loss of the marginal zone architecture and (d) Histological section of the lung of experimental rats showing normal alveolar duct and respiratory bronchiole

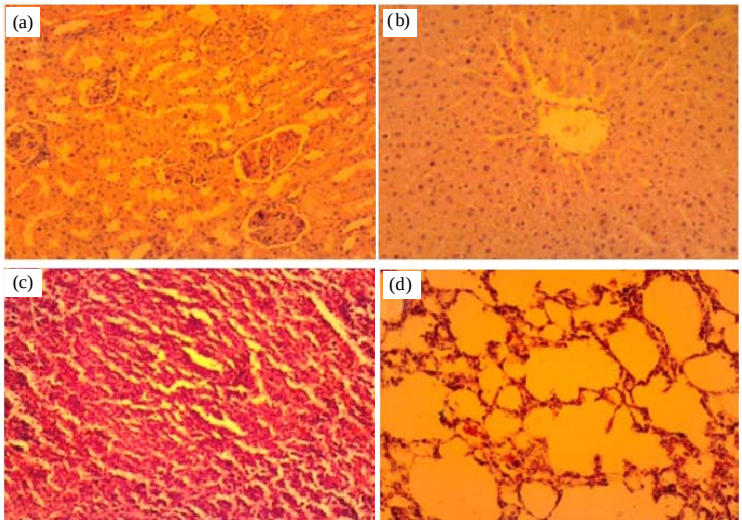

Fig. 4(a-d): Photomicrograph of the kidney, liver, spleen and lung of experimental rats (300 mg kg^{-1} treated) (H and E×100), (a) Normal histology of tubules, with some of the glomeruli nuclei appearing slightly more basophilia than normal with a well defined capsular space, (b) Liver section reveals normal histological structure of hepatocytes; hepatocytes polygonal in shape, tightly parked, containing basophilic central rounded nuclei separated by the hepatic sinusoids radiating from the central vein and with the presence of non-activated spindle shaped Kupffer cells within the sinusoids, (c) Section shows white pulp and the marginal zone of normal spleen and (d) Histological section of the lung of experimental rats shows normal alveolar duct and respiratory bronchiole

dosing subacute studies (Ghori *et al*., 2015; Boye *et al*., 2012; Dash and Murthy, 2011). However, no data is available on the safety of it ocular use.

Cage side observation for clinical and behavioral signs of toxicity did not show any manifest signs indicative of gross toxicity of various organ systems, such as; the nervous, endocrine, cardiovascular, respiratory systems etc. This presupposes that there was no marked damage to these systems to elicit these clinical or behavioral signs of safety concern.

The Draize test is the first approved modus of operandi for testing agents with a potential acute ocular toxicity *in vivo* (Wilhelmus, 2001). One principal prerequisite for this testing is to determine the pH of test agent (in this case HIE). The pH is considered a key physicochemical property to predict the probability of a test agent to cause ocular irritation and include redness, swelling, cloudiness, edema, hemorrhage, discharge and blindness, which are the bedrock of Draize test (Huhtala *et al*., 2008). The pH for the three doses were consistent with tolerable range (6.6-7.8) stipulated for topical eye preparations (Duval and Keshner, 2002). The application of solutions of HIE did not cause any significant ($p>0.005$) damage to the conjunctiva (notably redness of the palpebral conjunctiva, chemosis and mucus discharges), cornea opacity and iris (mainly congestion, swelling, circumcorneal injection, hemorrhages and their reaction to light). This may account for the convenient topical use HIE in the traditional management of certain ocular disorders, such as; conjunctivitis. It is important to stress that other properties of HIE, such as sterility and tonicity, may also warrant investigation, despite proof from the Draize test that it is a non-irritating agent.

The weight of the essential organs, such as the liver, kidney, spleen and lungs of rats exposed to different doses of HIE were comparable to the control group and were found to be normal. This result suggests that the changes were subtle and not marked enough to provoke grave structural changes in the rats. The hematological profile upon oral administration of HIE was unremarkable (Table 2). However, the biochemical analysis indicated concomitant elevation levels of direct bilirubin and alkaline phosphatase, ALP at all dose levels (Table 3) suggestive of a pathology. Diseases of the liver and bile, or the undue destruction of red blood cells, cause increased levels of bilirubin in the blood stream. This elevated level maybe manifest as jaundice and be seen in several forms of liver or biliary tract disease and is a nonspecific marker of liver disease. In this study, the elevated conjugated hyperbilirubinaemia is typical of parenchymal liver disease and biliary obstruction. This assertion of biliary obstruction is further supported by the elevated ALP, which is normally the case in number of disorders affecting the drainage of bile from the liver to the small intestine, such as gallstones (Limdi and Hyde, 2003).

The 100 mg kg^{-1} treated rats showed a rise in triglyceride and not cholesterol levels. This clearly indicates that the elevated triglyceride level does not constitute a risk factor for heart disease but rather could be associated with diabetes or pancreatitis. The concomitant albumin deficiency among the 100 mg kg^{-1} treated rats has been reported to be associated with increased plasma fibrinogen and triglyceride levels and may alter red cell membrane lipid composition. This clinical situation could be occasioned by medication intake, as in this study (Joles *et al*., 1997). The observed low levels of BUN in the rats are unusual and can be seen in severe liver disease, malnutrition and occasionally in over hydration. Although, this test is not usually used to diagnose or monitor these conditions. Histopathological evaluation revealed that HIE induced mild to moderate morphometric changes in the liver, kidney and spleen but not the lungs and confirms the findings obtained from the biochemical assessment. In this assessment, repeated doses of 100 mg kg^{-1} caused the most toxicity, both at the biochemical and histological level.

CONCLUSION

The topical ocular application in rabbits's eye indicated that HIE is safe to the ocular tissues however, repeated oral use for medium term (sub-chronic) use may not be safe.

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