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Effects of Methanol Root Extract of *Homalium letestui* on Oxidative Stress Markers and Histology of Livers and Kidneys of Wistar Rats

Ofonimeh Emmanuel Udofia
Department of Genetics and Biotechnology, Faculty of Biological Sciences, Akwa Ibom State University, Nigeria
EMail:emmaudofia2010@yahoo.com;ofonimeudofia19@gmail.com;PhoneNo:+23470346972

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Abstract

The effects of methanol extract derived from the root of Homalium letestui on oxidative stress markers and the histology of the liver and kidney were evaluated in Wistar rats. Phytochemical screening of the extract indicated the presence of alkaloids, tannins, flavonoids, saponins, and cardiac glycosides. Subsequently, the crude methanol extract was partitioned into four parts: butanol, dichloromethane, ethyl acetate, and aqueous. Sixty-six healthy albino mice weighing between 20 and 25 grammes were grouped into 11 groups of six mice each. The extract was orally administered to the experimental animals at doses of 250, 500, and 750 mg/kg body weight over a 14-day period. The study discovered a significant increase in catalase (CAT) enzyme activity ($p < 0.01$-0.001) across all doses (250-750 mg/kg) and fractions. Moreover, the extract and its fractions caused increases in the levels of superoxide dismutase (SOD) that were not statistically significant at the administered doses. While there was a decrease in malondialdehyde (MDA) concentration in all treated groups, the decrease was not statistically significant except for the dichloromethane fraction ($p < 0.001$). Histological examination of liver and kidney tissues revealed no obvious lesions. Overall, the findings indicate that the methanol extract and its fractions did not have detrimental effects on oxidative stress markers. Instead, they exhibited a tendency to increase the first line antioxidant enzymes. These results suggest that the extract may possess antioxidant properties and anti-histopathologic characteristics.

1.0 Introduction

Oxidative stress in living organisms arises from a fundamental disparity between the generation of reactive oxygen species (ROS) during metabolic processes and the capacity to counteract them through antioxidant defense mechanisms [1]. The overproduction of ROS within tissues can lead to cellular damage by modifying essential cellular components such as proteins, lipids, and nucleic acids [2]. Moreover, heightened levels of ROS can trigger the onset of inflammation by instigating the expression of proinflammatory cytokines, chemokines, and inflammatory transcription factors, resulting in tissue injury [3]. Inflammation represents a swift and coordinated series of events designed to enable tissues to respond to injuries or infections [4], and serves as a crucial defense mechanism that involves the recruitment of immune cells and the excessive production of inflammatory cytokines in response to ROS release [5]. To mitigate the detrimental consequences of ROS, human cells have developed an intricate antioxidant defense system comprising enzymatic

components responsible for converting ROS into less harmful molecules like oxygen and water [6]. Key endogenous enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) play a vital role in shielding aerobic cells from the deleterious impacts of ROS. These enzymes function by directly scavenging superoxide radicals and hydrogen peroxide, transforming them into inert species [7].

The extent of cell damage induced by excessive ROS generation hinges on the efficacy of cellular antioxidant enzymes in neutralizing these reactive species [8]. This intricate interplay between ROS production and antioxidant defense mechanisms underscores the critical importance of maintaining redox homeostasis to safeguard cellular integrity and function in living organisms. Recent studies have documented the significant impact of natural products with antioxidant activities on delaying oxidative stress and inflammatory tissue damage by reinforcing cell and tissue defenses, in addition to the protective effects of the cellular antioxidant defense system, whether enzymatic or non-enzymatic [9]. The utilization of natural product-based medicines in medical practices dates back centuries, offering a potentially safer alternative to allopathic drugs due to their reportedly fewer side effects and potential for long-term use [10]. Research by [10] highlighted that the active ingredients found in combinations of natural products can rival the potency of synthetic drugs, albeit requiring larger quantities or extended administration periods. Alarming statistics from [11] reveal that around 8% of hospital admissions in the United States are attributed to adverse reactions or side effects stemming from synthetic medications, leading to approximately 100,000 deaths annually due to associated toxicities. Despite the generally perceived safety of herbal medicines, it is crucial to contextualize their toxicity, as they, too, can exhibit side effects. Numerous natural compounds and their mimics exhibit valuable antioxidant properties that shield against oxidative damage in chronic diseases. To facilitate their optimal clinical application and efficacy enhancement, it is imperative to comprehensively understand and validate the bioactivities of these natural compounds alongside their underlying molecular mechanisms. This scientific groundwork is essential not only for clinical utilization but also to surmount regulatory challenges. The quest for natural products showcasing antioxidant capabilities has surged in recent decades. Plants, in particular, present a promising avenue for mitigating oxidative damage owing to their rich array of secondary metabolites, such as polyphenols, flavonoids, tannins, terpenoids, and anthraquinones [12]. [13] reported that natural compounds sourced from plants possess the ability to interact with reactive oxygen species (ROS), thereby interrupting the chain reaction before critical cellular molecules endure severe damage.

Homalium letestui is a medicinal plant utilized by traditional herbalists for treating various ailments. This forest tree, native to West African rainforests, can reach heights of 80-100 feet and boasts a straight, tall bole with a diameter reaching 70 cm. Despite its prevalence in deep rainforests, there is limited literature available regarding its medicinal applications [14]. In Nigerian folk medicine, *H. letestui* is revered for its medicinal properties, with the root noted for its aphrodisiac qualities. The Ibibios of Nigeria's Niger Delta region have long incorporated the plant's root and stem bark into decoctions, employing them to combat stomach ulcers, malaria, and various inflammatory conditions [15]. Furthermore, the plant has been employed in numerous parts of the world to address diverse health issues such as malaria, stomach ulcers, infertility, and diabetes. Notably, the bark sap is utilized as an enema, while the pulp is topically applied to alleviate oedema [16] [17]. Phytochemical analysis has revealed the presence of alkaloids, saponins, tannins, flavonoids, and cardiac glycosides in *H. letestui* [15] [16]. These compounds likely contribute to the plant’s therapeutic properties and underscore its potential as a valuable medicinal resource. Despite its extensive historical use in herbal medicine, the *in vivo* antioxidant properties of *H. letestui* remain inadequately explored. Moreover, little is known regarding potential side effects on cellular integrity, particularly in the liver and kidneys. To address these knowledge gaps, this study was designed to investigate the impact of oral administration of *H. letestui* methanol extract on oxidative stress markers and the histological architecture of liver and kidney tissues in Wistar rats. Expanding

insights into the antioxidant properties and potential physiological effects of *H. letestui* extract can enrich the existing literature on this traditional medicinal plant. By assessing its impact on oxidative stress markers and tissue morphology in animal models, this study aims to contribute valuable data to enhance our understanding of the therapeutic potential and safety profile of *H. letestui*.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Material

The plant material was sourced from Uyo Local Government Area of Akwa Ibom State, Nigeria and underwent identification and verification by Prof. Margaret Bassey, a taxonomist from the Department of Botany and Ecological Studies at the University of Uyo in Uyo, Nigeria. Subsequently, a voucher specimen referenced as UUPH A69(i) was placed in the Faculty of Pharmacy Herbarium at the University of Uyo.

2.2 Plant Extraction

Fresh roots of *Homalium letestui* were collected from their natural habitat, washed, and allowed to dry. Subsequently, the roots were cut into small pieces and air-dried over a period of three weeks. After drying, the roots were pulverized into a coarse powder using a mortar and pestle. The resulting powder was weighed, with 1 kg of it soaked in 99% methanol (Sigma Chemical, USA) for a duration of 72 hours. The resulting methanolic solution was filtered using Whatman filter paper No.4 and then concentrated by evaporation at 40ºC. The resulting extract was stored at -4ºC for use in subsequent experiments.

2.3 Phytochemical Screening

Phytochemical screening was performed to identify bioactive chemical elements such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides, and anthraquinones. The screening was done according to the standard methods by Sofowora [18] and Evans [19].

2.4 Fractionation (Partitioning)

To obtain their specific components, a 70 g weighted dry methanol extract was mixed with 10 mL of distilled water and sequentially divided into dichloromethane, ethyl acetate, n-butanol, and aqueous phases to isolate the respective fractions of these solvents. Subsequently, each fraction was dehydrated under reduced pressure using a rotary evaporator (Lab Tech EV 311, China) operating at 35°C. The methanolic extract and its fractions were preserved in a desiccator for future use.

2.5 Determination of Median Lethal Dose (LD_{50})

The LD50 (median lethal dose) was determined using the experimental protocol established by Miller and Tainter as documented by Randhawa [20]. Sixty-six healthy albino mice weighing between 20 and 25 grammes were segregated into 11 groups of six mice each. The extract was intraperitoneally (i.p) administered at varying doses ranging from 2000 to 5000 mg/kg. Physical signs of toxicity were observed, and the resulting data was utilized to plot a log probit versus concentration graph. The calculated LD50 value was found to be 335.0±183.33 mg/kg. The selection of the doses was based on the calculated median lethal dose (LD_{50}). Specifically, the low dose administered was 250 mg/kg, a level below the calculated LD_{50}. The middle dose was set at 500 mg/kg, representing twice the low dose, while the high dose was determined as 750 mg/kg, equivalent to three times the low dose.

2.6 Animal Treatments

Thirty adult male Wistar rats weighing between 170 and 200 grammes were obtained from the Animal House in the Department of Pharmacology and Toxicology at the University of Uyo, Nigeria. The male rats were housed individually and underwent a two-week acclimatization period, during which they had unrestricted access to Grower Marsh feed from Grand Bendel Ltd, Edo State, and water *ad libitum*. They were maintained under standard conditions of 12-hour light and dark cycles and were randomly selected, identified, and housed in cages before dosing. Rigorous measures were taken to ensure that the experimental animals fell within the appropriate size and weight range throughout the study duration.

The rats were randomly distributed into 8 groups, each consisting of 6 animals. Animals in Group 1 served as the control and were administered normal saline (10 ml). Groups 2, 3, and 4 received incremental oral doses of 250, 500, and 750 mg/kg of the crude methanol extract, respectively. Meanwhile, Groups 5, 6, 7, and 8 were treated with 500 mg/kg of the butanol, ethyl acetate, dichloromethane, and aqueous fractions, respectively. The administration of the extract and fractions was conducted daily over a 14-day period between the hours of 10 a.m. and 12 noon.

2.7 Assay of Oxidative Stress Markers

2.7.1 Malondealdehyde Test

The methodology described by Sharma and Krishna [21] was used to assess malondialdehyde (MDA) levels, serving as an indicator of lipid peroxidation. Specifically, 0.5 mL of tissue homogenate was combined with 3 mL of a TCA-TBA-HCL reagent (comprising 0.6% thiobarbituric acid, 0.25N hydrochloric acid, and 15% tricarboxylic acid). The mixture was then heated for 15 minutes at 100 ºC, followed by a cooling period. Subsequently, after centrifugation for 15 minutes at room temperature, the absorbance at 532 nm was determined.

2.7.2 Gluthathione Peroxidase

The technique outlined by Brehe and Bruch [22] was utilized for the quantification of glutathione peroxidase activity. To initiate the reaction, 200 µL of Buffer, 200 µL of Glutathione, 100 µL of Sodium Azide, and 100 µL of H_2O_2 were combined, followed by the addition of 100 µL of the sample. The resulting mixture was then allowed to incubate for 10 minutes at 37ºC, with the reaction ceasing upon the addition of 500 µL of TCA. Subsequent to centrifugation for 10 minutes, 500 µL of the resultant supernatant was collected. To this sample, 2000 µL of dibasic buffer 8.0 was added, along with 500 µL of DTNB. The mixture was thoroughly mixed and its absorbance was recorded at 412 nm.

2.7.3 Catalase Enzyme Activity

The procedure described by [23] relies on assessing the rate at which hydrogen peroxide (H_2O_2) decomposes after reacting with the oxidizing agent potassium dichromate in glacial acetic acid. Catalase, an enzyme, accelerates the rapid decomposition of H_2O_2 into water and oxygen molecules. The pace of this decomposition is directly linked to the quantity of catalase present in the analyzed specimen. Any surplus potassium dichromate in glacial acetic acid interacts with any remaining H_2O_2. The spectrophotometric measurement of the concentration of residual potassium dichromate at 570 nm serves as an indicator of catalase activity. The technique developed by [23] was employed for quantifying the activity of the catalase enzyme. Specifically, 1 mL of the H_2O_2 reagent (0.036%) was dispensed into a test tube and incubated at 37ºC for 3 minutes before adding 100 µL of the

sample, followed by boiling the solution for 10 minutes. The solution was then cooled using running tap water, and its absorbance was measured at 570 nm.

2.7.4 Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD), an enzyme, facilitates the dismutation of the superoxide anion, a highly reactive free radical, into oxygen and hydrogen peroxide. These enzymes play a crucial role in each cell's antioxidant defense system when encountering oxygen exposure. The specific type of SOD is determined by the metallic cofactor that the enzyme employs, as outlined by Misra and John [24]. Examples of potential cofactors include Cu, Zn, Mn, and Fe. A quantity of 4 mL of carbonate buffer was dispensed into a test tube, followed by the addition of 100 µL of the sample prior to incubating the mixture at 37 ºC. The reaction was initiated by promptly blending 100 µL of adrenaline and monitoring the absorbance at 480 nm at 30-second intervals over a 120-second duration.

2.8 Preparation of Liver and Kidney tissues

The liver and kidneys were meticulously dissected and weighed using a precise electronic digital balance. Tissue samples from the liver and the left kidney were randomly selected and preserved in 10% buffered formalin. Subsequently, these samples underwent dehydration and clearing in an escalating gradient series of ethanol and xylene, followed by infiltration with molten paraffin wax and embedding in paraffin blocks. Tissue sections of 5 µm thickness were then sliced and affixed onto clean frosted slide glasses before being stained with hematoxylin and eosin (H&E). Subsequently, the sections were scrutinized, and images were captured using a Leica DM 750 microscope furnished with a Leica ICC50W (Leica ICC50, China) High Definition Wireless camera module. These tissue slides were processed at the Histology Laboratory, University of Uyo Teaching Hospital in Uyo, Nigeria.

2.9 Statistical Analysis

The findings were presented as multiple comparisons of mean values ± standard error of the mean (SEM). The statistical significance was assessed through one-way ANOVA, followed by the Tukey-Kramer multiple comparisons post-test, conducted using GraphPad InStat Demo - (DATASET1.ISD). A probability level lower than 5% was deemed statistically significant

3.0 Results

The rats administered with the extract did not show any signs of toxicity. Moreover, there were no instances of morbidity or mortality observed during the study. Additionally, all groups of rats displayed a steady increase in body weight throughout the entire experiment timeline.

3.1 Effect of Extract on Body and Organ Weights of Male Rats

The effects of the extract on body and organ weights are shown in **Table 1**. The extract had no statistically significant effect on the final body weights of rats. Furthermore, the extract's effect on the kidney and liver was not statistically significant.

Table 1: Effect of Extract on Body and Organ Weight of Male Rats.

Dose (mg/kg)	Initial weight (g)	Final weight (g)	Liver (g)	Kidney (g)
N/S(10mL)	170.00±4.71	174.20±4.75	7.60±0.25	1.09±0.04
250	177.50±3.90	181.20±3.92	7.32±0.12	1.03±0.02
500	174.50±2.91	178.30±2.82	6.41±0.17	1.03±0.05
750	192.70±8.58	186.90±8.54	6.35±0.52	1.16±0.07

Values represent Mean± S.E.M. Significant at [a]p<0.05; [b]p<0.01; [c]p<0.001, when compared to control. n=6; N/S= Normal saline

3.2 Phytochemical Screening

Table 2 shows the results of the phytochemical screening of *H.letestui* methanol root extract. According to the results, the root extract contains alkaloids, saponins, and flavonoids. Tannins and cardiac glycosides were also found, although there were no anthroquinones.

Table 2: Phytochemical Screening of Methanol Root Extract of *Homalium letestui*

Metabolites	Test	Observation	Inference
Alkaloids	Dragendoff's	Orange precipitate	+
Saponins	Frothing Test	Persistent frothing	+
	Heamolytic Test	Clear red liquid fromed	+
Tannins	Ferric chloride	Dark green precipitate	+
Flavonoids	Ammonia Test	Two layers formed with yellow colouration at ammonia layer	+
	Sodium hydroxide Test	Yellow colouration	+
Cardiac	Salkowski's Test	Redish brown ring at Interphase	+
Glycosides	Keller Killiani Test	Brown ring at interphase	+
	Lieberman's Test	Violet to blue to green colour change	+
Anthraquinones	Borntrager's Test	No reddish colour	-
	Combined Anthraquinone Test	No red or pink colour	-

Key: + = present; - = absent

Table 3 shows the effect of methanol root extract of *H. letestui* and fractions on liver antioxidants in treated rats. Administration of the extract and its fractions caused increases in the levels of SOD that were statistically not significant at the doses administered (250-750 mg/kg). Catalase (CAT) enzyme activity increased significantly (p<0.01-0.001) at all the doses administered (250-750 mg/kg) and the various fractions. There was a decrease in the concentration of MDA in all the treated groups; this decrease was statistically not significant. However, the dichloromethane fraction was significant at p<0.001.

Table 3: Effects of Methanol Root Extract of *H. letestui* on Liver Antioxidants of Treated Rats

Dose mg/kg	MDA (nmol/mL)	CAT (µMH)	SOD (U/mL)	GPx (U/mL)
N/S(10mL)	1.99±0.04	38.52±1.14	8.57±0.23	1.88±2.00
250	1.65±0.04	38.25±0.50[c]	8.74±0.05	1.74±0.10
500	1.84±0.15	40.93±0.52[c]	8.76±0.05	1.55±0.06
750	1.93±0.10	40.67±0.14[c]	8.84±0.04	2.00±0.07
Aqueous (500)	1.97±0.02	41.15±0.03[c]	8.65±0.06	2.06±0.96
BUT(500)	1.99±0.03	41.05±0.10[c]	8.75±0.03	1.97±0.07
DCM (500)	1.48±0.01[c]	40.71±0.07[c]	8.59±0.03	1.66±0.08
E.A(500)	1.83±0.01	40.16±0.16[c]	8.91±0.08	1.85±0.20

Values represent Mean± S.E.M. Significant at [a]p<0.05; [b]p<0.01; [c]p<0.001, when compared to control. n=6. MDA= Malondialdehyde; SOD= Superoxide Dismutase; CAT= Catalase Activity; GPx= Gluthathione Peroxidase; DCM= Dichloromethane; E.A = Ethyl Acetate; N/S=Normal saline

3.3 Effect of *H. letestui* Methanol Root Extract and Fractions on Histology of the Kidney

The histopathological photomicrographs of kidney tissues of control group, extract treated groups and fractions of treated rats are shown in **Figures 1-2**. Histopathological examination of kidney tissue obtained from control animals and the fractions showed normal renal tissue histology evidenced with normal glomeruli and renal tubule, and without any alterations of tissue architecture.

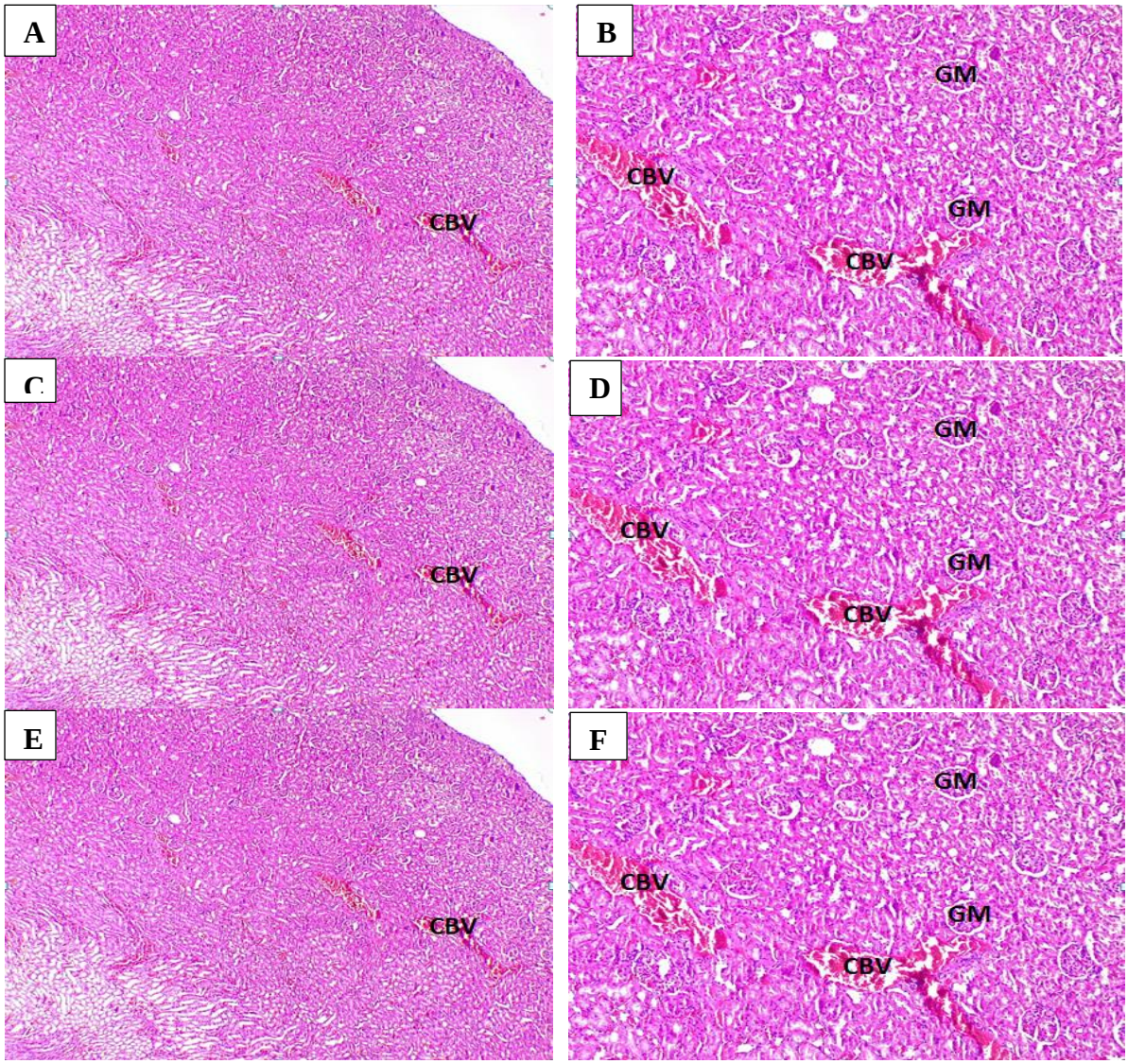


Figure 1: Renal section of rats treated with 10 mL/kg normal saline (A&B) at magnification (× 40) and (× 100), 250 mg/kg of extract (C&D) at magnification (× 40) and (× 100), and 500 mg/kg of extract (E&F) at magnification (× 40) and (× 100), stained with H & E technique.

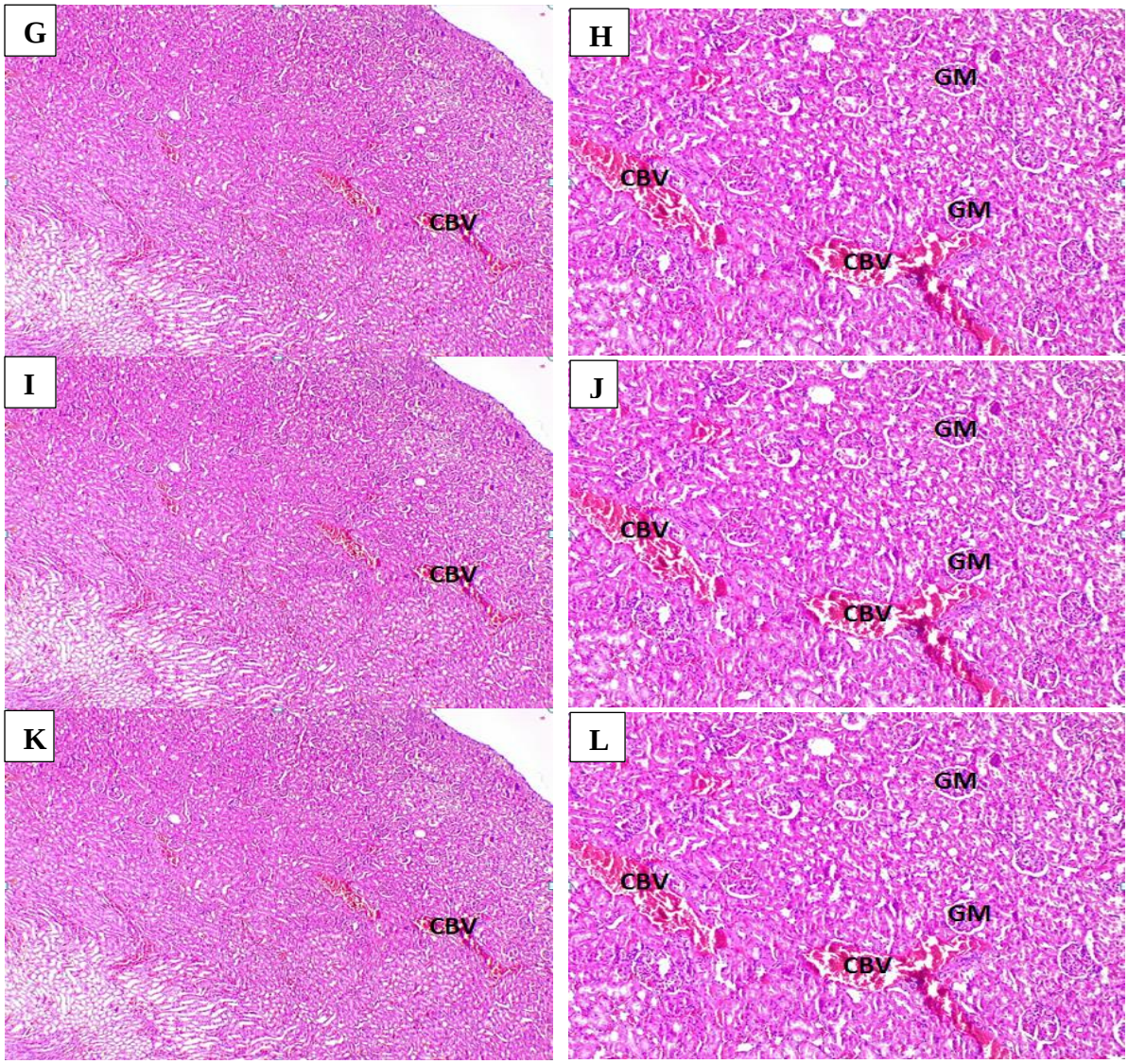


Figure 2: Renal section of rats treated with 750 mg/kg of extract (G&H) at magnification (× 40) and (× 100), and 500 mg/kg of butanol fraction (I&J) at magnification (× 40) and (× 100) and 500mg/kg of dichloromethane fraction (K&L) at magnification (× 40) and (× 100), stained with H & E technique.

3.4 Effect of *H. letestui* Methanol Root Extract on Histology of the Liver

Histopathological examination of the sections of the liver from the control and the treated groups showed preserved and normal cellular histoarchitecture with array of hepatocytes, sinusoid and central vein. The fractions (Dichloromethane, Butanol and Aqueous) also had normal cellular histoarchitecture with array of hepatocytes, sinusoid and central vein. However, ethyl acetate fraction of the extract showed highly predominant dilated congested blood vessels and enlarged bile duct (**Figures 3 and 4**).

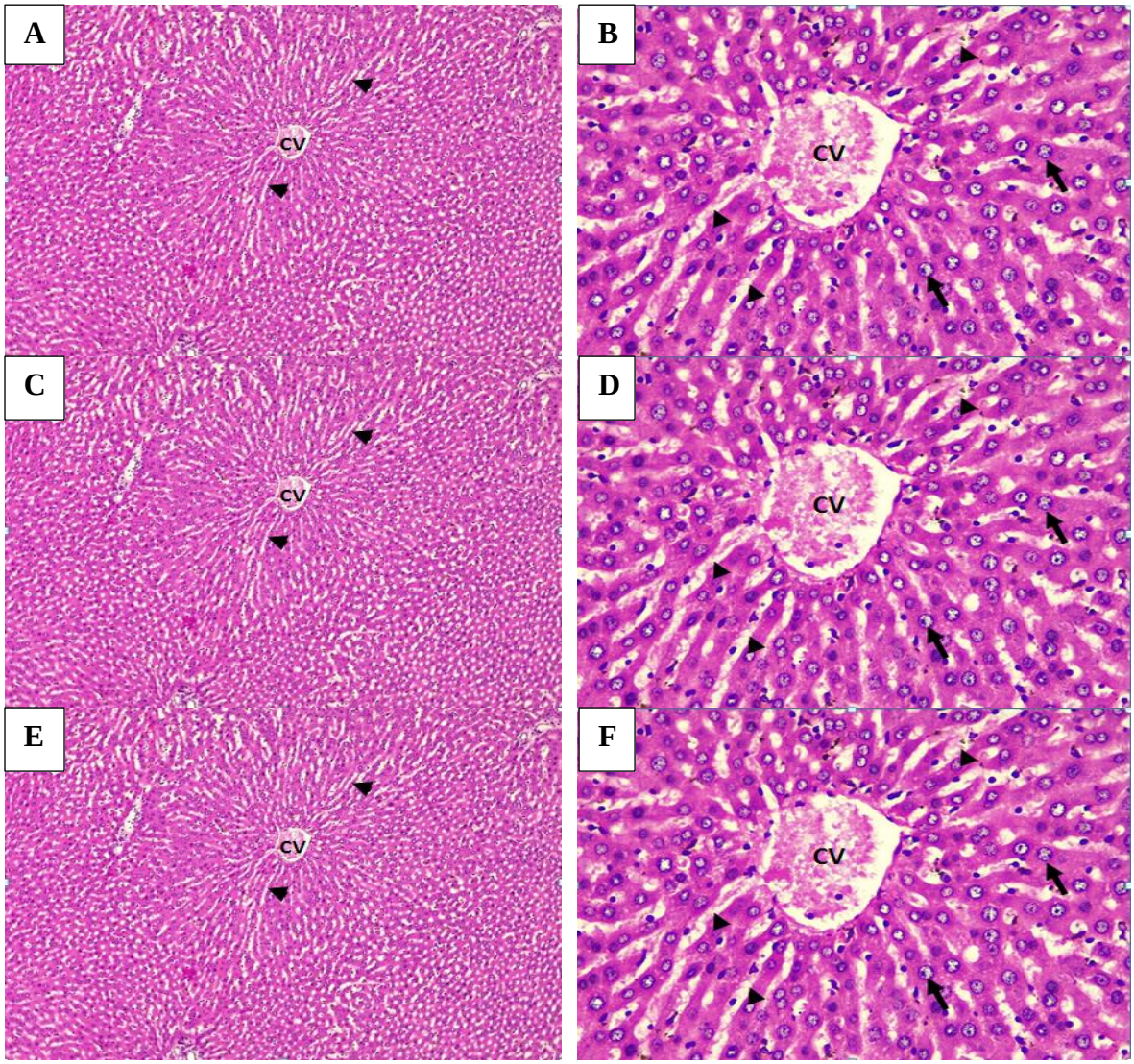
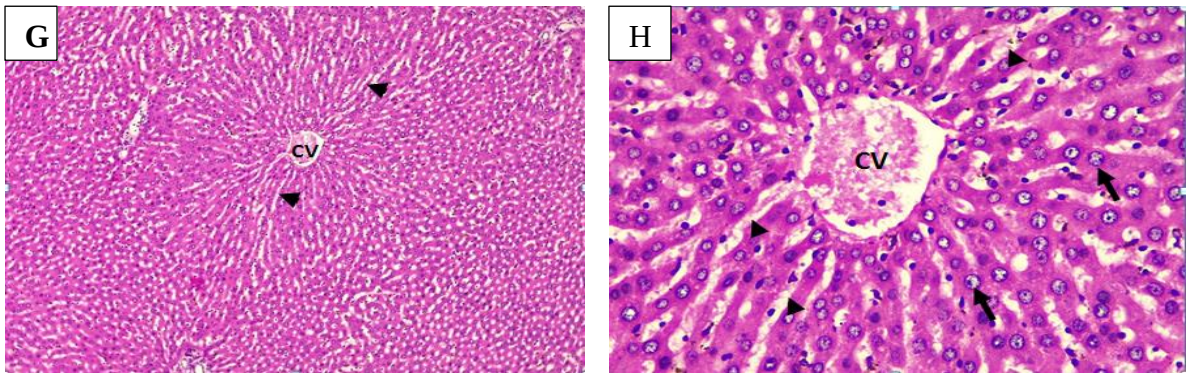


Figure 3: Histology section of liver tissues of rats treated with 10 mL/kg of normal saline (A&B) at magnification (× 100) and (× 400), 250 mg/kg of the extract (C&D) and 500 mg/kg of extract (E&F) at magnification (× 100) and (× 400), stained with H & E technique.



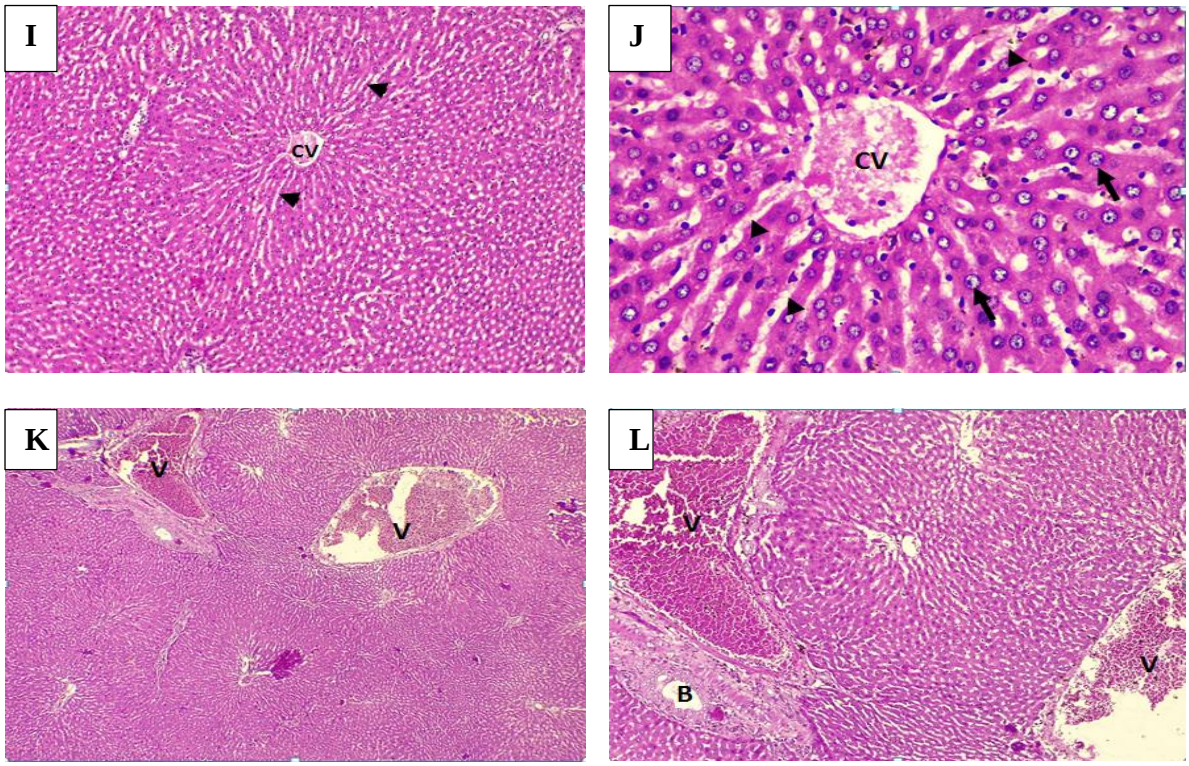


Figure 4: Histology section of liver tissues of rats treated with 750 mg/kg of the extract (G&H) at magnification (× 100) and (× 400), 500 mg/kg of dichloromethane fraction of the extract (I&J) at magnification (× 100) and (× 400) and 500 mg/kg of ethyl acetate fraction (K&L) at magnification (× 100) and (× 400), stained with H & E technique.

4.0 Discussion

The investigation focused on the impact of orally administering *H. letestui* extract on oxidative stress markers and the histology of liver and kidney tissues in treated Wistar rats. The 14-day treatment with methanol root extract of *H. letestui* demonstrated excellent tolerance among all treated rats. Notably, the extract did not affect food intake at any dosage levels and did not disrupt body growth patterns. Analysis of organ weight alterations in toxicological assessments serves as a vital indicator of toxicity [25]. Notably, no significant shifts in liver or kidney weight were observed in rats treated with the extract across all dosages compared to the control group. This consistency aligns with findings from prior subchronic studies by [26], indicating a lack of toxic effects on the studied organs as evidenced by the absence of weight variations. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, and cardiac glycosides in the *H. letestui* extract. These identified phytochemicals have been recognized for their protective properties in mitigating excessive levels of reactive oxygen species (ROS). Previous research has highlighted the efficacy of certain alkaloids in safeguarding macromolecules against oxidative harm [27]. Given their antioxidant prowess, alkaloids possess the potential to combat neurodegenerative diseases (NDs) by targeting oxidative stress mechanisms, as supported by findings from [29]. Studies have further illustrated that alkaloids sourced from Huperzia selago and Diphasiastrum complanatum shield cells from oxidative stress, DNA and mitochondrial damage, and apoptosis induced by sodium nitroprusside (SNP) [27]. The alkaloids present in the plant extract likely play a role in enhancing cellular antioxidant capacity, thereby contributing to overall cellular health maintenance. Flavonoids, a class of polyphenolic compounds characterized by a benzo-γ-pyrone structure prevalent in plants, play a significant role in pharmacological activities [29]. Extensive research has

explored the health benefits of these compounds, primarily attributed to their antioxidant properties. The functional hydroxyl groups present in flavonoids enable them to scavenge free radicals and/or chelate metal ions, thereby exerting their antioxidant effects [30][31]. Their protective mechanisms involve reducing excessive levels of reactive oxygen species (ROS) and enhancing renal enzymatic and non-enzymatic antioxidants through various pathways, such as modulation of the Nrf2-antioxidant pathway [32][33]. By suppressing ROS synthesis, inhibiting enzymes, chelating trace elements involved in free radical generation, scavenging ROS, and enhancing antioxidant defenses, flavonoids contribute significantly to cellular health maintenance [34].

In a similar vein, saponins have emerged as potent antioxidants known for protecting cells by minimizing ROS production during oxidative stress [35]. Studies by [36] have demonstrated that garlic saponins function as antioxidants, shielding rat pheochromocytoma PC12 cells from hypoxia-induced ROS damage via redox-sensitive signaling pathways mediated by ROS. Saponins exert their protective effects by scavenging free radicals, thereby thwarting lipid oxidation through a chain-breaking reaction. Based on their antioxidative properties, saponins hold potential as candidates for both pharmaceutical and nutraceutical applications [37]. The findings underscore the pivotal roles of flavonoids and saponins as essential bioactive compounds that effectively combat oxidative stress and bolster cellular defense mechanisms. Their antioxidant properties not only offer therapeutic potential but also pave the way for developing novel strategies to address oxidative-related disorders. Further exploration into the mechanistic actions of these compounds may elucidate additional avenues for therapeutic interventions targeting oxidative stress-induced pathologies. The antioxidant experiments conducted in this study have highlighted the potent free radical scavenging activity of the extract from *H. letestui*. Free radicals, characterized by the presence of unpaired electrons in atomic orbitals, are highly reactive and unstable molecular species that can either donate or accept electrons from other molecules, functioning as either oxidants or reductants [25]. This reactivity results in oxidative stress, a foundational pathology observed in a wide array of disease states. Oxidative stress stems from the disrupted equilibrium between the generation and accumulation of reactive oxygen species (ROS) in cells and tissues and the system's ability to neutralize these reactive byproducts. While ROS play crucial physiological roles like cell signaling and are typically produced as byproducts of oxygen metabolism, external stressors such as UV radiation, pollutants, heavy metals, and xenobiotics contribute to an overproduction of ROS, leading to cellular and tissue damage (oxidative stress). Various antioxidants, including vitamin E, flavonoids, and polyphenols, have garnered attention in recent research for their potential anti-oxidative stress properties [38].

Malondialdehyde (MDA) serves as a notable marker for oxidative stress as it is produced as a final product of polyunsaturated fatty acids peroxidation in cells. Elevated levels of free radicals trigger an excess production of MDA, underscoring the role of MDA as an indicator of oxidative stress and antioxidant status in individuals, particularly those with cancer [39]. The decrease in first-line antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) resulting in an increase in free radicals contributes to lipid peroxidation and aberrant metabolism. Elevated MDA levels, coupled with decreased first-line antioxidants, confirm the presence of oxidative stress. Antioxidant enzyme markers like SOD, GPx, and CAT signify the defense mechanisms against oxidative tissue damage [40].

The research findings provide valuable insights into the antioxidative potential of *H. letestui* extract as a free radical scavenger and emphasize the broader impact of oxidative stress on disease pathogenesis. Understanding the intricate balance between ROS production and antioxidant defenses is crucial in developing targeted therapeutic interventions for managing oxidative stress-related disorders. Further investigation into the mechanistic roles of antioxidants and their impact on oxidative stress pathways may pave the way for innovative treatment strategies in various disease conditions. The results of this study demonstrated that the administration of methanol root extract led to a reduction in malondialdehyde (MDA) levels in treated rats, although this decline was not

statistically significant across all groups that received the extract. The non-significant decrease in MDA levels suggests a potential inhibitory effect on free radical production within the cells due to the extract's intervention. Moreover, the levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) exhibited an increase in all experimental groups, albeit not reaching statistical significance. Notably, catalase enzyme activity significantly rose in all treated groups, indicating that the antioxidant defense mechanisms within the cells remained robust. This observation highlights the potential of the extract to bolster the overall antioxidant capacity of the cells, even though the SOD and GPx levels did not show significant changes. Furthermore, the histological analysis of the eviscerated organs, specifically the liver and kidney, utilizing the hematoxylin and eosin (H&E) staining technique, revealed no detectable histologicallesions in the tissues of the experimental animals. The cellular integrity and architecture of the tissues remained unaltered compared to their respective controls. This preservation of tissue morphology could be attributed, at least in part, to the extract's ability to stabilize cellular membranes, as suggested by previous studies [41] [42]. However, a notable finding emerged from the study where the administration of 500 mg/kg of the ethyl acetate fraction resulted in the pronounced presence of dilated congested blood vessels and enlarged bile ducts, as illustrated in Figure 3. This observation indicates a potential adverse effect associated with this specific fraction at the given concentration, warranting further investigation into its effects on cellular structures and functions. The present study underscores the potential therapeutic implications of the methanol root extract in modulating oxidative stress markers and maintaining cellular integrity. The observed improvements in antioxidant enzyme activity and tissue histology suggest a protective role of the extract, although caution is advised when assessing the effects of specific extract fractions at varying doses. Future research endeavors should delve deeper into elucidating the precise mechanisms underlying the observed effects and exploring the optimal dosage and formulation of the extract for therapeutic applications. A more in-depth inquiry is also essential to evaluate the ramifications of prolonged or chronic administration of *H. letestui* root extract of different fractions on experimental animals. Such an investigation would serve to ascertain the safety profile of the extract following extended periods of treatment.

5.0 Conclusion

The current study delved into investigating the impact of the methanol root extract of *Homalium letestui* on oxidative stress markers and the histology of liver and kidney tissues in treated animals. The findings revealed that the extract did not induce significant alterations in the concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Nevertheless, there was a noteworthy increase in the activity of catalase (CAT) enzyme across all experimental groups and fractions. Importantly, the histological examination of the eviscerated organs—specifically the liver and kidney—did not reveal any histological lesions in the tissues. This observation indicates that the cellular integrity and architecture of the organs remained unaffected by the extract treatment. These results suggest that the root extract of *Homalium letestui* may possess potential antioxidant properties capable of mitigating oxidative stress, which is recognized as a primary contributor to numerous degenerative diseases. Additionally, the study hints at the safety profile of the plant extract at certain doses, as no visible signs of toxicity were observed at the administered doses, and the cellular architecture of the liver and kidney tissues remained intact.

In light of these findings, it can be inferred that the root extract of *Homalium letestui* holds promise as a natural agent for combating oxidative stress-related conditions. Further research is warranted to elucidate the underlying mechanisms of its antioxidant effects and to determine the optimal dosage for therapeutic applications. The safety and efficacy demonstrated in this study underscore the potential of this plant extract as a valuable candidate for further exploration in the realm of oxidative stress management and preventative healthcare strategies.

Competing Interests

Author has declared that no competing interests exist.

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