Effects Of Alcoholic Leaf Extract Of Hunteria Umbellata On Various Parameters In Wistar Female Rats Exposed To Trichloroethylene (TCE)

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Abstract

This investigation examined the effects of alcoholic *Hunteria umbellata* leaf extract on several parameters in Wistar female Albino rats exposed to trichloroethylene (TCE). The study assessed body weight changes, antioxidant activity, lipid profile, hematological parameters, and kidney biomarkers. Regarding body weight, the treatment groups showed a significant increase compared to the negative control, which exhibited weight loss. The administration of *Hunteria umbellata* extract resulted in weight gain in a dose-dependent manner. Antioxidant activity analysis demonstrated that TCE exposure led to a decrease in catalase (CAT) and superoxide dismutase (SOD) levels while malondialdehyde (MDA) levels increased. However, treatment with *Hunteria umbellata* extract showed a significant improvement in antioxidant activity, with increased levels of CAT and SOD and decreased MDA levels.

The extract had favorable effects on the lipid profile, raising levels of high-density lipoprotein (HDL) and lowering those of low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), cholesterol, and triglyceride (TAG). Hematological parameters analysis showed that the extract administration had positive effects on red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC) count, and platelet levels. It also influenced the levels of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Furthermore, the study assessed kidney biomarkers, including sodium (Na+), chloride (Cl-), potassium (K+), bicarbonate (HCO3-), urea, and creatinine levels. Treatment with Hunteria umbellata extracts improved biomarker levels, indicating potential kidney protection against TCE-induced damage. In conclusion, the alcoholic leaf extract of Hunteria umbellata exhibited beneficial effects on body weight, antioxidant activity, lipid profile, hematological parameters, and kidney biomarkers in TCE-induced Wistar female Albino rats. These results raise the possibility that Hunteria umbellata extract has potential protective effects against TCE-induced toxicity. More study is required to clarify the underlying mechanisms and evaluate their safety and effectiveness in human beings.

Keywords: *Hunteria umbellate,* Trichloroethylene, antioxidant, Silymarin, Hematology

Introduction

The use of herbal medicine has a long-standing history in the treatment of various illnesses and diseases. It has been practiced for many years and continues to be widely used today (Kokwaro, 2012). Prior to the emergence of orthodox medical practices, people had accumulated a wealth of empirical knowledge about the therapeutic properties of local plants. Through a process of trial, error, and success, herbalists and their apprentices have gained significant understanding of medicinal plants (Iwu *et al.*, 2013). Initially, plant-based medicines were often used in their natural form, without significant processing. Many active remedies were employed based on empirical observations of their effectiveness within traditional societies across the globe (Holm *et al.*, 2012). Since time immemorial, medicinal plants have been used as a medicine source in almost all cultures (Lemma, 2015).

A *Hunteria umbellata*, a member of the Apocynaceae family, effectively treats several diseases. The diminutive plant, commonly called Acadci by the Hausa, Mkpokiri by the Igbo, and Abeere by the Yoruba, grows in tropical climates (Ibeh *et al.*, 2017). *Hunteria umbellata*, a plant native to Sierra Leone, has been traditionally utilized for its medicinal properties. In Sierra Leone, the bark of *Hunteria umbellata* is processed into a bitter tonic and employed as a stomachic aid, as well as a topical lotion to

alleviate fever (Burkill, 2014). Similarly, in Cote d'Ivoire, a fresh extract derived from the root-bark of this plant is applied to treat sores caused by leprosy (Burkill, 2014). Furthermore, in Germany, extracts of *Hunteria umbellata* are utilized for phytotherapeutic purposes, including the reduction of heart rate, aphrodisiac effects, and management of blood pressure and lipid content (Adegoke & Alo, 2017).

Hunteria umbellata has a trunk diameter of up to 40 cm (16 inches) and can grow as a small tree or shrub up to 22 meters (72 feet) tall. The herb is used to cure a wide range of illnesses. Fever, leprosy sores, stomach, and liver issues are just a few of its medical applications(Bouquet & Debray, 2015; Ballay, 2014). *Hunteria umbellata* has numerous alkaloids that have been identified, but little is known about their pharmacological effects on the brain and the heart. *Hunteria umbellata* all have sympathomimetic effects, cardiovascular effects, and potent and long-lasting hypotensive activity (Endress *et al.*, 2017). The liver, being the largest organ in the human body, serves a vital role in carrying out a variety of essential function. Repeated exposure to toxins will eventually result in hepatotoxicity. Carbon tetrachloride (CCl₄), acetaminophen, trichloroethylene (TCE), nitrosamines, and polycyclic aromatic hydrocarbons are some of the toxicants that have been previously reported.

The main effect of TCE-induced hepatotoxicity, which is fat buildup, is that it affects both lipid synthesis and lipid breakdown after activation. TCE prevents the formation of triacylglycerol and contributes to the impairment of triacylglycerol transport through very low-density lipoprotein (VLDL) (Boll *et al.*, 2015). This study looks at the effect of *Hunteria umbellata* alcoholic leaf extract on some biochemical parameters in Trichloroethylene (TCE)-induced hepatotoxicity Albino rat.

Laboratory Animals

A total of fifty (50) female albino rats were obtained from Abia State University's animal house in Uturu for this experiment. The rats, weighing between 105g and 210g, were transported to the Department of Biochemistry Animal House at Abia State University, where the experimental procedures were conducted. Clean cages were provided for the rats, and they were fed with commercial feed manufactured by the Nigerian flour mills. A period of fourteen days was allocated for the rats to acclimatize before the commencement of the experiments. Subsequently, the rats were divided into five (5) groups, each consisting of ten female albino rats.

Route of extract Administration

Extracts were administered orally with gavages according to body weight.

Ethical Clearance

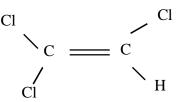
Ethical principles in accordance with institutional and international guidelines for care and use of laboratory animals were strictly adhered to. With Institutional ethical clearance number: ABSU/RP/98

Experimental Design

The animals were randomly grouped into five groups of five (5) animals each.

Group 1: Normal control was given commercial feed and water throughout the experiment. GROUP 2 were induced with trichloroethylene for liver toxicity and was not treated till the end of the study; GROUP 3: This group also consists of five (5) female albino rats, liver toxicity trichloroethylene was induced, and they were treated with the standard drug (silymarin). GROUP 4 was induced with TCE liver toxicity trichloroethylene and treated with 100mg/kg of *Hunteria umbellata* alcoholic leaf extract. GROUP 5: Induced with trichloroethylene for liver toxicity and treated with 200mg/kg of *Hunteria* alcoholic leaf extract. GROUP 5: Induced with trichloroethylene for liver toxicity and treated with 200mg/kg of *Hunteria* alcoholic leaf extract. This study was approved by the Abia State University Ethical Committee on the use of laboratory animals (ABSU/RP/98), and all treatments were done by the Guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

Trichloroethylene (TCE) Induction



Because of its polarity, trichloroethylene (TCE) was diluted with olive oil at a 3:5 ratio (i.e., 3ml:5ml, respectively). Utilizing 1.0ml/kg body weight, the dosage for administration was calculated. Except for the usual control, 0.3ml of TCE was administered to each group of female albino rats based on their body weight. The albino rat was carefully intraperitoneally injected with TCE at a dose calculated by body weight.

Blood Collection Procedure

After twenty-eight (28) days since administration, the animals were subjected to fasting on the following day. They were then administered chloroform anesthesia and sacrificed. A cardiac puncture was performed on each animal using a syringe and needle to obtain blood samples. The collected blood was transferred into sterile sample bottles for clinical chemistry analysis. Subsequently, the unlabeled sample bottle containing the blood was placed in a centrifuge and spun at 3000 rpm for 20 minutes. The serum was separated from the clot using a Pasteur pipette and

transferred into sterile sample test tubes for biochemical parameter analysis. The blood from the hematological sample bottle was utilized for hematological analysis.

Biochemical Assay Procedure:

The blood samples were subjected to centrifugation at 3000 rpm for 10 minutes to separate the serum. Prior to centrifugation, the samples were allowed to clot at room temperature. The serum levels of three enzymes, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were measured using the Fortress Diagnostic Reagent Kit. The measurement of malondialdehyde (MDA), a marker for thiobarbituric acid reactive substances, in the serum was conducted using the method described by Ohkawa et al. (1979). The activity of superoxide dismutase (SOD) was determined by assessing its ability to inhibit the autoxidation of pyrogallol, following the protocol established by Marklund and Marklund (1974). Catalase activity was measured by evaluating the rate of hydrogen peroxide decomposition, following the procedure outlined by Aebi (1984). The method devised by Sedlak and Lindsay (1968) was employed for this purposes used to measure reduction of Ellman's reagent at 412 nm in order to quantify the level of reduced glutathione (GSH).

The Fortress Reagent kit determined Glutathione peroxidase activity, following the manufacturer's guidelines. Total cholesterol, triglycerides and HDL-cholesterol levels were determined using diagnostic kits provided by Randox Laboratories, England. LDL-cholesterol was estimated based on the principles described by Friedewald *et al.* (1972).

The spectrophotometric determination of urea, creatinine, sodium ion, potassium ion, chloride, calcium Ca2⁺, and bicarbonates was performed using the standard ready-to-use kits obtained from Rondox Laboratory Ltd., located in Co. Antrim, United Kingdom.

Results

Table 1 shows the mean body weights, percentage weight gain, and percentage weight loss of rats with 28 days of treatment with alcoholic leaf extract of *Hunteria umbellata* on TCE-induced Wistar female Albino rats. The Normal control, Positive control, and treatment groups experienced a notable increase in the average body weight (ranging from 200ml/kg to 600ml/kg) compared to the negative control, which showed a percentage weight loss.

	CONTROL	NEG. CONTROL	STANDARD DRUG (Silymarin)	Hunteria umbellata (100mg/kg)	Hunteria umbellata (200mg/kg)
DAY 1	180.40±23.81	185.42±39.48	211.13±7.30	144.53±10.50	204.92±9.65
DAY 7	190.91±34.75	169.91±23.54	189.26±26.00	156.23±16.62	211.13±7.30
DAY 14	195.41±31.54	169.91±23.54	216.93±27.78	150.09±25.33	215.32±6.04
% WL		-15.51			
% WG	15.01		5.80	5.56	10.4

Table 1 presents the impact of Hunteria umbellata alcoholic leaf extract on the body weight (in grams) of female Wistar albino rats.

Values are mean ± Standard Deviation (SD) n=5.

Animals were divided into five (5) groups; Group 1: Normal control, Group 2: Negative control, Group 3: Positive control TCE induced animals, given oral administration of hepatoprotective drug "silymarin"), Group 4: TCE (Trichloroethylene) induced animals given 100 mg/kg body weight of *Hunteria umbellata* alcoholic leaf extract, Group 5: TCE induced animals given 200mg/kg body weight of *Hunteria umbellata* alcoholic leaf extract.

The findings indicate that the control group, positive control group, and treatment groups (ranging from 100mg/kg to 200mg/kg) exhibited a percentage weight gain, in contrast to the negative group which experienced a percentage weight loss.

Note: %WL= percentage weight loss

%WG= percentage weight

Table 2: Acute Toxicity (LD₅₀) results of the aqueous seed extract of *Hunteria umbellata* on wistar rat

Dose mg/kg	Observations				
Phase I					
10	Scratching of the mouth				
100	Scratching of the mouth and calmness				
1000	Calmness and slow movement				
Phase II					
1600	Mouth scratching and restlessness				
2900	Mouth scratching , restlessness and climbing on other rats				
5000	Restlessness, and Apnea				

Group	ТР	AST	ALT	ALP	ALB	BIL
Control	8.21± 0.20 ^a	40.25 ±1.71 ^a	17.75 ± 2.63 ^a	60.00 ± 4.24 ^a	4.20 ±0.21a	0.55 ±0.15 ^a
Negative control	6.89 ±0.26 ^b	64.00±4.83 ^b	55.00 ±3.83 ^b	62.75±10.05 ^a	3.66±0.06 ^b	0.88±0.48 ^a
Standard drug	6.76±0.36 ^c	58.75±2.63 ^c	49.25 ±1.50 ^c	60.25 ±5.74 ^a	3.75±0.16 ^c	0.88 ±0.16 ^a
(Silymarin)						
Hunteria	7.04 ±0.23 ^d	52.75±2.63 ^d	49.25±2.99 ^d	58.25±7.93 ^a	3.98±0.09 ^a	0.87±0.12 ^ª
umbellata						
(100mg/kg)						
Hunteria	7.46 ±0.35 ^a	51.82 ± 1.00 ^a	48.31±1.00 ^e	57.12±1.00 ^b	3.87±1.01 ^d	0.83±0.01 ^b
umbellata						
(200mg/kg)						

TABLE 2: Effect of Alcoholic Leaf Extract of *Hunteria umbellata* On Indices of Antioxidant Activity of TCE (Trichloroethylene) INDUCED WISTAR FEMALE ALBINO RATS.

The presented values are expressed as the mean \pm standard deviation (SD) for a sample size of n=5. Values within the same row that share the same letter of the alphabet are not significantly different from each other (p>0.05). The abbreviations used in the table are as follows: TP represents Total Protein, AST refers to Aspartate Aminotransferase, ALT represents Aspartate Alanine Transaminase, ALP represents Alkaline Phosphatase, ALB denotes Albumin, and Bil stands for Bilirubin.

TABLE 2 Effect of Alcoholic Leaf Extract of *Hunteria umbellata* on Indices of Antioxidant Activity of TCE (Trichloroethylene) Induced Wistar Female Albino Rats

Parameters CONTROL N		NEGATIVE	STANDARD	Hunteria	Hunteria
		CONTROL	DRUG	umbellata	umbellata
			(Silymarin)	(100mg/kg)	(200mg/kg)
GSH	51.09±6.27 ^a	42.07±3.19 ^b	42.54±2.30 ^b	42.48±2.77 ^b	54.54±3.00 ^a
SOD	21.98±1.21 ^a	20.38±1.34 ^a	20.59±1.58 ^a	26.10±2.59 ^a	26.11±2.48 ^b
CAT	17.66±1.85ª	11.72±1.40 ^b	13.17±1.01 ^b	13.20±0.66 ^b	15.35±0.95 ^a
MDA (mMol/L)	0.50±0.09 ^a	0.91±0.04 ^b	0.90±0.06 ^b	0.93±0.06 ^b	0.95±0.02 ^b

Values represent the mean \pm SD for n=5. Values in the same row, bearing the same letter of the alphabet are not significantly different from each other (p<0.05).GSH= Glutathione, SOD= Superoxide dismutase, CAT= Catalase, MDA= Malondialdehyde.

The result shows that the administration of TCE (Trichloroethylene) caused a decrease in the serum CAT and SOD levels when the Negative control, Positive control (Standard), 100mg/bdw, and 200mg/ bdw, values were compared with the Normal control group Normal control. Also, there were significant increases (p>0.05) in the level of MDA and GSH, Normal control was compared with the treatment group. Administration of TCE causes a decrease in the serum of GSH levels when the Negative control, Positive control, and 100mg/kg are compared with the control except for the 200mg/kg, which has a significant increase (P>0.05)

compared with the control. There is also a decrease in the serum of CAT levels when the Negative control, Positive control, 100mg/kg, and 200mg/kg are in comparison to the control group, there was a significant increase the level of SOD and MDA, the control was compared with the treatment groups.

	Hunteria umbellata leaf extract						
Parameters	Control	Negative control	Silymarin STD	Hunteria umbellata (100mg/kg)	Hunteria umbellata (200mg/kg)		
HDL	50.50±2.50 ^a	50±7.07 ^a	52.5±3.5 ^a	45.5±3.5 ^b	73.70±2.90 ^c		
LDL	23.00±0.00 ^a	43±0.00 ^b	65±0.00 ^c	59.5±1.5 ^c	32.90±1.90 ^a		
VLDL	11.00±0.00 ^a	43±0.00 ^b	10.5±0.5 ^a	12±0.00 ^a	35.70±1.90 ^c		
cholesterol	44.00±1.00 ^a	66±1.41 ^b	86±1.00 ^b	82±2.00 ^b	80±4.10 ^b		
TAG	55.50±1.50 ^a	62.5±2.12 ^b	52.5±2.5 ^a	60.5±1.5 ^b	142.30±3.40 ^a		

 TABLE 3 Effect of Alcoholic Leaf Extract of Hunteria Umbellata on Lipid

 Profile on TCE (Trichloroethylene) Induced Wistar Female Albino Rats.

The values presented in the table indicate the mean \pm standard deviation (SD) for a sample size of n=5. Values within the same row that share the same letter of the alphabet are not significantly different from each other (P<0.05). The abbreviations used in the table are as follows: HDL represents High Density Lipoprotein, LDL refers to Low Density Lipoprotein, VDL represents Very Low Density Lipoprotein, CHOL denotes Cholesterol, TAG stands for Triacylglycerol, and STD represents Standard Drug.

We demonstrate the protective effect of the *Hunteria umbellata* against trichlorethylene-induced toxicity on hematological parameters in female albino rats. In female rats treated with standard drug (mg/kg), 100 mg/kg, and 200 ml/kg, there were no significant differences in RBCs, MCVs, and monocytes compared to normal negative controls (p >0.05). There was a statistically significant 200 mL/kg increase in PCV, Hb, WBC, MCHC, platelets, and lymphocytes compared to positive and negative controls (P > 0.05). In addition, neutrophils, lymphocytes, WBC, PCV, and hemoglobin were significantly increased in ml/kg and 100 ml/kg standard drug compared to negative controls.

TABLE 5: The impact of alcoholic leaf extract of *Hunteria umbellata* on hematological parameters was examined in Trichloroethylene (TCE) induced Wistar female albino rats..

		Negative	Silymarin	Extract at	Extract at
Parameter	eter Control Control		(STD)	100mg/kg	200mg/kg
RBC (x10 ¹² /L)	7.05±0.272	6.11±0.221 ^a	6.05±0.150	6.47±0.197	6.47±0.197
PCV (%)	40.89±2.224	43.70±3.237	39.34±3.692	43.37±4.318 41.23±1.88	
HB (g/dL)	(g/dL) 14.02±0.443 43.54±1.61		14.14±0.714 14.57±0.377		14.33±0.284
WBC (x10 ⁹ /L)	9.52±0.937	9.56±0.791	9.15±0.305	9.5±0.365	9.97±0.183
Platelet (x10 ⁹ /L)	562.33±41.60	522.00±11.54	600.33±90.16	551.67±53.13	532.67±33.64
MCV (fl)	64.407±0.382	64.807±1.020	66.067±7.094	64.437±2.74	66.587±1.92
MCH (pg)	21.493±0.753	21.513±0.098	24.04±3.496	21.703±0.399	22.663±0309
MCHC (g/L)	21.83±0.97	21.85±0.36	24.04±3.56	21.37±0.57	22.99±0.13

Hunteria umbellata (mg/kg)

Values represent the mean \pm SD for n=5, Values in the same row bearing the same alphabets are not significantly different from each other (P > 0.05). RBC, Red Blood Cells; PCV, Packed Cell Volume; HB, Haemoglobin; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell., STD: Standard Drug.

We demonstrate the protective effect of *Hunteria umbellata* against trichlorethylene-induced toxicity on hematological parameters in female albino rats. In female rats treated with standard drugs (mg/kg), 100 mg/kg, and 200 ml/kg, there were no significant differences in RBCs, MCVs, and monocytes compared to normal negative controls (p >0.05). There was a statistically significant 200 mg/kg increase in PCV, Hb, WBC, MCHC, platelets, and lymphocytes compared to negative controls (P > 0.05). In addition, neutrophils, lymphocytes, WBC, PCV, and hemoglobin were significantly increased in ml/kg and 100 ml/kg standard drugs compared to negative controls.

TABLE 6: Effect of Alcoholic leaf extract of *Hunteria umbellata* on Kidney biomarker on Trichloroethylene- induced Wistar Female Albino Rats.

Group	Na+ (mEq/L)	Cl- (mEg/L)	K+ (mEq/L)	HCo3- (mEg/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	131.7± 2.8ª	94.1 ± 6.3 ^a	4.96 ± 0.26 ^a	22.05 ± 1.09 ^a	15.94 ±0.76 ^a	0.43 ±0.05 ^a
TCE	167.6 ±0.83 ^c	104.6±4.06 ^b	6.08 ±0.36 ^b	39.04±0.23 ^b	27.55±3.64 ^b	1.90±0.15 ^c
Standard drug	144.8±5.71 ^b	99.2 ±2.29 ^c	4.94±0.23 ^a	32.25 ±5.74 ^c	24.64±1.49c	0.99 ±005 ^b
(Silymarin)						
Hunteria umbellata	143.5 ±4.22 ^b	97.2±1.28 ^c	4.69±0.27 ^c	34.25±7.93 ^c	23.21±0.68 ^c	0.92±0.04 ^b
(100mg/kg) <i>Hunteria umbellata</i> (200mg/kg)	138.3 ±2.17ª	99.4 ± 2.18 ^c	4.88±0.17ª	23.66±0.77ª	23.66±0.77 ^c	0.89±0.05ª

Values represent the mean \pm SD for N=3. Values in the same row bearing the same alphabets are not significantly different from each other (P > 0.05).

The table presents the effects of alcoholic leaf extract of *Hunteria umbellata* on kidney biomarkers in female albino rats exposed to Trichloroethylene (TCE). The TCE group exhibited significant deviations in most biomarkers, indicating kidney damage. However, the standard drug (Silymarin) group and both doses of *Hunteria umbellata* extract (100mg/kg and 200mg/kg) showed improved biomarker levels, suggesting potential kidney protection. The values with the same alphabet in the last line were not significantly different. Overall, *Hunteria umbellata* extract may have protective effects on kidney biomarkers, but further research is required to understand its benefits and safety better.

Discussion

Elevated concentrations of plasma lipids, particularly cholesterol, and triglycerides, are known risk factors for cardiovascular problems (Brown & Goldstein, 1992). Lipids are transported in the bloodstream by lipoproteins, complexes of lipids, and proteins (Nwanjo, 2005). High LDL and low HDL cholesterol levels are vital to developing hyperlipidemia (Ugwu *et al.*, 2013). According to Ezekwesili *et al.* (2008), lowering LDL and raising HDL levels can help avoid or delay the onset of chronic illnesses linked to hyperlipidemia. *Hunteria umbellata* is a widely used medicinal plant with potential health benefits.

The study on *Hunteria umbellata* leaf extract results demonstrates a significant difference (p<0.05) in the HDL, VLDL, and TAG concentrations between the standard control group and Group B1. This suggests that the extract can improve the blood lipid profile. Moreover, there were no statistically significant differences observed in the concentrations of HDL, VLDL, and TAG between the standard control group and Group 1. The negative control group showed the highest concentrations of VLDL and TAG. Moreover, there was a significant difference in cholesterol levels between the standard control group and the other groups. However, a significant difference in HDL values was observed between vitamin E and other groups. Free radicals are continuously created in biological systems and linked to tissue damage and several disorders (Halliwell & Gutteridge, 1999).

While synthetic medications are effective in protecting against oxidative damage, they are frequently associated with adverse side effects. Consequently, incorporating natural antioxidants from food supplements and traditional treatments can serve as an alternative approach to address this concern (Yazdanparast & Ardestani, 2007). The present study aimed to investigate the effects of Hunteria umbellata on antioxidant enzymes in TCE-induced female albino rats.

The protection against reactive oxygen species (ROS) is greatly aided by antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) (Surapaneni & Venkataramana, 2007).

Malondialdehyde (MDA), a byproduct of lipid peroxidation, indicates the extent of oxidative damage and cell injury (Surapaneni & Venkataramana, 2007). Glutathione (GSH) is a non-enzymatic antioxidant protecting cells from oxidative damage (Kadiska *et al.*, 2000). Decreased levels of GSH are associated with hepatic damage (Dambach *et al.*, 2006). Catalase (CAT) is an antioxidant enzyme that breaks down hydrogen peroxide, protecting tissues from highly reactive hydroxyl radicals (Nandy *et al.*, 2012). Superoxide dismutase (SOD) acts as the first line of defense against damage caused by reactive oxygen species (Kangralkar *et al.*, 2010).

The rats exposed to TCE in this study exhibited alterations in their antioxidant defense system, including changes in CAT activity. These changes may be attributed to oxidative stress and the formation of free radicals (Naazeri *et al.*, 2014). The results indicate that Hunteria umbellata extract may benefit lipid profile along with antioxidant defense in TCE-induced rats. However, further research is needed to understand the underlying mechanisms and confirm the findings.

The study suggests that *Hunteria umbellata* extract may improve lipid profile parameters and modulate antioxidant defense in TCE-induced rats. The findings support the potential health benefits of Hunteria umbellata and its potential application in managing hyperlipidemia and oxidative stress-related disorders. However, more research is needed to clarify the precise mechanisms of action and assess the efficiency and safety of the extract.

Additionally, the significant increase in malondialdehyde (MDA) levels in the TCE-induced group may result from protein deterioration and membrane-bound enzymes' direct free radical inactivation. Results for body weight and liver antioxidant capacity in the TCE-induced group of female albino rats showed that the treatment groups (Silymarin standard "Positive control" and *Hunteria umbellata* alcoholic leaf extract "100mg/kg and 200mg/kg") increased liver antioxidant capacity while decreasing it in the Negative control group.

Hematological parameters play a crucial role in assessing an animal's physiological well-being, as they provide valuable insights into the animal's health status (Khan & Zafar, 2005). Blood, being a target organ for various toxins, serves as an essential marker to reflect an animal's health condition when exposed to different substances and conditions (Olafedehan *et al.*, 2010).

Red blood cells, or erythrocytes, are particularly important components of blood. They act as carriers of hemoglobin, a protein that binds with oxygen during respiration, forming oxyhemoglobin (Chineke & Ikeobi,

2006). Red blood cells play a vital role in transporting both oxygen and carbon dioxide throughout the body, as emphasized by Isaac *et al.* (2013).

The red blood cell count (RBC test) is a critical parameter for evaluating the status of red blood cells in the blood. Deviations from the normal RBC count can provide essential information about an animal's health. An unusually high RBC count might indicate dehydration, renal disease, bone marrow disorders like polycythemia, or living at high altitudes. Conversely, a reduced RBC count signals a decrease in oxygen delivery to the body tissues and a decrease in the removal of carbon dioxide, which could be caused by anemia, bone marrow disorders, nutritional deficiencies, exposure to toxins, or thyroid conditions (Ugwuene, 2011; Soetan & Ajibade, 2013; Isaac *et al.*, 2013).

In the study, the administration of the plant extract at both 100mg/kg and 200mg/kg doses resulted in a decrease in certain red blood cell-related parameters, namely RBC count, PCV, and hemoglobin (HB) levels, compared to the Control group. Notably, the decrease was more pronounced in the group receiving the lower dose of 100mg/kg of the extract.

The observed results align with the understanding of hemoglobin's vital role in oxygen transport and carbon dioxide removal (Isaac *et al.,* 2013). The decrease in RBC count and related parameters suggests that the plant extract may be affecting the production or functioning of red blood cells, leading to a reduced capacity to carry oxygen.

In contrast, the white blood cell count (WBC) and platelet count showed no significant differences among the groups, indicating that the plant extract had limited impact on these specific blood parameters. Similarly, mean corpuscular volume (MCV) showed minor variations without a consistent pattern of change across the groups. However, the mean corpuscular hemoglobin (MCH) showed a slight increase in the group receiving the higher dose of 200mg/kg of the plant extract compared to the other groups.

Overall, the results suggest that the plant extract may affect certain red blood cell-related parameters, particularly RBC count, PCV, and HB, at both dosages. However, it does not appear to have a substantial influence on other blood parameters, such as WBC count, platelet count, MCV, and mean corpuscular hemoglobin concentration (MCHC).

Therefore, understanding hematological parameters, especially the red blood cell count, is essential for assessing an animal's overall health. These parameters serve as valuable tools for researchers and veterinarians to monitor an animal's well-being and detect any potential health issues resulting from exposure to toxins or other health-related conditions. By closely examining these indicators, professionals can take appropriate measures to safeguard the health and well-being of animals under their care. The results from the table suggest that the alcoholic leaf extract of *Hunteria umbellata* shows potential protective effects on kidney biomarkers in female albino rats exposed to TCE. The TCE group exhibited significant deviations in most kidney biomarkers compared to the control group, indicating kidney damage or dysfunction caused by TCE exposure. However, the administration of *Hunteria umbellata* extract at both 100mg/kg and 200mg/kg doses resulted in improvements in Na⁺, Cl⁻, K⁺, and HCO3-levels, which became closer to those of the control group. Furthermore, the levels of urea and creatinine, indicators of kidney function, decreased in the treated groups, suggesting potential beneficial effects.

Comparatively, the standard drug (Silymarin) group showed significant improvement in most kidney biomarkers compared to the TCE group, with levels of Na⁺, Cl⁻, and K+ approaching those of the control group and reduced levels of HCO3⁻, urea, and creatinine. This indicates a protective effect of Silymarin on kidney function in the presence of TCE-induced toxicity.

The lack of significant differences among the treatment groups for specific biomarkers, as indicated by the same alphabet in the last line, suggests that the treatments did not significantly affect those specific parameters. Additional research is needed to discover the best dosages, examine the potential mechanisms of action of Hunteria umbellata extract, and assess its safety profile for kidney protection.

Overall, the results provide promising evidence that *Hunteria umbellata* extract, particularly at 100mg/kg and 200mg/kg, could offer protective effects against TCE-induced kidney damage.

Conclusion

We have shown in this study that the preventive effects of *Hunteria umbellata* alcoholic leaf extract offset the TCE-induced toxicity in female albino rats. The majority of the results point to the alcoholic leaf extract of *Hunteria umbellata* as being protective against TCE-induced toxicity in female albino rats. SOD, MDA, and GHS levels rose due to its administration, while catalase levels dropped. These results suggest that *Hunteria umbellata* alcoholic leaf extract may protect against lipid peroxidation by scavenging the harmful radicals produced by TCE and, as a result, normalizing the quantity and distribution of lipids in female albino rats' systemic circulation.

Although further tests are necessary to isolate and characterize the bioactive compounds present in the alcoholic leaf extract of *Hunteria umbellata*, the elevated levels of antioxidants observed in this study could be associated with the hepatoprotective properties of the extract against cellular oxidative damage caused by Trichloroethylene (TCE). However, to

gain a comprehensive understanding of the protective and antioxidant mechanisms of *Hunteria umbellata* alcoholic leaf extract against TCE toxicity, molecular studies involving pro-inflammatory and inflammatory bioassays are recommended. Additionally, investigating which transcription factors are up-regulated or down-regulated during the protective phase would be beneficial.

Moreover, the results suggest that *Hunteria umbellata* extract may have protective effects on kidney biomarkers in female albino rats exposed to TCE. Treatment with the extract at doses of 100mg/kg and 200mg/kg led to improvements in Na+, Cl-, K+, and HCO3- levels, approaching those of the control group, while simultaneously reducing urea and creatinine levels. The standard drug, Silymarin, also exhibited protective effects on kidney function. However, further research is needed to comprehensively understand the mechanisms of action, determine optimal dosages, and assess the safety of *Hunteria umbellata* extract for kidney protection.

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Conflict of interest statement:

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Data availability statement

We are devoted to supporting open science practises and transparency in government. We want to help the scientific community and promote more study and collaboration in the field by sharing our data. Please be aware that any use of the data must comply with all applicable ethical and legal rules and must be supported by proper citation and author attribution.

Bibliography

- 1. Adegoke, E.A.; Alo, B. (2017). Abereamines, water-soluble seed alkaloids from *Hunteria umbellata*. *Phytochemistry*. 25(6): 1461-1468.
- 2. Adeneye, A.A.; Adeyemi, O.O.; Agbaje, E.O. (2015). Further evaluation of the antihyperglycaemic effect of *Hunteria umbellata* seed extract in experimental diabetes. *Ethnopharmacological journal*. 126: 238-336
- Adewale, A.A.; Anthony, C.P.; Zainab, F.; Anne, M.; Williams, Z.S.; Olaide, A.O.; Oluwatoyin, A.E. (2013). African journal, traditional complement. *Alternative medicine*. 10: 189-202
- 4. Aebi H. Catalase in vitro. Methods in Enzymology, 1984; 105: 121 126.
- Al-Harbi, N.O.; Iman, F.; Nadeem, A.; Al-Harbi, M.M.; Igbal, M.; Ahmad, F.S. (2014). International immunopharmacology. 21, 383-388
- 6. Ballay, H. (2014). Medicinal Plant, Geographical location. 18-20
- 7. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. Churchill Livingstone, Elsevier, China. 2008.
- 8. Boll, M.; Weber, L.W.D.; Becker, E.; Stampfl, A.; Naturforsch, Z. (2015). Biomolecules and Biochemical changes. *Medicinal Plant*. 56: 111-121

- 9. Boone, M.J. (2016). Hunteria umbellata, plant resources of tropical Africa. *Medicinal Plants*. 220-225
- Bouquet, A.; Debray, M. (2015). Plant Medicinal. *Research Documents*. 32: 231
- 11. Burkill, H.M. (2014). The useful plants of west tropical Africa. Royal Botanic Gardens, *united kingdom*.1:612.
- 12. Burkill, H.M. (2016). The useful plants of west tropical Africa. Royal Botanic Gardens. *United Kingdom*. 5: 686.
- Dalziel, J.M. (2015). The useful plants of west tropical Africa .Crown Agents for overseas Governments and Administrations, *London united Kingdom*. 612.
- Endress, M.E.; Sennblad, B.; Nilsson, S.; Civeyrel, L.; Chase, M.W.; Huysmans, S.; Grafstorm, E.; Bremer, B. (2017). A phylogenetic analysis of Apocynaceae and some related taxonomy in Gentianales. 7: 59-102
- 15. Falodun, A.; Nworgu, Z.A.M.; Ikponmwonsa, M.O. (2016). Phytochemical components of *Hunteria umbellata* and its effect on isolated non-pregnant rat uterus in oestrus. *Pharmaceutical science*. 19: 256-258
- Harward, J.B. (2018). Alkaloids present in *Hunteria umbellate*, Leaf extract. *Medicinal Plants*. 336-337
- 17. Herzog, F. (2018). Multipurpose shade trees in coffee and cocoa plantation in Coted'Ivoire. *Agroforestry Systems*. 27: 259-267
- 18. Holm, G.; Herbst, V.; Teil, B. (2012). Brogenkunde. *Plant medicine*. 67:263-269.
- 19. Hong, B.O.; Shao, H.A.; Betteridge, D.J. (2018). What is oxidative stress metabolism. 49: 3-8
- 20. Ibeh, I.N.; Idu, M.; Ejimadu, I.M. (2017). Medicinal Plant Botanical Classification and traditional name. *Research journal*. 15: 4-7
- Ibeh, I.N.; Idu, M.; Ejimadu, I.M. (2017). Toxicological assessment of Abeere, Mkpokiri and Adaci, *Hunteria umbellata* (Apocynaceae). *Biosciences*. 23: 22-27
- 22. Igbe, I.; Omogbai, E.K.I.; Ozolua, R.I.; Okpo, S.O.; Obasuyi, O. (2019). Hyperglycaemic activity of aqueous seed extract of *Hunteria umbellata* in normal and streptozotocin-induced diabetic rats. Antipyretic and analgestic effects of the aqueous extract of the fruit pulp of *Hunteria umbellata*. Pharmaceutical Resources. 8: 331-336
- 23. Iwu, M.M.; Duncan, A.R.; Okunji, C.O. (2013). New Antimicrobials of plant origin. 40-45.
- 24. Kokwaro, J.O. (2012). Medicinal plants of East Africa. Second Edition. 20-22.
- 25. Lemma, A. (2015). The potentials and challenges of Endod, the Ethiopian soap-berry plant for control of schistosomiasis. 25-28.
- 26. Momodu, O.; Enogieru, A.; Omoniyi, S.; Om'Iruabohs, F.A.E. (2014). Clinical anatomy. Medicinal Plant. *Express Journals*.13: 66-73
- Mulhall, J.; King, R.; Glina, S.; Hvidsten, K. (2018). Importance of and satisfaction with sex among men and women worldwide. *Sex medication*. 5: 78-95
- Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 1974; 47: 469 – 474.

- 29. Neuwinger, H.D. (2016). African traditional medicine. *Medpharm scienctific Stuttgart Germany*. 598.
- Nwaogwugwu, J.C.; Egege, A.N.; Akubugwo, E.I. (2019). Biochemical changes of ethanolic leaf extract psidum Guajava on Strytozocin induced Diabetic rats. *Public Health*. 3(1): 000133
- 31. Oliver Bever, B. (2015). Medicinal plants in tropical West Africa, Cambridge University press .*United Kingdom*. 375
- 32. Oluwemimo, A.; Usifoh, C.O. (2018). The anthelmintic activity of *Hunteria umbellata* K. Schum. (family: Apocynaceae) extracts. *Journal of Scientific and Industrial Research*. 44(5): 286- 290.
- Ohkawa H, Ohishi N, Yogi K. Assay for lipid peroxidation in animal tissues by barbituric acid reaction. Annals of Biochemistry, 1979; 95: 351 – 358.
- Omino, E.A. (2016). A contribution to the leaf anatomy and taxonomy of Apocynaceae in Africa. Wageningen Agricultural university. Netherlands. 178.
- 35. Raman, A.; Mallam, V. (2017). Enhanced in vitro activity of glucokinase enzyme in the presence of extracts of Hunteria umbellate seeds, a traditional Nigerian treatment For diabetes. *Journal of pharmacy and pharmacology*. 46: 1046.
- 36. Rehan, A.; Stephen, B.; Buttler, J.C. (2018). Liver in toxication. *Pharmacological journal*. 143: 311-319
- 37. Renner, U. (2015). Hunteriamine, use of alkaloids for hypotensive treatment , Hunteria eburnean, *physiological chemical*. 331: 105-108
- 38. Richard, A.H. (2017). Royal Botanic Garden, Hunteria umbellata. 5-7
- 39. Richard, A.H. (2018). Botanic gardens conservation international. *Hunteria umbellate*. 3-5
- Schreckinger, M.E.; Wang, J.; Yousef, G.; Lila, M.A.; Gonzalez de Mejia, E. (2015). Antioxidant capacity and in vitro inhibition of adipogenesis and inflammation by phenolic extracts of vaccinium floribundum and Aristotelia chilensis. Agricultural food chemical. 58: 8966-8976.
- Sedlak J, Lindsay RH. Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry, 1968; 25: 1192 – 1205.
- 42. Tolman, K.G.; Dalpiaz, A.S. (2013). Occupational and environmental Hepatotoxicity in Drug- induced liver disease. 659-675
- 43. Vercauteren, J.; Kerharo, J.; Morfaux, A.M.; Massiot, G.; Le Men-Oliver, L.S.; Le Men, J. (2018). Alkaloids present in *Hunteria umbellata*, Leaf extract. *Phytochemistry*. 19: 1959-1961
- 44. Weber, L.W.; Boll, M.; Stampfl, A. (2013). Critical review on toxicology. *Plant toxicity*. 33, 105-136.