

Original Research Article

Curculigopilosa rhizome reverses the activities of some oxidative stress marker enzymes and lipid profiles dysfunction in drug toxicity-induced rats

ABSTRACT

Exposure to drug toxicity results in stress and dysfunction of metabolizing enzymes that cause illnesses and diseases. Consumption of medicinal plant rhizomes is a regime for managing the complications. The effects of an aqueous extract of *Curculigopilosa* (*C. pilosa*) rhizome on the activities of some oxidative stress marker enzymes (catalase, glutathione, and superoxide dismutase), as well as the concentration of lipid profiles (cholesterol, triglycerides, and phospholipids) of p-hydroxyacetanilide (pPHA)-induced toxicity in rats, were evaluated. Forty rats were randomly grouped into eight groups (n = 5). The control group; aqueous extract of *C. pilosa* rhizome group; 750 mg/kg and 1000 mg/kg per body weight of pPHA group; the preventive groups (aqueous extract of *C. pilosa* rhizome and 750 mg/kg per body weight of pPHA; aqueous extract of *C. pilosa* rhizomes and 1000 mg/kg per body weight of pPHA); and the curative groups (750 mg/kg per body weight of pPHA and aqueous extract of *C. pilosa* rhizome; 1000 mg/kg body weight of pPHA and aqueous extract of *C. pilosa* rhizome). The oxidative stress marker enzymes and lipid profiles were analysed spectrophotometrically in the serum, kidney, brain, and liver of the animals on the seventh and fourteenth days after the administrations. The results show that pPHA decreases the oxidative stress marker activities and the lipid profile concentrations in all the compartments, but the pre- and post-treatment with an aqueous extract of *C. pilosa* rhizome improved the activities of the stress marker enzymes and the lipid profiles dysfunction. The result suggests that an aqueous extract of *C. pilosa* rhizome has preventive and curative therapeutic potential for pPHA-induced toxicity.

Keywords: Preventive, curative, p-hydroxyacetanilide, spectrophotometrically, aqueous

1. INTRODUCTION

Analgesics are among the most popular drugs that are being abused, which brings about drug toxicity. The p-hydroxyacetanilide (pPHA) compound, also called acetaminophen, paracetamol, or Tylenol, is an analgesic drug that was derived by oxidizing two analgesics, phenacetin and acetanilide, and is widely used as a therapeutic drug for pain. However, high doses (abuse) of pPHA are toxic, which is harmful to the body and influences organs (liver, kidney, ocular, etc.), damage, blood, and central nervous system dysfunction. Stress and malfunction of the metabolizing enzymes necessary for normal body function can occur due to drug toxicity, which causes illnesses and diseases [1-3]. The metabolism of a drug may

generate a reactive intermediate that can reduce molecular oxygen directly to generate reactive oxygen species, which are a byproduct of normal metabolism and have roles in cell signalling and homeostasis. When the cellular antioxidant capacity (such as ascorbic acid, vitamin E, and glutathione) and antioxidant enzymes (such as thioredoxins, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, etc.) that regulate cellular levels exceed that of reactive oxygen species, oxidative stress can result. Oxidative stress causes damage that comes from the significant modification of intracellular targets such as deoxyribose nucleic acid, proteins, and lipids, which modulate survival signalling cascades. These lead to a wide range of diseases, including cardiovascular diseases, chronic obstructive pulmonary disease, diabetes, cataracts, and cancer, to mention a few [4-7]. Also, oxidative stress resulted in damage to cellular biomembranes caused by radical-mediated lipid peroxidation, which converts unsaturated lipids into polar lipid hydroperoxides. Any small changes in the abundance, composition, or location of lipids (cholesterol, triglycerides, phospholipids, and free fatty acids) can have profound effects on cellular viability and functions. Therefore, hydroxyl radical attack on the fatty acyl chains of phospholipids and triglycerides caused lipid peroxidation, which affects cellular function, and the disorder in homeostasis of these lipids resulted in dysfunction [7-10]. Lipid dysfunction has been considered a global public health challenge and a contributor to complications in the endocrine, central nervous, hepatic, and renal systems [8,11,12]. Medicinal plant products and their derivatives have been considered the origin of therapeutic elements since ancient times. The use of medicinal plants as herbal remedies has also been widely embraced in many developed countries, with complementary and alternative medicines now becoming mainstream globally [13-15]. The *Curculigopilosa* (*C. pilosa*) Schum and Thom plant belongs to the family Hypoxidaceae. The rhizomes of the *C. pilosa* plant possess medicinal properties (bioactive constituents) that are used as food for adults and infants, as well as in the management and treatment of several diseases [16-21], but the mechanism of action is yet to be understood. Therefore, this study aims to investigate the effect of an aqueous extract of *C. pilosa* rhizome on the activities of some oxidative stress marker enzymes (catalase, reduced glutathione, and superoxide dismutase), as well as the concentration of lipid profiles (cholesterol, triglycerides, and phospholipid) of para-hydroxy acetanilide (pPHA)-induced toxicity in rats.

2. METHODOLOGY

2.1 Collection of plant material and processing

The plant material, *C. pilosa* rhizome, was purchased from Lusada market, Ado-Odo/Ota L.G.A., Ogun State, in the south-western part of Nigeria. The plant was identified, authenticated, and stored in the herbarium. The rhizome extract was performed according to previous method [16].

2.2 Experimental Animals and Study Design

Forty (40) healthy female albino rats of an average weight of 100-150g were housed in plastic cages and allowed to feed and water freely to acclimatize with the environment for two weeks under standard environmental conditions ($25 \pm 21^{\circ}\text{C}$; 12/12 h light/dark cycle). After which, they were divided into eight groups (n = 5) as described:

Table 1: The grouping and animal treatments

Groups	Treatment dose
I	Control given water (7 days)

II	Oral administration of 300 mg/kg body weight of an aqueous extract of <i>C. pilosa</i> rhizome (7 days)
V	Oral administration of 750 mg/kg of p-hydroxyacetanilide (pPHA) per body weight (7 days)
VI	Oral administration of 1000 mg/kg of p-hydroxyacetanilide (pPHA) per body weight (7 days)

Preventive group

III	Oral administration of 300 mg/kg body weight of an aqueous extract of <i>C. pilosa</i> rhizome (7 days) and 750 mg/kg p-hydroxyacetanilide (pPHA) per body weight (7 days)
IV	Oral administration of 300 mg/kg body weight of an aqueous extract of <i>C. pilosa</i> rhizome (7 days) and 1000 mg/kg of p-hydroxyacetanilide (pPHA) per body weight (7 days)

Curative group

VII	Oral administration of 750 mg/kg of p-hydroxyacetanilide (pPHA) per body weight (7 days) and 300 mg/kg body weight of an aqueous extract of <i>C. pilosa</i> rhizome (7 days)
VIII	Oral administration of 1000 mg/kg of p-hydroxyacetanilide (pPHA) per body weight (7 days) and 300 mg/kg body weight of an aqueous extract of <i>C. pilosa</i> rhizome (7 days)

At the end of the administration, the animals were fasted overnight and sacrificed under light (ketamine) anaesthesia. Blood was collected by cardiac puncture into heparinized tubes, and organs (brain, liver, and kidneys) were excised. The serum, brain, liver, and kidney were processed according to the method of [22].

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2.3 Biochemical Analysis

Catalase, superoxide dismutase, and reduced glutathione activities were measured following the methods of [23,24]. The lipid profiles (cholesterol, triglyceride, and phospholipid) were determined using Randox commercial kits.

2.4 Statistical Analysis

The statistical analysis was performed using SPSS version 20.0. The results were expressed as mean \pm SEM. A one-way analysis of variance (ANOVA) was carried out at $P < 0.05$ among the groups.

3. RESULTS AND DISCUSSION

The present study was conducted to investigate the effect of analgesic pPHA-induced toxicity on some oxidative stress markers and lipid metabolizing enzymes in the serum, kidney, brain, and liver of a female rat treated with an aqueous extract of *Curculigopilosa* rhizome. The study reveals that the hallmark of pPHA-induced toxicity in rats is a significant ($P < 0.05$) reduction in the activities of oxidative stress marker enzymes (catalase (Table 2), superoxide dismutase (Table 3), and reduced glutathione (Table 4)) in all the compartments.

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Table 2: The effect of *C. pilosa* rhizome extract on catalase activity in PHA-induced toxicity rats

Treatment dose	Catalase activity			
	Serum (U/ml)	Brain (U/g wet tissue)	Liver (U/g wet tissue)	Kidneys (U/g wet tissue)

Control	96.53±43.67 ^a	157.89±1.71 ^a	138.86±7.99 ^a	134.41±1.24 ^a
<i>C. pilosa</i>	367.99±92.29 ^b	177.81±4.86 ^b	171.17±1.72 ^b	139.01±1.15 ^b
Cp + 750 mg/kg	132.94±66.05 ^{b*}	164.61±1.19 ^{b*}	158.03±0.97 ^{b*}	123.81±0.67 ^{b*}
Cp + 1000 mg/kg	71.96±13.66 ^{b**}	158.91±3.18 ^{b**}	135.83±2.07 ^{b**}	134.58±2.65 ^{b**}
750 mg/kg	109.57±19.14 ^c	129.63±1.29 ^c	61.97±2.27 ^c	114.58±1.01 ^c
750 mg/kg + Cp	152.71±33.74 ^{c*}	135.63±1.46 ^{c*}	86.35±1.85 ^{c*}	124.58±1.01 ^{c*}
1000 mg/kg	108.69±7.76 ^d	90.10±0.96 ^d	46.03±0.51 ^d	100.58±3.24 ^d
1000 mg/kg + Cp	142.79±20.17 ^{d*}	105.90±2.16 ^{d*}	67.99±1.06 ^{d*}	128.58±0.58 ^{d*}

Values in columns are mean ± S.E.M for 5 rats in each group. Values having different superscripts within a column differ significantly from each other ($P < 0.05$). Cp = aqueous extract of *C. pilosa* rhizome

Table 3: The effect of *C. pilosa* rhizome extract on superoxide dismutase activity in PHA-induced toxicity rats

Treatment dose	Superoxide dismutase activity			
	Serum (U/ml) x 10 ²	Brain (U/g wet tissue) x 10 ²	Liver (U/g wet tissue) x 10 ³	Kidneys (U/g wet tissue) x 10 ³
Control	0.19±0.04 ^a	0.32±0.04 ^a	0.38±0.07 ^a	0.30±0.01 ^a
<i>C. pilosa</i>	0.25±0.03 ^b	0.61±0.03 ^b	0.78±0.25 ^b	0.70±0.09 ^b
Cp + 750mg/kg	0.10±0.05 ^{b*}	0.47±0.19 ^{b*}	0.48±0.14 ^{b*}	0.50±0.03 ^{b*}
Cp + 1000mg/kg	0.21±0.02 ^{b**}	0.08±0.06 ^{b**}	0.40±0.06 ^{b**}	0.36±0.06 ^{b**}
750mg/kg	0.23±0.03 ^c	0.14±0.01 ^c	0.43±0.08 ^c	0.22±0.02 ^c
750mg/kg + Cp	0.54±0.06 ^{c**}	0.25±0.06 ^{c**}	0.51±0.09 ^{c**}	0.27±0.05 ^{c**}
1000mg/kg	0.23±0.02 ^d	0.13±0.06 ^d	0.41±0.06 ^d	0.19±0.01 ^d
1000mg/kg + Cp	0.33±0.03 ^{d**}	0.36±0.07 ^{d**}	0.58±0.02 ^{d**}	0.28±0.03 ^{d**}

Values in columns are mean ± S.E.M for 5 rats in each group. Values having different superscripts within a column differ significantly from each other ($P < 0.05$). Cp = aqueous extract of *C. pilosa* rhizome.

These enzyme reductions occur when pPHA is activated by cytochrome P450, amidases, and peroxidases to form the toxic reactive metabolite N-acetyl-p-benzoquinoneimine. At toxic doses of pPHA, there is excessive production of N-acetyl-p-benzoquinoneimine, causing a reduction in glutathione activity (Table 4), which leads to hepatotoxicity, as observed in previous research [16,25]. However, due to the inadequate glutathione activity, the production of reactive oxygen species like superoxide, hydrogen peroxide, and hydroxyl radicals has been increased by N-acetyl-p-benzoquinoneimine, which leads to a reduction in the enzymatic defense systems: catalase (Table 2) and superoxide dismutase (Table 3) activities, respectively. Therefore, these enzyme reductions caused an imbalance in the formation and removal of free radicals (oxidative stress). But, in a condition with severe oxidative stress, the generated oxidant compounds will react with cell components such as lipid, protein, deoxyribose nucleic acid, and cell membrane and lead to pathological complications and oxidative damage, including atherosclerosis, vascular diseases, diabetes, and cancers, to mention a few [1,7,16,26-31].

Table 4: The effect of *C. pilosa* rhizome extract on glutathione activity in PHA-induced toxicity rats

Treatment dose	Glutathione activity			
	Serum (mmol/L)	Brain (mmol/g tissue)	Liver (mmol/g tissue)	Kidney (mmol/g tissue)
Control	3.52±0.49 ^a	14.67±1.04 ^a	23.69±0.39 ^a	9.33±0.38 ^a
<i>C. pilosa</i>	3.38±0.36 ^b	15.67±1.05 ^b	24.70±0.46 ^b	14.47±0.93 ^b

Cp + 750mg/kg	4.00±0.51 ^{b*}	13.61±0.27 ^{b*}	22.80±0.31 ^{b*}	13.14±0.34 ^{b*}
Cp + 1000mg/kg	5.86±0.34 ^{b**}	13.55±0.67 ^{b**}	17.90±0.57 ^{b**}	11.87±0.38 ^{b**}
750mg/kg	6.85±0.43 ^c	12.13±0.67 ^c	20.89±0.24 ^c	15.30±1.19 ^c
750mg/kg + Cp	4.87±0.58 ^{c**}	13.19±0.53 ^{c**}	22.90±0.17 ^{c**}	14.05±0.62 ^{c**}
1000mg/kg	8.27±0.47 ^d	11.33±0.46 ^d	18.29±0.40 ^d	12.50±0.51 ^d
1000mg/kg + Cp	6.64±0.42 ^{d**}	13.15±0.45 ^{d**}	21.59±0.24 ^{d**}	11.70±0.48 ^{d**}

Values in columns are mean ± S.E.M for 5 rats in each group. Values having different superscripts within a column differ significantly from each other ($P < 0.05$). Cp = aqueous extract of *C. pilosa* rhizome

Table 5: The effect of *C. pilosa* rhizome extract on serum lipid profile concentrations of PHA-induced toxicity rats

Treatment dose	Serum		
	Cholesterol concentration (mg/dl)	Triglyceride Concentration (mg/dl)	Phospholipids Concentration (mg/dl)
Control	88.54±1.73 ^a	71.11±8.49 ^a	285.92±2.25 ^a
<i>C. pilosa</i>	94.41±2.27 ^b	52.22±2.42 ^b	248.46±1.40 ^b
Cp + 750mg/kg	86.48±8.44 ^b	55.96±4.11 ^b	270.30±4.40 ^{b*}
Cp + 1000mg/kg	84.99±5.19 ^{b**}	58.19±4.24 ^{b**}	286.30±6.08 ^{b**}
750mg/kg	69.90±4.75 ^c	66.58±2.57 ^c	297.81±3.43 ^c
750mg/kg + Cp	74.42±4.04 ^{c**}	47.14±4.51 ^{c**}	290.09±2.98 ^{c**}
1000mg/kg	54.99±8.05 ^d	87.05±5.51 ^d	319.78±8.88 ^d
1000mg/kg + Cp	59.30±5.70 ^{d**}	49.81±3.90 ^{d**}	289.03±4.56 ^{d**}

Values in columns are mean ± S.E.M for 5 rats in each group. Values having different superscripts within a column differ significantly from each other ($P < 0.05$). Cp = aqueous extract of *C. pilosa* rhizome

This study also observed a reduction in the cholesterol, triglycerides, and phospholipid concentrations caused by pPHA-induced toxicity (Table 5), which is similar to the result from other research [32]. The reduction might be due to high activation of enzymes like cytochrome P450, lipoxigenases, and cyclooxygenases in oxidized lipids, and high levels of two free radicals, or reactive oxygen species, namely, hydroxyl radical and hydroperoxyl, that can inflict direct damage to lipids. The hydroxyl radical is produced from oxygen in cell metabolism and under a variety of stress conditions and causes oxidative damage to cells because it unspecifically attacks biomolecules. It is assumed that hydroxyl radicals in biological systems are formed through redox cycling by the Fenton reaction, where free iron reacts with hydrogen peroxide, and the Haber-Weiss reaction, which results in the production of free iron when superoxide reacts with ferric iron. In addition to the iron redox cycling transition-metals, including copper, nickel, cobalt, etc., can also be responsible for hydroxyl radical formation in living cells. The hydroperoxyl radical is a protonated form of superoxide that yields hydrogen peroxide, which can react with redox-cycling active metals to further generate hydroxyl radicals through Fenton or Haber-Weiss reactions. It is a much stronger oxidant than superoxide anion-radical and could initiate lipid peroxidation through the chain oxidation of polyunsaturated phospholipids, thereby leading to impairment of membrane function. Phospholipids and cholesterol are targets of damaging and potentially lethal peroxidative modification [29,33]. Lipid peroxidation occurs in a process in which oxidants such as free radical species attack lipids containing carbon-carbon double bond(s) that involve hydrogen abstraction from a carbon with oxygen insertion, resulting in lipid peroxy radicals and hydroperoxides, by enzymes such as selenium-dependent glutathione

peroxidases and selenoprotein, which catalyze the reduction of hydrogen peroxide or organic hydroperoxides to water or the corresponding alcohols, respectively, using glutathione as a reductant. The presence of selenocysteine in the catalytic center of glutathione peroxidases, as the catalytic moiety, makes fast reactions with the hydroperoxide and reducibility by glutathione. And selenoprotein reduced phospholipid hydroperoxide using glutathione or thioredoxin as co-substrate [29,34-36]. This process continues to attack the lipids and cause damage until there is an alleviation of the stress response. A standard therapeutic for pPHA overdose is N-acetyl cysteine, a scavenger of reactive oxygen species. However, due to the strait therapeutic opening, rapid disease progression, and severe adverse effects, the therapeutic effectiveness of N-acetyl cysteine is still scanty. Therefore, new treatments that are better than N-acetyl cysteine as regards therapeutic efficacy and safety are required [37-39]. Recently, components of medicinal plants were found to be promising therapeutics as herbal medicines. In this present study, the pre- and post-treatment of *C. pilosa* rhizome aqueous extract has a significant ($P < 0.05$) therapeutic potential (curative and preventive) effect on the induced oxidative stress damage in the tissues, where there is an up-regulation of the activities of catalase (Table 2), superoxide dismutase (Table 3), and glutathione (Table 4), respectively, which are similar to the results of previous research [13,25,40], and lipid concentrations (Table 5).

Table 6: The effect of *C. pilosa* rhizome extract on glutathione activity in PHA-induced toxicity rats

Treatment dose	Glutathione activity			
	Serum (mmol/L)	Brain (mmol/g tissue)	Liver (mmol/g tissue)	Kidney (mmol/g tissue)
Control	3.52±0.49 ^a	14.67±1.04 ^a	23.69±0.39 ^a	9.33±0.38 ^a
<i>C. pilosa</i>	3.38±0.36 ^b	15.67±1.05 ^b	24.70±0.46 ^b	14.47±0.93 ^b
Cp + 750mg/kg	4.00±0.51 ^{b*}	13.61±0.27 ^{b*}	22.80±0.31 ^{b*}	13.14±0.34 ^{b*}
Cp + 1000mg/kg	5.86±0.34 ^{b**}	13.55±0.67 ^{b**}	17.90±0.57 ^{b**}	11.87±0.38 ^{b**}
750mg/kg	6.85±0.43 ^c	12.13±0.67 ^c	20.89±0.24 ^c	15.30±1.19 ^c
750mg/kg + Cp	4.87±0.58 ^{c**}	13.19±0.53 ^{c**}	22.90±0.17 ^{c**}	14.05±0.62 ^{c**}
1000mg/kg	8.27±0.47 ^d	11.33±0.46 ^d	18.29±0.40 ^d	12.50±0.51 ^d
1000mg/kg + Cp	6.64±0.42 ^{d**}	13.15±0.45 ^{d**}	21.59±0.24 ^{d**}	11.70±0.48 ^{d**}

Values in columns are mean ± S.E.M for 5 rats in each group. Values having different superscripts within a column differ significantly from each other ($P < 0.05$). Cp = aqueous extract of *C. pilosa* rhizome

The presence of bioactive constituents (phenolics, flavonoids, tannins, and cardiac glycosides) found in the aqueous extract of *C. pilosa* rhizome [29,41,42] is responsible for protective effects mechanisms against pPHA-induced oxidative stress. These compounds are attributed to the extract's free radical scavenging activity, suppression of reactive oxygen species synthesis, and improvement of antioxidative defense enzyme systems to suppress radical damage [43-44]. The scavenging of free radicals is effected by the bioactive components in the aqueous extract of the *C. pilosa* rhizome by reducing the activity of cytochrome P450. This decreases the toxic metabolite N-acetyl-p-benzoquinoneimine and improves the cellular state of glutathione, which then eliminates free radical species like hydrogen peroxide and superoxide radicals and boosts the activities of catalase and superoxide dismutase. As well as the bioactives, reducing the activities of enzymes responsible for the production of hydroperoxides and improving the lipid concentration. This has justified the use of an aqueous extract of *C. pilosa* rhizome in traditional medicine for the treatment of various clinical conditions, such as inflammation, in the management of health issues.

4. CONCLUSION

The generation of reactive oxygen species that cause oxidative stress and lipid dysfunction during high doses or abuse of pPHA is known to be detrimental to health. This study observed that it causes a reduction in the activities of catalase, superoxide dismutase, and glutathione, as well as cholesterol, triglycerides, and phospholipid concentrations.

An aqueous extract of *C. pilosa* rhizome has many bioactive constituents (antioxidants) that have contributed to the positive effects on the serum, brain, liver, and kidney catalase, superoxide dismutase, and glutathione activities, as well as the cholesterol, triglycerides, and phospholipid concentrations. This can be attributed to its function during pre- and post-treatment in preventing, managing, or treating health issues that occur during the toxicity of induced oxidative stress.

ETHICAL APPROVAL

All authors hereby declare that the Guide for the Care and Use of Laboratory Animals and Principles of Laboratory Animal Care were followed. All experiments have been examined and approved by the appropriate ethics committee of the university.

REFERENCES

1. Guengerich FP. A history of the roles of cytochrome P450 enzymes in the toxicity of drugs. *ToxicolResearch*. 2021;37(1):1-23. <https://doi.org/10.1007/s43188-020-00056-z>
2. Sayed HY, Zidan AH. Histopathological and biochemical effects of acute and chronic tramadol drug toxicity on liver, kidney, and testicular function in adult male albino rats. *J Forensic, Toxicology and Medicolegal Analysis*. 2016. 1(2):40-45.
3. Ghosh S, Derle A, Ahire M, More P, Jagtap S, Phadatare SD. *et al*. Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. *PLoS One*. 2013; 8(12):e82529. <https://doi.org/10.1371/journal.pone.0082529>.
4. Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*. 2021;20(9):689-709. <https://doi.org/10.1038/s41573-021-00233-1>
5. Adu OB, Adeyemo GA, Falua OB, Fajana OO, Ogunrinola OO, Saibu GM. *et al*. The effect of *Thaumatococcus daniellii* leaf extracts on immunological and oxidative stress markers in Rat. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 2021;7(4):6-14. <https://doi.org/10.9734/AJBGMB/2021/v7i430179>
6. Tewari D, Samoila O, Gocan D, Mocan A, Moldovan C, Devkota HP. *et al*. Medicinal plants and natural products used in cataract management. *Frontiers in Pharmacology*. 2019;10:466. <https://doi.org/10.3389/fphar.2019.00466>
7. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. *J Toxicol*. 2012;2012:645460. <https://doi.org/10.1155/2012/645460>.
8. Ogunrinola OO, Kanmodi RI, Ogunrinola OA, Adegbulugbe JA, Adu OB. Cholesterol and triglyceride concentrations of lipopolysaccharides- induced inflammatory male rat in response to *Petiveria alliacea* L. Leafs extract. *J. Appl. Sci*. 2022;22:100-106. <https://doi.org/10.3923/jas.2022.100.106>
9. Hauck AK, Bernlohr DA. Oxidative stress and lipotoxicity. *Journal of lipid research*. 2016;57(11):1976-1986. <https://doi.org/10.1194/jlr.R066597>
10. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *European J Medicinal Chemistry*. 2015;97:55-74. <https://doi.org/10.1016/j.ejmech.2015.04.040>

11. Ahmed M. Non-alcoholic fatty liver disease in 2015. *World J. Hepatol.* 2015;7:1450. <https://doi.org/10.4254/wjh.v7.i11.1450>
12. Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: Inflammatory resolution versus thrombotic chaos. *Ann. New York Acad. Sci.* 2012;1254:18–32. <https://doi.org/10.1111/j.1749-6632.2012.06480.x>
13. Samarghandian S, Farkhondeh T, Samini F, Borji A. Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney. *Biochemistry Research International.* 2016. <https://doi.org/10.1155/2016/2645237>
14. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology.* 2013;4:177. <https://doi.org/10.3389/fphar.2013.00177>
15. WHO (2022). WHO establishes the Global Centre for Traditional Medicine in India: Maximizing potential of traditional medicines through modern science and technology. <https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india>
16. Ogunrinola OO, Awote OK, Ogunrinola OA, Popoola HA, Ajetunmobi GO, Mbahokafor CA. The Rhizome of *Curculigopilosa* Exerts Hepatoprotective and Nephroprotective Effects in Rats Exposed to Acetaminophen Toxicity. *South Asian Research Journal of Natural Products.* 2023;5(4):9-15.hal-04020143
17. Karigidi KO, Olaiya CO. Effects of *Curculigopilosa* supplementation on antioxidant and antidiabetic activities of yam flour. *Journal of Food Science Technol.* 2020. <https://doi.org/10.1007/s13197-020-04872-x>
18. Karigidi KO, Ojebode ME, Anjorin OJ, Omiyale BO, Olaiya CO. Antioxidant Activities of *Curculigopilosa* and *Gladilouspittascinus* against Lipid Peroxidation in Rat's Liver and Heart. *J Herbs, Spices Medicinal Plants.* 2018. <https://doi.org/10.1080/10496475.2018.1510457>
19. Adefegha SA, Oyeleye SI, Oboh G. African crocus (*Curculigo pilosa*) and wonderful kola (*Buchholziacoriacea*) seeds modulate critical enzymes relevant to erectile dysfunction and oxidative stress. *J Complement Integrat Med.* 2018. <https://doi.org/10.1515/jcim-2016-0159>
20. Olaiya CO, Idowu PA, Karigidi K. Antioxidative and Antimicrobial Activities of Corn Steep Liquor Anti-diabetic Herb Extracts. *Ann Food Sci Technol.* 2016;17(2):272–279.
21. Shaba EY, Mann A, Yisa J. Antimicrobial and Cytotoxic Activities and GC-MS Analysis of Phytocomponents of Methanolic Extract of *Curculigo pilosa* (schum and Thonn) Engl. (hypoxidaceae) Rhizomes. *Br J Pharm Res.* 2014;4(12):15521567. <https://doi.org/10.9734/BJPR/2014/10189>
22. Ogunrinola OO, Fajana OO, Adu OB, Otutuloro AM, Moses TA. The effect of *Vernonia amygdalina* leaves on lipid profile in cadmium-induced rat. *MOJ Toxicl.* 2019;5:83-87.
23. Ogunrinola OO, Wusu DA, Fajana OO, Olaitan SN, Smith ZO, Bolaji ARI. Effect of low level cadmium exposure on superoxide dismutase activity in rat. *Tropical Journal of Pharmaceutical Research.* 2016;15(1):115-119. <https://doi.org/10.4314/tjpr.v15i1.16>
24. Senthilkumar M, Amaesan N, Sankaranarayanan A. Determination of Glutathione Activity. In: *Plant-Microbe Interactions. Springer Protocols Handbooks.* Humana, New York, NY. 2021. https://doi.org/10.1007/978-1-0716-1080-0_27.
25. Akgun E, Boyacioglu M, Kum S. The potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats. *Experimental Animals.* 2021;70(1):54-62. <https://doi.org/10.1538/expanim.20-0075>.
26. Khan S, Rehman MU, Khan MZI, Muhammad K, Haq IU, Khan MI. In vitro and in vivo antioxidant therapeutic evaluation of *Dodonaea viscosa* Jacq. *Biorxiv.* 2022:2022-04. <https://doi.org/10.1101/2022.04.17.488588>
27. Azarmehr N, Afshar P, Moradi M, Sadeghi H, Sadeghi H, Alipoor B. *et al.* Hepatoprotective and antioxidant activity of watercress extract on acetaminophen-induced hepatotoxicity in rats. *Heliyon.* 2019;5(7).

- <https://doi.org/10.1016/j.heliyon.2019.e02072>
28. Parikh H, Pandita N, Khanna A. Phytoextract of Indian mustard seeds acts by suppressing the generation of ROS against acetaminophen-induced hepatotoxicity in HepG2 cells. *Pharmaceutical Biology*. 2015;53(7):975-984.
<https://doi.org/10.3109/13880209.2014.950675>
 29. Ayala A, Munoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*. 2014. <https://doi.org/10.1155/2014/360438>
 30. Aycan IO, Tufek A, Tokgoz O, Evliyaoglu O, Firat U, Kavak GO. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. *International Journal of Surgery*. 2014;12(3):213-218. <https://doi.org/10.1016/j.ijso.2013.12.013>
 31. Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense system, and schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 2013;46:200–206. <https://doi.org/10.1016/j.pnpbp.2013.02.015>
 32. Canayakin D, Bayir Y, KilicBaygutalp N, SezenKaraoglan E, Atmaca, HT, KocakOzgeris FB. Paracetamol-induced nephotoxicity and oxidative stress in rats: The protective role of *Nigella sativa*. *Pharmaceutical Biology*. 2016;54(10):2082-2091. <https://doi.org/10.3109/13880209.2016.1145701>
 33. Gianazza E, Brioschi M, Fernandez AM, Banfi C. Lipoxidation in cardiovascular diseases. *Redox Biology*. 2019;23:101119. <https://doi.org/10.1016/j.redox.2019.101119>
 34. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V. *et al.* Oxidative stress: Harms and benefits for human health. *Oxidative medicine and cellular longevity*. 2017. <https://doi.org/10.1155/2017/8416763>
 35. Itri R, Junqueira HC, Mertins O, Baptista MS. Membrane changes under oxidative stress: The impact of oxidized lipids. *Biophysical reviews*. 2014;6:47-61. <https://doi.org/10.1007/s12551-013-0128-9>
 36. Volinsky R, Kinnunen PKJ. Oxidized phosphatidylcholines in membrane-level cellular signaling: from biophysics to physiology and molecular pathology. *FEBS Journal*. 2013;280(12):2806–2816. <https://doi.org/10.1111/febs.12247>
 37. Chang L, Xu D, Zhu J, Ge G, Kong X, Zhou Y. Herbal therapy for the treatment of acetaminophen-associated liver injury: Recent advances and future perspectives. *Frontiers in Pharmacology*. 2020;11:313. <https://doi.org/10.3389/fphar.2020.00313>
 38. Du K, Ramachandran A, Jaeschke H. Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. *Redox Biol*. 2016;10:148–156. <https://doi.org/10.1016/j.redox.2016.10.001>
 39. Tien YH, Chen BH, Wang Hsu GS, Lin WT, Huang JH, Lu YF. Hepatoprotective and anti-oxidant activities of *Glossogyneteniuifolia* against acetaminophen-induced hepatotoxicity in mice. *Am. J. Chin. Med*. 2014;42 (6):1385–1398. <https://doi.org/10.1142/s0192415x14500876>
 40. Sani MF, Kouhsari SM, Moradabadi L. Effects of three medicinal plants extracts in experimental diabetes: Antioxidant enzymes activities and plasma lipids profiles in comparison with metformin. *Iranian J. Pharmaceutical Research*. 2012;11(3):897. PMID: 24250517; PMCID: PMC3813132
 41. Sharaibi JO, Joshi RK, Makinde SO, Oluwa KO. Phytochemical constituents and free radical scavenging activity of fresh and dried samples of *Curculigopilosa* (Schum. &Thonn.) Engl. (Hypoxidaceae). *International Journal of Pharmacognosy and Life Science*. 2020;1(1):33-37. <https://doi.org/10.33545/27072827.2020.v1.i2a.16>
 42. Sofidiya MO, Oduwale B, Bamgbade E, Odukoya O, Adenekan S. Nutritional composition and antioxidant activities of *Curculigopilosa* (Hypoxidaceae) rhizome. *African Journal of Biotechnology*. 2011;10(75):17275-17281. <https://doi.org/10.5897/AJB11.1335>

43. Kumar S, Mishra A, Pandey AK. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using in vitro models. BMC Complementary and Alternative Medicine. 2013;13 <https://doi.org/10.1186/1472-6882-13-120>
44. Mishra A, Kumar S, Pandey AK. Scientific validation of the medicinal efficacy of *Tinosporacordifolia*. The Scientific World Journal. 2013;2013:8. <https://doi.org/10.1155/2013/292934.292934>

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