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Pulicaria odora as a Natural Antioxidant Remedy for Metribuzin-induced Toxicity

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ABSTRACT

Background: Pesticides like metribuzin are environmental pollutants that induce toxicity and oxidative stress in organisms.

Methods: This study evaluates the protective role of *Pulicaria odora* extract against metribuzin-induced toxicity in Wistar rats. The leaves of *P. odora* were extracted using methanol-water (70:30, v/v), yielding phenolic (2.77 mg GAE/g) and flavonoid (0.77 mg QE/g) contents. Its antioxidant capacity, assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

Result: Rats treated with metribuzin (13.2 mg/kg for 36 days) exhibited significant activity, including weight loss, altered hematological parameters and elevated hepatic (GPT, GOT) and renal (urea, creatinine) biomarkers. Co-administration of *P. odora* extract (34.2 mg/kg) significantly reversed these effects, restoring biomarkers and alleviating hepatic and renal toxicity by reducing transaminases, urea, creatinine, cholesterol and triglycerides. The extract's antioxidant properties, attributed to its polyphenolic content, mitigated oxidative stress and stabilized cellular functions without adverse behavioral effects. This study highlights *P. odora* potential as a natural agent against herbicide-induced toxicity. Its bioactive compounds and mechanisms warrant further investigation, alongside exploring therapeutic applications in other *Pulicaria* species.

Key words: Antioxidants, Metribuzin, Polyphenols, *Pulicaria odora,* Toxicity.

INTRODUCTION

The Asteraceae family comprises over 100 species, with *Pulicaria* ranking as the third-largest genus, widely distributed across Africa, Asia and Europe (Naqvi *et al*., 2020). Traditionally, *Pulicaria* species have been used to treat musculoskeletal pain, inflammation, menstrual cramps, digestive disorders, dysentery and diarrhea (Liu *et al*., 2010). Recent studies highlight their diverse bioactive properties, including the cytotoxic effects of *P. undulata* (Hegazy *et al*., 2021), anti-inflammatory potential of *P. jaubertii* (Hussain *et al*., 2023), antibacterial efficacy of *P. odora* (Touati *et al*., 2018) and antioxidant and hepatoprotective effects of *P. crispa* and *P. incisa* (El-Sabagh *et al*., 2021).

The extensive use of herbicides like metribuzin poses significant environmental and public health risks, particularly through their persistence, non-target exposure, and potential adverse effects on animals and humans (Kumar *et al*., 2024). Metribuzin, a selective systemic herbicide, is widely applied in agricultural and domestic settings, as well as for pest control in residential and commercial facilities and in veterinary medicine for managing ectoparasites (Gitsopoulos *et al*., 2024; Close and Banasiak, 2022). Studies have shown that metribuzin accumulates in the kidneys, liver, heart, adrenal glands and body fat of mammals and fish (Chiali *et al*., 2013; Pelić *et al*., 2020; Sousa *et al*., 2020), leading to oxidative stress and the formation of reactive oxygen species (ROS), which contribute to its toxicity and pose risks to human health (Gupta *et al*., 1999). Conversely, increased consumption of antioxidant-rich plant products has been shown to reduce environmental toxicant levels in the body by scavenging free radicals (Djordjević *et al*., 2024).

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P. odora, locally known as "ouden halouf," is an annual plant that can grow up to 100 cm tall and produces yellow flowers in humid conditions. It is traditionally valued for treating stomach ulcers, wounds, gastric disorders and abdominal pain (Djilali, 2020). Phytochemical studies on *Pulicaria* species have identified various bioactive compounds, including monoterpene glucosides (El-Ghaly, 2016), diterpenes, sesquiterpenes, flavonoids, polyphenols, triterpenes and steroids (de la Luz Cádiz Gurrea *et al*., 2019).

This study is driven by the limited information regarding the phytochemical composition and bioactive properties of *P. odora*. The aim is to investigate the plant's biological efficacy in mitigating the harmful effects of agricultural toxins, particularly metribuzin and to evaluate its antioxidant and anti-inflammatory properties. Through *in vivo* studies on rats, this research seeks to demonstrate *P. odora*'s potential as a protective or supportive treatment to reduce tissue damage caused by exposure to hazardous substances.

MATERIALS AND METHODS

Pulicaria odora was collected in May 2022 at peak flowering from the Ain Kechra region in Skikda, northeastern Algeria, at an altitude of 282 meters (36°46′03.4″N, 6°26′52.8″E). The plant was identified and authenticated by Dr. Sahraoui, a botanist from the Faculty of Sciences, University of August 20, 1955, Skikda. After collection, the stems were separated and the leaves were thoroughly rinsed with tap water to remove surface impurities. The cleaned material was air-dried in a shaded, room-temperature environment for approximately 15 days. Once dried, the plant material was ground into a fine powder using a laboratory mill in preparation for extraction and subsequent analyses.

Extraction of plant

Fifty grams of powdered *P. odora* leaf material was immersed in 500 mL of a methanol-water solution (70:30, v/v) and continuously stirred using a magnetic stirrer for 24 hours at room temperature (20°C) to facilitate thorough extraction. Following the maceration process, the solution was filtered through Whatman No. 1 filter paper. The filtrate was then concentrated by vacuum evaporation at 40°C to remove the solvent, resulting in a concentrated extract. The dried extracts were carefully transferred into glass containers and stored at 4°C for subsequent analysis.

Determination of total phenolic content

To determine the total phenolic content, 500 µL of 10% Folin-Ciocalteu reagent was added to a solution containing 100 µL of the extract diluted in pure methanol. After a reaction time of 5 minutes, 400 µL of 7% sodium carbonate (Na, CO*f*) solution was added to the mixture. The solution was then incubated at room temperature for 30 minutes in a low-light environment to prevent photodegradation. Following incubation, the absorbance of the solution was measured at 375 nm using a Shimadzu UV-1601 UV/Vis spectrophotometer (Germany). The results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g), quantitatively measuring the total phenolic content.

Quantification of flavonoid content

The flavonoid content of *P. odora* extract was quantified using the aluminum chloride colorimetric method, as Hegazy and Ibrahium (2012) described. In this procedure, 1 mL of the extract was mixed with 1 mL of a 2% aluminum chloride (AlCl*f*) solution. The mixture was then incubated for 10 minutes at room temperature to allow for the formation of a stable complex. After incubation, the absorbance was measured at 430 nm using a Shimadzu UV-1601 UV/Vis spectrophotometer (Germany). The total flavonoid content was expressed in micrograms of quercetin equivalents per gram of dry weight (µg QE/g DW).

DPPH radical scavenging activity

The free radical scavenging activity of *P. odora* leaf extract was assessed using the DPPH assay described by Jeong *et al.* (2004). This method mixed 100 µL of diluted leaf extract with 1 mL of a freshly prepared 0.05 mM DPPH solution in methanol. The mixture was incubated for 30 minutes at room temperature in a dark environment to prevent light-induced degradation of the DPPH radical. After incubation, the absorbance was measured at 517 nm using a spectrophotometer to quantify the decrease in DPPH concentration. The scavenging activity was expressed as percent inhibition of DPPH, calculated using the following equation:

$$\text{Inhibition } \% = \frac{A_0 - A_1}{A_0} \times 100$$

Where,

A_0= The absorbance of the control (DPPH solution without extract).

A_1= The absorbance with the extract.

FRAP assay for ferric reducing antioxidant capacity

The ferric reducing antioxidant power (FRAP) of *P. odora* leaf extract was assessed following the protocol by Hemma *et al*. (2018). This assay evaluates the reduction power of the sample by measuring its ability to reduce Fe^{3+} to Fe^{2+}. In this procedure, 400 µL of various concentrations of leaf extract or a standard solution were combined with 400 µL of phosphate buffer (0.2 M, pH 6.6) and 400 µL of potassium ferricyanide solution (1% aqueous $K_3Fe(CN)^6$). The mixture was incubated at 50°C for 20 minutes. After incubation, 400 µL of trichloroacetic acid solution (10% in water) was added, followed by centrifugation at 3000 rpm for 10 minutes to separate the phases. A portion of the supernatant (400 µL) was then diluted with 400 µL of distilled water and 80 µL of $FeCl_3$ solution (0.1% in water) was added to the mixture. The absorbance of the final solution was measured at 700 nm to determine the reduction power. Ascorbic acid (AA) was used as a positive control in the assay.

Animal study

Thirty-six male albino Wistar rats, six weeks old and weighing between 150 and 180 grams, were obtained from the Pasteur Institute of Algeria. The study was conducted in the animal laboratory of the Science Department on August 20, 1955, at the University of Skikda, following institutional and national guidelines on laboratory animal care and the World Medical Association's ethical guidelines (2016). The rats were housed in polyethylene cages lined with sterilized softwood shavings under controlled conditions of 25±2°C, 45-55% humidity and a 12-hour light/dark cycle. They had ad libitum access to food and water. Before the experiment, the rats were acclimatized to laboratory conditions for two weeks. All animal handling procedures adhered to the European Ethics Committee's guidelines (06 EC/DCMB/FNSL/EU2021).

Animal treatment and experimental design

Following an acclimatization period, the rats were randomly allocated into four experimental groups, each comprising nine animals. Group GT, designated as the negative control, received mineral water orally. Group GP was administered an oral dose of *P. odora* extract at 34.2 mg/kg body weight daily. Group GM received metribuzin at a daily dose of 13.2 mg/kg body weight. Finally, Group GMP was treated with a combined daily regimen of metribuzin (13.2 mg/kg body weight) and *P. odora* extract (34.2 mg/kg body weight). After 36 days of treatment, the rats were euthanized and blood samples were collected for biochemical and hematological analysis. The serum was separated from the collected blood immediately post-euthanasia.

Hematological analysis

Hematological parameters were evaluated using whole blood samples and analyzed with a fully automated blood cell counter (Mindray BC 31). The parameters measured included red and white blood cell counts, hemoglobin (Hb) concentration, hematocrit, mean corpuscular values, lymphocyte counts and platelet levels.

Biochemical analysis

Serum samples were analyzed for clinical biochemistry using the Mindray BS-330E automated instrument. The biochemical parameters measured included glucose, urea, creatinine, triglycerides (TG), cholesterol, glutamic-oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).

Statistical analysis

All data were analyzed in triplicate and the results are presented as mean ± standard deviation (SD). IC_{50} values for antioxidant assays were determined using linear regression analysis. Statistical analyses were performed using GraphPad Prism version 9.5.1. Differences between groups were assessed using ANOVA followed by Tukey's test, with statistical significance set at $P<0.05$, $P<0.01$ and $P<0.001$.

RESULTS AND DISCUSSION

Extraction yields

The use of hydro-methanol as the solvent in maceration extraction enabled the calculation of the extraction rate. The total polyphenol yield obtained from *P. odora* leaves was 17.1% (w/w dry plant material). In comparison, methanolic crude extracts of *P. crispa* and *P. undulata*, wild species from Sudan, yielded 22.6% and 23%, respectively (Hegazy *et al.,* 2021). This slight variation in yield percentage may be attributed to various factors, such as the extraction method, particle size, duration of extraction, solvent type and solvent volume (de la Luz Cádiz Gurrea *et al.,* 2019).

Total flavonoids and total phenolic contents

To standardize the hydro-methanolic extract of *P. odora* leaves, the total phenolic content was measured using the folin-ciocalteu method and the UV-Vis colorimetric method was employed to quantify the total flavonoid content. Results showed a flavonoid concentration of 0.77 mg quercetin equivalent per gram of dry extract and a phenolic content of 2.77 mg gallic acid equivalent. Compared to these findings, Touati *et al.* (2018) reported lower total polyphenol and flavonoid levels in the methanolic extract of *P. odora* leaves, at 90±0.63 µg GAE/g DWE and 11.34±3.15 µg QE/g DWE, respectively. Additionally, the authors observed that *P. odora* leaves contain significantly higher levels of flavonoids and total phenolics than the roots. Prior studies also indicate that plants within the *Pulicaria* genus typically contain high levels of phenolics and flavonoids. For example, Senhaji *et al.* (2017) identified total phenolics and flavonoids in the ethyl acetate extract of *P. murritanica* at 72.88±0.21 mg GAE/g DWE and 38.95±0.75 mg QE/g DWE, respectively. Similarly, Malarz *et al.* (2023) reported that hydroalcoholic extracts of *P. inuloides* contain 43.81±2.36 mg GAE/g DWE of total polyphenols. Various factors may explain these differences, including genetic variation, plant maturity, storage duration and environmental conditions such as high temperatures, intense sunlight, drought and salinity, which are known to enhance secondary metabolite production, particularly polyphenols (Astill *et al.,* 2001; Martínez *et al.,* 2021; Turkmen *et al.,* 2009).

Antioxidant activity

Two methods were employed to assess the antioxidant effects of *P. odora* cured extracts: DPPH radical scavenging activity and ferric ion ($FeCl_3$) reducing power. The hydro-methanolic leaf extract of *P. odora* demonstrated dose-dependent DPPH radical scavenging, with inhibition rates of 44.02%, 48%, 48.6%, 50.27%, 54.27%, 59.24% and 63.4% at concentrations of 80, 100, 120, 140, 160, 180 and 200 µg/mL, respectively (Fig 1). A strong positive correlation was observed between extract concentration and radical inhibition, indicating increased antioxidant activity with higher concentrations. The IC_{50} for ascorbic acid was 103.33 µg/mL, while the IC_{50} for the *P. odora* extract was 123.69 µg/mL.

To further validate antioxidant activity, the reducing power test was conducted. Results showed a direct correlation between absorbance at 700 nm and extract concentration, where increased absorbance reflected the reduction of ferric ions by extract components. The inhibition percentages for the crude extract were 11.33%, 14.62%, 32.52%, 48.44%, 75.34% and 90.2%, compared to ascorbic acid's 34.66%, 45.66%, 57.03%, 69.62%, 79.83% and 97.21% at 15.62, 31.25, 62.5, 125, 250 and 500 µg/mL, respectively (Fig 2). The IC50 values were 42.56 µg/mL for ascorbic acid and 192.24 µg/mL for the crude extract. Similar antioxidant responses have been reported for medicinal plant extracts, where phenolic-rich extracts demonstrated substantial DPPH and FRAP activities and improved antioxidant status in experimental rats (Azyu *et al.*, 2025).

Sub-acute toxicity study

The current study investigates the potential detoxifying effects of *P. odora* on metribuzin-induced toxicity in Wistar albino rats. A comprehensive evaluation of physiological parameters, including body weight, hematological indices and biochemical markers, was conducted to elucidate the impact of both *P. odora* and metribuzin. Behavioral observations indicated that the oral administration of *P. odora* extract did not result in mortality or exhibit any toxicological symptoms, thereby underscoring the extract safety profile and potential therapeutic efficacy.

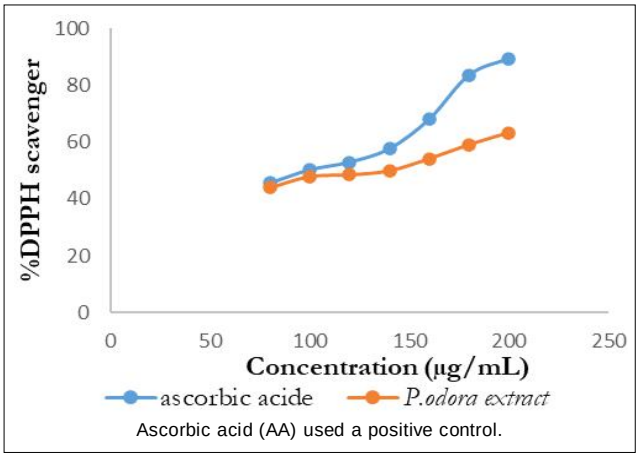
Growth parameters

Fig 3 displays rat body weight data across the experimental groups, highlighting differences among the four groups. At the beginning of the study, the initial body weight of the experimental rats averaged 170±10.2 g. A significant reduction in average body weight was observed in the GM group, which received metribuzin alone, compared to the control group ($p<0.001$). This weight loss is likely due to decreased food intake during the study period. These results align with findings by Samir and Asma (2018), who reported similar weight reduction effects following metribuzin exposure in rabbits over 6 to 18 days. In contrast, co-administration of *P. odora* leaf extract with metribuzin in the GPM group effectively prevented weight loss, showing no statistically significant difference from the control group. The phenolic compounds in *P. odora* extract are likely instrumental in counteracting rat metribuzin-induced oxidative stress (Zeng *et al.,* 2021).

Hematological and immune response to metribuzin and *P. odora* extract in rats

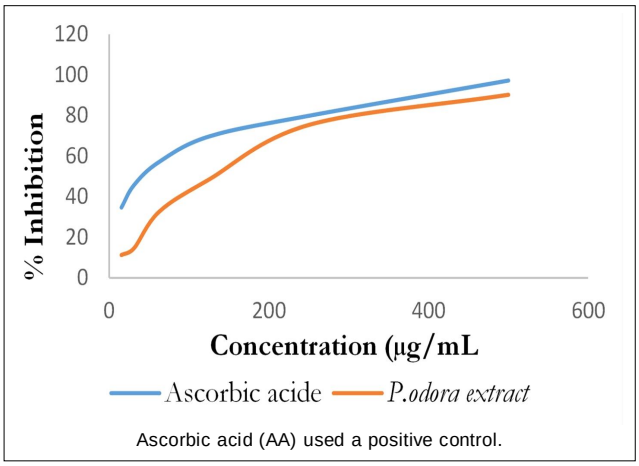
Fig 4 illustrates the hematological responses to metribuzin and *P. odora* extract treatments, highlighting notable changes in these parameters. In the GM group, which received metribuzin alone, significant elevations were observed in red blood cell (RBC) counts and hematocrit levels ($P<0.01$), along with a more pronounced increase in white blood cell (WBC) counts, lymphocytes, hemoglobin (Hb), mean corpuscular volume (MCV) and platelets (PLTs) ($P<0.001$) compared to the control. In contrast, co-treatment with *P. odora* extract in the GMP group led to a marked reduction in platelets, Hb, WBC, RBC and MCV levels relative to the GM group, while lymphocyte and hematocrit levels remained unaffected. Rats administered only *P. odora* extract (GP) showed a significant increase in RBC counts ($P<0.01$) and a minor but significant decrease in MCV ($P<0.05$) when compared to the control group, with other hematological parameters showing no significant differences.

The observed elevation in RBC counts, MCV, hematocrit and Hb levels in the GM group may reflect a physiological stress response to metribuzin exposure, potentially due to compensatory mechanisms aimed at countering herbicide-induced oxidative stress. This response likely involves increased oxygen transport to cells, evidenced by the rise in RBC counts, possibly through enhanced release or synthesis of erythrocytes from hematopoietic tissues. Similar responses have been



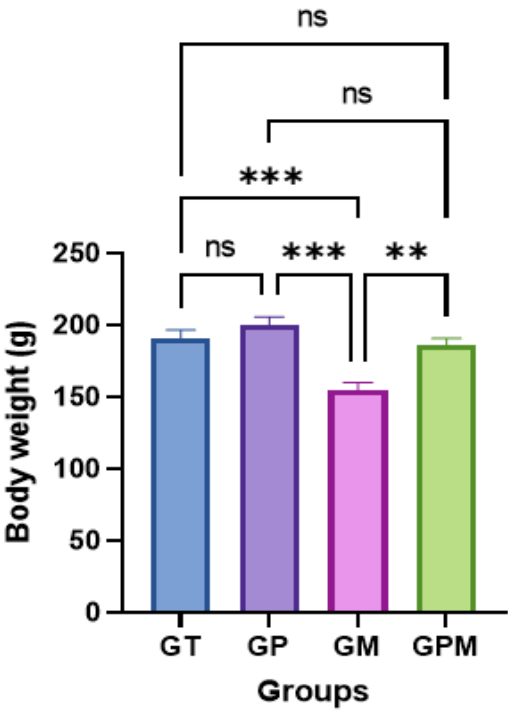
Ascorbic acid (AA) used a positive control.

Fig 1: DPPH radicals scavenging activity of *P. odora.*



Ascorbic acid (AA) used a positive control.

Fig 2: Antioxidant activity of methanol extract from leaves of *P.odora* using FRAP assay.



GT (Control), GP (*P. odora* at 1 mg/kg BW/day), GM (Metribuzin at 1 mg/kg BW/day) and GPM (*P. odora* + Metribuzin at 1 mg/kg BW/day). Statistical analysis revealed that differences were significant at P<0.05, **P<0.01 and ***P<0.001, with "ns" indicating non-significant differences. Values are expressed as Mean±SEM.

Fig 3: Average body weight of male albino wistar rats (n = 9) over a 36-day treatment period, across four groups.

reported by Lutnicka *et al.* (2019) in *C. carpio* exposed to linuron. Furthermore, (Kadeche *et al*., 2016) and Samir *et al.* (2020) observed declines in RBC, Hb and hematocrit levels and reduced erythrocyte counts in metribuzin-treated rats, particularly with prolonged or high-dose exposure, attributing these effects to oxidative damage and hematotoxicity.

Metribuzin-treated rats in the present study exhibited significantly increased lymphocyte, platelet and WBC counts (P<0.001), likely indicating an immune response to herbicide-induced toxicity. Elevated WBC and lymphocyte levels may suggest an immune mobilization against tissue damage, consistent with findings from Samir *et al.* (2020), who reported similar leukocyte increases in response to metribuzin's cytotoxic effects. The rise in platelet count may stem from metribuzin-related tissue damage, as platelets contribute to inflammatory responses by releasing pro-inflammatory mediators to recruit immune cells (Chen *et al*., 2020). Additionally, metribuzin's oxidative stress-inducing properties (Almeida *et al*., 2019) may further explain the increase in platelet levels as a compensatory response to RBC suppression.

Treatment with *P. odora* extract alone induced minor changes in RBC counts and MCV relative to the GP and control groups, similar to findings by Mansouri *et al.* (2015) in studies with *Foeniculum vulgare* extracts. This effect may result from erythropoietin stimulation by the liver and kidneys in response to *P. odora*. The phenolic constituents of *P. odora* likely contribute antioxidant effects, stabilizing cell membranes and protecting against free radical damage (Koren *et al*., 2010). Although changes in platelet, MCV and RBC levels were not statistically significant, they suggest a potential protective effect of *P. odora*. Moreover, Hb and WBC levels in the GM group

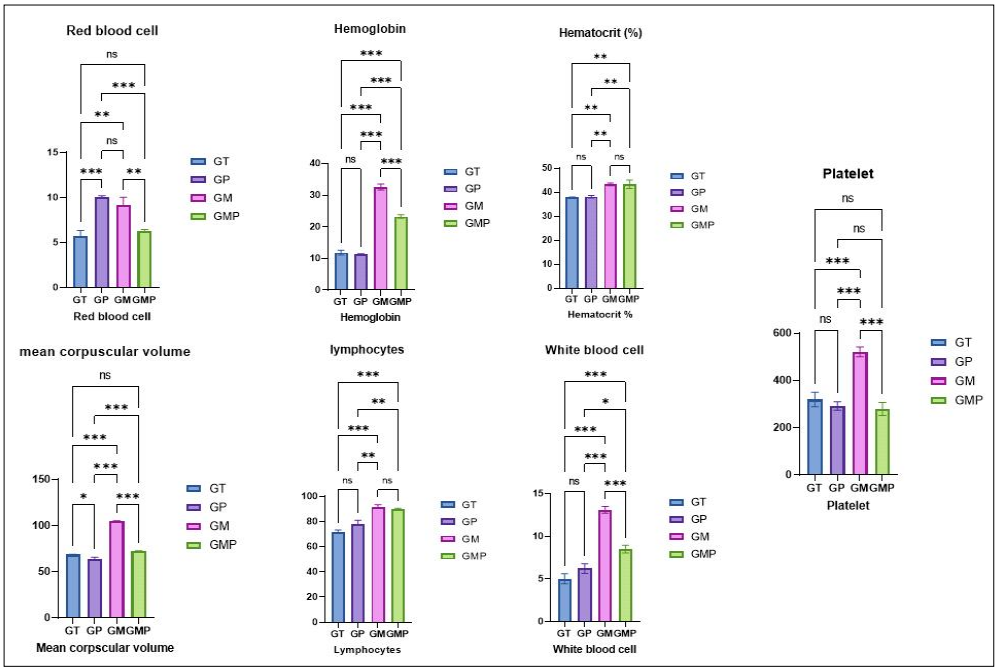


Fig 4: The hematological profile of rats intoxicated with metribuzin and after treatment with methanolic extract of *P. odora*.

showed a significant reduction (P<0.001) relative to controls, while these levels remained elevated in the GP group, further supporting *P. odora*'s stabilizing role.

Collectively, these results imply that oxidative stress plays a central role in metribuzin toxicity, with *P. odora* extract, rich in polyphenols and flavonoids, offering significant antioxidant and free radical-scavenging properties that may counteract metribuzin-induced damage. This aligns with findings by Kadeche *et al.* (2017) on vanillin's protective effect against metribuzin-induced hematological alterations, underscoring *P. odora*'s potential as a therapeutic agent against herbicide toxicity.

Biochemical effects of metribuzin and *P. odora* extract in rats

In this study, exposure to metribuzin (GM group) significantly increased (P<0.001) several biochemical markers, including blood glucose, total cholesterol, triglycerides, glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), urea and creatinine, relative to the control group (Fig 5). These elevations suggest a toxic impact on liver and kidney function, as evidenced by raised transaminase levels and markers of renal function. Interestingly, the co-treatment group (GMP), which received *P. odora* extract alongside metribuzin, exhibited significant reductions in GPT, GOT and creatinine (P<0.001), urea (P<0.01), as well as total cholesterol and triglycerides (P<0.05), compared to the GM group. This indicates that *P. odora* may confer a protective effect, mitigating metribuzin-induced hepatic and renal toxicity. However, co-treatment did not significantly affect blood glucose levels, suggesting limited influence on glucose metabolism. Additionally, the group receiving only *P. odora* extract (GP group) showed a mild, statistically insignificant increase in GPT, GOT and triglycerides compared to the control group, with no significant changes in other biochemical parameters, indicating that *P. odora* alone does not markedly disrupt biochemical homeostasis. Changes in hematological indices and serum biomarkers of hepatic and renal function are commonly used to evaluate the systemic effects and safety of bioactive plant extracts in Wistar rats (Al-Ghamdi *et al.,* 2026). Collectively, these findings support the potential protective role of *P. odora* against metribuzin-induced toxicity.

This study also noted a marked increase in blood glucose levels in rats exposed to metribuzin (13.2 mg/mL over 36 days), consistent with previous findings indicating that chronic low-level metribuzin exposure elevates plasma glucose, possibly due to reduced glucose uptake and impaired tissue functionality (Chiali *et al.,* 2013). Similar glucose elevations were reported in *Cyprinus carpio* after atrazine exposure (Blahova *et al.,* 2014), suggesting that pesticides may disrupt carbohydrate metabolism. Potential mechanisms include increased glycogenolysis, elevated adrenocorticotropic and glucagon hormones, or insulin dysfunction (Mehra *et al.,* 2014). Conversely, studies on long-term, low-dose metribuzin exposure in rats (1/20 to 1/5 of LD_{50}, twice weekly) reported decreased glucose levels, possibly due to depleted carbohydrate reserves or inhibition of glucose release from hepatic tissue (Maksymiv *et al.,* 2015; Samir *et al.,* 2020). Metribuzin exposure also increased lipid parameters, particularly triglycerides and total cholesterol, potentially due to enhanced cholesterol synthesis in the

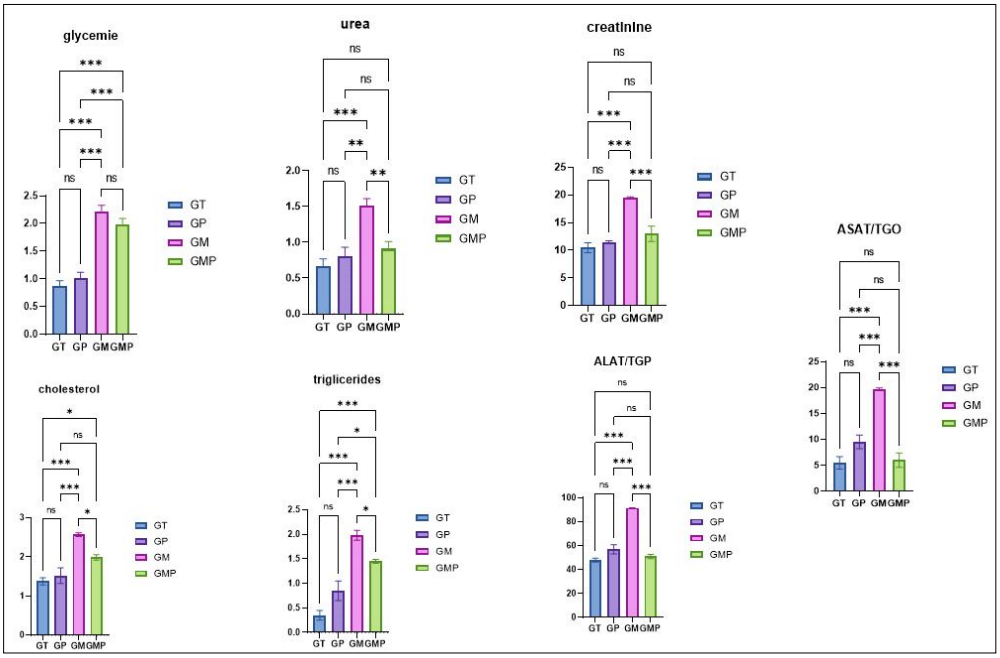


Fig 5: Intoxicated rats' biochemical parameters with metribuzin and after treatment with a methanolic extract of *P. odora*.

liver or compromised liver cell membrane integrity. Elevated triglycerides may result from decreased lipase activity and increased mobilization of adipose stores (Chiali *et al.,* 2013; El-Demerdash and Nasr, 2014). Some studies, however, have reported reduced plasma cholesterol under metribuzin exposure, potentially due to diminished cholesterol synthesis or tissue accumulation.

Increased urea and creatinine levels in metribuzin-treated rats indicate renal impairment and reduced glomerular filtration rate, consistent with findings in albino rats exposed to metribuzin (Khozimy *et al.,* 2017). Elevated liver biomarkers GPT and GOT indicate hepatotoxicity, aligning with Samir and Asma (2018), who observed increased transaminase activity in metribuzin-exposed rabbits and fetuses. The herbicide's residual accumulation in the liver, as confirmed by HPLC analysis, underscores hepatic vulnerability to metribuzin toxicity. Similarly, Sena *et al.* (2021) found heightened GPT and GOT in *Xenopus laevis* exposed to atrazine. Rats treated with *P. odora* extract alone (GP group) exhibited slight, non-significant increases in triglycerides, GOT and GPT, potentially indicating activation of lipid metabolism-related enzymes. However, no studies to date have specifically examined the biochemical effects of *P. odora*, underscoring the need for further investigation.

Importantly, co-treatment with *P. odora* extract and metribuzin mitigated the metabolic disturbances induced by metribuzin, restoring levels of GPT, GOT, urea, creatinine, cholesterol and triglycerides to near normal. This protective effect is likely due to the natural antioxidants, particularly polyphenols, in *P. odora*, which may counteract metribuzin-induced biochemical imbalances. These findings align with previous studies on *P. crispa* extract, which lowered cholesterol, urea and creatinine in diabetic rats (Daradka *et al.,* 2021) and support evidence of the benefits of *Pulicaria* genus on liver and kidney health (Bakr *et al.,* 2021; Alharthi *et al.,* 2023). Similar antioxidant compounds, such as vanillin and quercetin, have demonstrated efficacy in ameliorating metribuzin-induced biochemical changes (Abarikwu 2014; Kadeche *et al.,* 2017), suggesting that *P. odora*'s quercetin content may contribute to its protective properties.

CONCLUSION

The findings of this study demonstrate that *P. odora* extract possesses significant protective effects against metribuzin-induced toxicity in Wistar rats. The extract's antioxidant properties, attributed to its phenolic and flavonoid content, played a key role in reducing oxidative stress and mitigating the physiological and biochemical alterations induced by metribuzin. Co-administration of *P. odora* extract resulted in a marked improvement in hematological and biochemical parameters, including liver and kidney function markers. The absence of adverse effects on behavioural assessments further supports the safety and potential therapeutic value of *P. odora*. These results suggest that *P. odora* may serve as a promising natural agent for combating herbicide-induced toxicity. Further research is warranted to explore its bioactive compounds, mechanisms of action and the therapeutic potential of other species within the *Pulicaria* genus.

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Disclaimers

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Authors' contributions

Conceptualization: A. Benmahammed and ABA, Data curation: ABA and F. Bouleknafet, Formal analysis: F.A. Al-Mekhlafi and N. Abutaha, Funding acquisition: M.A. Wadaan and H.M. Alharbi Investigation: M.A. Wadaan, Methodology: A. Benmahammed and ABA, Project administration: F. Bouleknafet and S. Lambiase Resources: F.A. Al-Mekhlafi. Supervision: ABA. Writing-original draft: ABA, F.A. Al-Mekhlafi and F. Bouleknafet Writing-review and editing: N. Abutaha, F.A. Al-Mekhlafi and H.M. Alharbi.

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Data availability statement

All the data is available within the manuscript.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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