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Evaluation of the Protective Impact of *Tridax procumbens* on Mancozeb-Induced Oxidative Stress in Albino Wistar Rats

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Abstract

The present study aimed to assess the oxidative stress response and antioxidant profile in the tissues of Wistar rats following subacute oral exposure to mancozeb, and to evaluate the protective efficacy of aqueous and methanolic extracts of *Tridax procumbens* against mancozeb-induced toxicity. Thirty-six adult Wistar rats were randomly divided into six groups. Group I served as the control and received distilled water; Group II was administered mancozeb (500 mg/kg body weight); Groups III and IV received *Tridax procumbens* aqueous and methanolic extracts, respectively (300 mg/kg body weight); Groups V and VI received the same extracts in combination with mancozeb. All treatments were given orally for 28 consecutive days. Mancozeb exposure significantly elevated oxidative stress markers, including Lipid Peroxidation (LPO), Nitric Oxide (NO), and superoxide radicals in the liver, kidney, brain, and testes. Additionally, antioxidant enzyme levels (SOD and CAT) and non-enzymatic antioxidants (GSH, total thiols, and tissue protein) were markedly altered. Co-administration of *Tridax procumbens* extracts significantly ameliorated these biochemical disruptions, restoring antioxidant balance. Histopathological evaluations corroborated these findings, revealing tissue degeneration in mancozeb-treated rats, while co-treatment with *Tridax procumbens* extracts showed substantial restoration of normal histoarchitecture. Subacute oral administration of mancozeb induced significant oxidative stress, biochemical alterations, and histological damage in vital organs of Wistar rats. However, co-treatment with aqueous and methanolic extracts of *Tridax procumbens* effectively mitigated these toxic effects, likely due to their potent antioxidant properties.

Major Findings: *Tridax procumbens* may serve as a promising natural therapeutic agent against pesticide-induced oxidative damage.

Keywords: Antioxidant Profile, Mancozeb, Methanol Extract, Oxidative Stress, *Tridax procumbens*, Wistar Rats

1. Introduction

The rapidly growing human and animal populations have exerted immense pressure on global food systems, necessitating substantial increases in agricultural productivity to achieve food security. This demand calls for a productive, competitive, and sustainable agricultural sector, underpinned by advanced practices such as the use of high-yielding crop varieties, precision irrigation, soil fertility optimisation, and improved pest management strategies. However, despite technological advancements, crop yields continue to suffer substantial losses due to pest infestations.

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The pre-harvest pests alone account for approximately 35% of potential crop yield losses globally[1,2]. To combat these losses, farmers increasingly rely on chemical pesticides, with an estimated annual expenditure of USD 40 billion worldwide[3,4]. Mancozeb (MNZ), a widely used carbamate fungicide, offers broad-spectrum protection against fungal pathogens across a variety of crops[5]. While it is valued for its low acute toxicity and rapid excretion in mammals, chronic exposure to MNZ has been linked to significant toxic effects, including genotoxicity, oxidative damage, endocrine disruption, and reproductive toxicity, as evidenced in both male and female rodent models[6-12].

Emerging evidence highlights that the adverse effects of MNZ are primarily mediated through the generation of Reactive Oxygen Species (ROS), which overwhelm antioxidant defence systems and result in cellular damage across critical organs such as the liver, kidney and reproductive organs[13]. In light of this, there has been a growing interest in the use of herbal supplements with antioxidative properties to mitigate such chemically induced toxicity. *Tridax procumbens,* a member of the Asteraceae family, is a traditional medicinal herb known for its immunomodulatory, antioxidant, hepatoprotective, and anti-inflammatory properties[14-18]. Several studies have demonstrated that the bioactive compounds in medicinal plants, particularly flavonoids, exert protective effects by modulating redox homeostasis and suppressing oxidative stress pathways[19]. Despite this promise, the protective role of *Tridax procumbens* against MNZ-induced oxidative stress remains underexplored. Hence, the present study provides a novel approach by investigating the protective role of *Tridax procumbens* against mancozeb-induced oxidative stress in albino Wistar rats. While the genotoxic and histopathological effects of MNZ on vital organs are well-documented, there is a paucity of research evaluating herbal interventions against oxidative damage. By focusing on *Tridax procumbens*, a plant with established antioxidant potential, this research uniquely explores its therapeutic potential in reversing or attenuating MNZ-induced oxidative alterations, thus contributing to safer agricultural chemical use and integrative toxicology.

2. Materials and Methods

2.1 Animal Procurement

Thirty-six healthy albino Wistar rats, each weighing approximately 120-150 g, were procured from NIPER, Mohali. Before initiating the experiment, the animals underwent a 15-day acclimatisation period under standard laboratory conditions, maintained at a temperature of 27–30°C. During this time, they were provided with commercial rat pellets and had free access to water. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) under reference number GADVASU/2019/IAEC/50/03, dated 23/07/2019.

2.2 Experimental Design

After a period of acclimatisation, the albino Wistar rats were randomly divided into six groups of 6 animals each. Group I served as the control, while Group II was the toxic dose group, where animals were orally administered mancozeb dissolved in tap water at a dose of 500 mg/kg BW per day. Animals of Groups III and IV received oral administration of aqueous and methanol extracts of *Tridax procumbens* at a dose of 300 mg/kg body weight, respectively. Animals in Group V were orally supplemented with a combination of mancozeb (500 mg/kg BW) and the aqueous extract of *Tridax procumbens* (300 mg/kg BW), while those in Group VI received mancozeb (500 mg/kg BW) along with the methanol extract of *Tridax procumbens* (300 mg/kg BW). All the doses were dissolved in distilled water, and the treatment was given for a period of 28 consecutive days.

2.3 Observation

Throughout the experimental period, the animals were closely observed for the appearance of any toxic symptoms.

Table 1. Dose groups

Groups	Treatment received	Dosage
I	Distilled water	Ad-libitum
II	Mancozeb	500mg/Kg BW/day
III	*Tridax procumbens* aqueous extract	300mg/Kg BW/day
IV	*Tridax procumbens* Methanol extract	300mg/Kg BW/day
V	Mancozeb+*Tridax procumbens* aqueous extract	500mg/Kg BW+ 300mg/Kg BW/day, respectively
VI	Mancozeb+*Tridax procumbens* methanol extract	500mg/Kg BW+ 300mg/Kg BW/day, respectively

The nature, duration, and severity of any observed toxicity, as well as any mortality, were carefully recorded. Blood samples were collected from the retro-orbital sinus of the animals into heparinised vials on days 0 and 29 of the study. Erythrocyte lysates were subsequently prepared for the assessment of various biochemical parameters.

On the 29th day of the treatment period, all animals were euthanised, and vital organs, including the liver, kidneys, brain, and testes, were harvested. These tissues were used for the estimation of oxidative stress-related parameters. Additionally, small portions of each organ were fixed in 10% neutral buffered formalin for histopathological analysis.

2.4 Test Parameters

A range of biochemical and oxidative stress parameters were evaluated in this study. Haemoglobin content was measured using a standard method[20]. Lipid peroxidation was assessed by determining Malondialdehyde (MDA) levels through the Thiobarbituric Acid (TBA) assay[21]. Glutathione peroxidase activity was estimated using a standard procedure[22], while Superoxide Dismutase (SOD) activity was evaluated based on the enzyme's ability to inhibit pyrogallol auto-oxidation[23]. Catalase (CAT) activity was assessed by monitoring the decomposition of hydrogen peroxide[24]. Glutathione levels were determined according to an established method[25]. Superoxide anion radical generation and Nitric Oxide (NO_2) levels were measured spectrophotometrically, based on the reduction of Nitroblue Tetrazolium (NBT)[26,27]. Total protein content was quantified using a standard method with bovine serum albumin (BSA) as the reference standard[28]. Total thiol (-SH) group concentration was determined by the formation of a coloured complex with Dithiobisnitrobenzoic acid (DTNB)[29]. On the 29th day of the treatment period, all rats were sacrificed, and tissues, including liver, kidney, brain, and testes, were collected for the estimation of oxidative stress markers. Portions of these organs were also fixed in 10% neutral buffered formalin for histopathological examination, and tissue sections were stained using the standard haematoxylin and eosin technique[30].

2.5 Statistical Analysis

The significance of differences between the control and treatment groups was assessed using Student's t-test[31].

3. Results

3.1 Animal Behaviour and Toxicity Reaction Observation

Oral administration of mancozeb and the plant extracts to normal Wistar rats did not result in any mortality. However, subacute exposure to mancozeb at a dose of 500 mg/kg body weight for 28 consecutive days led to mild signs of toxicity. Rats treated with mancozeb exhibited a moderate level of anorexia, along with mild symptoms such as lacrimation, listlessness, hind limb weakness, and sluggish movement, which became noticeable after 15 days of treatment.

Table 2. Effect on Lipid peroxidation, superoxide radical, nitric oxide and tissue protein in albino Wistar rats.

Lipid peroxidation (nmol MDA/g tissue)				
Group	**Liver**	**Kidney**	**Brain**	**Testes**
I	796.21±26.34^{a}	764.42±50.03^{a}	716.08±11.95^{a}	776.98±34.04^{a}
II	910.79±22.12^{b}	860.04±23.08^{a}	858.17±14.64^{b}	954.59±26.97^{b}
III	794.34±24.35^{a}	799.68±15.61^{a}	772.70±13.92^{a}	802.88±25.45^{a}
IV	805.02±19.19^{a}	797.54±19.31^{a}	765.22±25.98^{a}	789.78±32.86^{a}
V	891.83±31.37^{ab}	861.91±61.60^{a}	896.13±30.62^{b}	895.83±47.69^{ab}
VI	819.71±27.29^{ab}	810.63±34.04^{a}	866.72±24.10^{b}	846.42±31.81^{ab}
Superoxide radical (pmole/min /g of tissue)				
Group	**Liver**	**Kidney**	**Brain**	**Testes**
I	0.61±0.03^{a}	0.50±0.08^{ab}	0.46±0.11^{ab}	0.86±0.14^{ab}
II	0.85±0.24^{b}	1.53±0.32^{c}	0.87±0.08^{ab}	1.01±0.24^{b}
III	0.63±0.03^{a}	0.93±0.06^{ab}	1.02±0.12^{b}	0.46±0.04^{a}

Table 2. Continued...

IV	0.91±0.03^{ab}	1.02±0.12^{bc}	0.93±0.31^{ab}	0.40±0.06^{a}
V	0.61±0.07^{a}	0.46±0.03^{a}	0.36±0.03^{a}	0.46±0.10^{a}
VI	0.56±0.08^{a}	0.54±0.05^{ab}	0.58±0.09^{ab}	0.36±0.02^{a}
Nitric oxide (nmole /g of wet tissue)				
I	17.56±1.23^{a}	16.79±1.54^{a}	12.81±1.24^{a}	16.28±0.18^{bc}
II	29.11±2.52^{b}	25.68±3.18^{b}	26.11±1.72^{b}	21.69±1.52^{d}
III	20.08±0.31^{a}	18.79±1.47^{ab}	12.83±0.34^{a}	13.91±0.73^{ab}
IV	19.80±1.82^{a}	18.47±2.23^{ab}	9.29±2.12^{a}	15.18±1.15^{abc}
V	19.23±2.27^{a}	17.01±2.16^{a}	10.22±0.89^{a}	12.24±0.48^{a}
VI	23.39±1.25^{ab}	23.20±1.11^{ab}	13.52±0.64^{a}	18.35±0.85^{cd}
Tissue protein (mg/g of tissue)				
I	150.08±1.10^{c}	125.88±11.06^{b}	105.06±16.35^{b}	101.76±16.26^{ab}
II	79.74±14.58^{a}	83.63±14.44^{a}	69.14±4.40^{a}	84.36±12.45^{a}
III	116.63±1.65^{b}	107.33±1.59^{ab}	67.08±4.54^{a}	112.05±3.22^{ab}
IV	118.49±1.98^{b}	81.53±9.92^{a}	77.98±1.41^{ab}	107.62±6.60^{ab}
V	119.27±1.41^{b}	111.59±4.05^{ab}	86.09±6.23^{ab}	110.5±6.06^{ab}
VI	127.59±2.46^{b}	124.68±5.81^{b}	76.23±6.03^{ab}	131.29±4.88^{b}

Note: The presented values are expressed as mean ± SE, with a sample size of n = 6.
Means sharing at least one common superscript do not exhibit significant difference ($p<0.05$). Group I – Control, II –Mancozeb, III – *Tridax procumbens* aqueous extract, IV – *Tridax procumbens* methanol extract, V- Mancozeb + *Tridax procumbens* aqueous extract, VI- Mancozeb + *Tridax procumbens* methanol extract.

Table 3. Effect on Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), reduced glutathione and total thiol levels in albino Wistar rats

Superoxide Dismutase (U/mg of wet tissue)				
Group	**Liver**	**Kidney**	**Brain**	**Testes**
I	1.56±0.06^{b}	0.78±0.04^{bc}	2.22±0.41^{c}	1.72±0.28^{b}
II	7.12±1.46^{a}	5.914±1.43^{a}	5.72± 0.40^{a}	4.84±0.85^{a}
III	1.01±0.05^{b}	1.57±0.06^{bc}	3.34±3.34^{b}	1.37±0.21^{bc}
IV	0.89±0.10^{b}	2.61±0.50^{b}	3.38±0.08^{b}	1.93±0.15^{b}
V	0.53±0.14^{b}	1.43±0.09^{bc}	2.19±0.17^{c}	1.06±0.14^{bc}
VI	0.12±0.03^{b}	0.56±0.08^{c}	2.24±0.32^{c}	0.41±0.09^{c}
Glutathione Peroxidase (U/mg protein)				
I	74.06±17.76^{b}	90.80±20.20^{b}	61.82±10.38^{bc}	42.92±13.50^{ab}
II	20.11±3.89^{a}	22.69±7.59^{a}	29.00±7.21^{ab}	33.99±7.02^{ab}
III	27.04±6.64^{a}	17.74±3.35^{a}	23.64±4.05^{a}	33.85±10.26^{ab}
IV	25.14±8.10^{a}	24.42±7.93^{a}	37.41±12.16^{ab}	15.10±2.61^{a}
V	35.02±4.57^{a}	32.67±6.48^{a}	30.81±6.56^{ab}	21.26±5.51^{a}
VI	56.42±3.89^{ab}	58.42±9.49^{ab}	81.74±8.77^{c}	60.68±4.81^{b}
Catalase (µmol of H_2O_2 decomposed/min/mg protein)				
I	0.75±0.12^{a}	2.76±0.95^{a}	0.92±0.17^{a}	1.09±0.24^{a}
II	3.63±0.79^{c}	3.21±0.93^{a}	2.90±0.97^{ab}	6.18±1.55^{b}
III	1.71±0.20^{ab}	1.66±0.83^{a}	2.49±0.51^{ab}	2.30±0.43^{a}
IV	2.01±0.61^{abc}	2.17±0.31^{a}	2.07±0.42^{ab}	1.44±0.20^{a}
V	1.03±0.28^{a}	1.01±0.09^{a}	2.45±0.60^{ab}	2.22±0.36^{a}

Table 3. Continued...

VI	2.86±0.12^{bc}	1.70±0.24^{a}	4.06±0.53^{b}	0.65±0.20^{a}
Reduced glutathione (nmol/g of tissue)				
I	196.77±55.35^{a}	158.00±34.02^{a}	110.95±18.93^{a}	161.91±38.49^{a}
II	64.98±8.48^{b}	102.22±16.78^{b}	67.43±8.69^{b}	52.92±9.25^{b}
III	115.88±22.63^{ab}	102.56±12.77^{b}	118.72±22.62^{a}	113.74±21.15^{a}
IV	108.19±31.07^{ab}	102.49±12.71^{b}	108.11±21.86^{a}	109.43±16.92^{a}
V	170.46±36.15^{a}	143.32±31.73^{a}	117.80±24.09^{a}	118.01±28.54^{a}
VI	185.02±42.29^{a}	151.98±32.24^{a}	124.33±24.70^{a}	123.35±26.58^{a}
Total thiol (nmole /g of tissue)				
I	3.24±0.22^{bc}	2.52±0.02^{b}	2.55±0.27^{b}	1.69±0.16^{ab}
II	1.57±0.12^{a}	1.97±0.11^{a}	1.58±0.14^{a}	1.30±0.15^{a}
III	2.31±0.03^{ab}	2.65±0.09^{b}	2.04±0.07^{ab}	2.07±0.06^{ab}
IV	3.94±0.44^{c}	2.88±0.17^{bc}	1.61±0.12^{a}	1.79±0.06^{ab}
V	3.72±0.40^{c}	2.58±0.15^{b}	1.74±0.07^{a}	2.69±0.68^{b}
VI	3.60±0.48^{c}	3.38±0.21^{c}	2.15±0.18^{ab}	2.39±0.13^{ab}

Note: The presented values are expressed as mean ± SE, with a sample size of n = 6.
Means sharing at least one common superscript do not exhibit significant differences ($p<0.05$). Group I – Control, II –Mancozeb, III – *Tridax procumbens* aqueous extract, IV – *Tridax procumbens* methanol extract, V- Mancozeb + *Tridax procumbens* aqueous extract, VI- Mancozeb + *Tridax procumbens* methanol extract.

In contrast, administration of both aqueous and methanolic extracts of *Tridax procumbens* at a dose of 300 mg/kg body weight caused no mortality or visible signs of toxicity. Additionally, there were no notable changes in behaviour, indicating a high safety margin for the plant extracts at the tested dose.

4. Discussion

The present study demonstrated the protective role of aqueous and methanolic extracts of *Tridax procumbens* against mancozeb-induced oxidative stress in Wistar rats. Oral administration of mancozeb significantly increased Lipid Peroxidation (LPO), evidenced by elevated MDA levels, particularly in the liver and testes. LPO, a key indicator of oxidative stress, reflects membrane damage, loss of fluidity, receptor dysfunction, and increased membrane permeability[32,33]. However, co-treatment with *T. procumbens* extracts restored MDA levels to near-normal in the liver and testes, indicating its antioxidative effect. No significant recovery was observed in kidney tissue[34]. These results are consistent with earlier studies showing increased LPO in human lymphocytes and erythrocytes exposed to mancozeb, which could be mitigated by antioxidants like quercetin[35].

Oxidative stress results from excessive generation of ROS such as superoxide anions (O_2^-), hydroxyl radicals (-OH), and Hydrogen Peroxide (H_2O_2)[36]. In this study, mancozeb exposure (Group II) led to significantly increased superoxide radical levels in liver and kidney tissues. This increase was effectively attenuated in *T. procumbens*-treated groups (V and VI), suggesting its role as a free radical scavenger[37]. Similar trends were noted for Nitric Oxide (NO), a moderately reactive free radical, whose production increased markedly in mancozeb-treated rats. Treatment with *T. procumbens* significantly reduced NO levels across the liver, kidney, brain, and testes[38-40]. The extracts' antioxidative action is likely due to their flavonoids and phenolic compounds, which can scavenge free radicals and inhibit lipid peroxidation. Prior research has shown that *T. procumbens* extracts can restore antioxidant enzyme activities impaired by oxidative challenges[41-43].

Endogenous antioxidant enzymes play a pivotal role in ROS detoxification. SOD and catalase are primary enzymatic defense against ROS[44]. SOD activity increased significantly in mancozeb-treated animals, possibly as a compensatory mechanism against elevated superoxide radicals. However, *T. procumbens* extracts normalized SOD activity across tissues, suggesting mitigation of ROS overload[45]. Similarly, catalase activity increased in the liver and testes after mancozeb exposure, and this was restored to baseline levels upon extract treatment[46,47]. This pattern supports the role of *T. procumbens* in modulating oxidative enzyme response under pesticide stress. GPx, another key antioxidant enzyme, showed decreased

activity in the liver, kidney, and brain of mancozeb-treated rats, likely due to depletion of glutathione cofactor under oxidative conditions. Methanolic extract of *T. procumbens* (Group VI) reversed this decline, reinforcing its potential in supporting enzymatic antioxidant systems[48,49]. The observed protective effects can be attributed to phytochemicals such as luteolin, glucotureolin, β-sitosterol, and quercetin present in *T. procumbens*, which possess known antioxidative properties.

Total tissue protein levels, which decreased in liver, kidney, and brain following mancozeb administration, were restored with *T. procumbens* treatment, especially the methanolic extract in the kidney and testes. The protein loss may be due to oxidative degradation or impaired synthesis caused by free radical-mediated DNA and protein damage[50-52]. Restoration of protein levels indicates a protective role of the extract against oxidative tissue injury.

Thiol-disulfide homeostasis is vital for cellular redox balance and signalling. Mancozeb exposure led to significant depletion of total thiol content in liver, kidney, brain, and testes, suggesting oxidative damage[53]. Treatment with *T. procumbens* extracts helped maintain thiol levels, particularly in the testes, which are more susceptible due to high polyunsaturated fatty acid content[54]. These findings align with other pesticide-induced oxidative stress models showing reduced thiol and antioxidant enzyme levels[55]. Glutathione (GSH), a key intracellular antioxidant, was significantly depleted by mancozeb in all examined tissues. GSH is crucial for detoxification, ROS scavenging, and maintaining redox balance[56-58]. Treatment with *T. procumbens* extracts (Groups V and VI) restored GSH levels to near-normal, reaffirming its protective role. These results are consistent with previous studies reporting GSH depletion in mancozeb-exposed animal and cell models[60-63], and its restoration upon administration of *T. procumbens* or similar phototherapeutics[62,63].

5. Histopathology

The histopathological changes in liver, kidneys and brain tissue of rats of different groups are demonstrated in Figures 1-3.

Histological examination of liver tissues from the mancozeb-treated group revealed marked necrosis and cellular degeneration, characterised by cytoplasmic vacuolization and infiltration of mononuclear cells. These pathological alterations were notably ameliorated following treatment with aqueous and methanolic extracts of *Tridax procumbens*. In the kidney, mancozeb exposure led to evident congestion and degeneration of the proximal convoluted tubules, changes that were substantially reversed upon administration of the plant extracts. In the brain, mancozeb induced distinct microscopic alterations, particularly in the cerebellum and the white matter of the pons. The cerebellar sections exhibited degenerative changes, including irregular arrangement and necrosis of Purkinje cells. Additionally, vacuolization was observed in the white matter of the pons. These structural abnormalities were significantly improved following treatment with both aqueous and methanolic extracts of *T. procumbens*.

Previous studies have reported similar findings, with high doses of mancozeb causing focal necrosis, fatty infiltration, and dilation of sinusoidal spaces in liver tissue[64]. In the brain, Purkinje cells appeared necrotic, irregularly distributed, and devoid of axons, while the kidney exhibited tubular necrosis and epithelial alterations in the proximal convoluted tubules. Moreover, oral administration of mancozeb has been associated with congestion and inflammatory cell infiltration in the portal areas of the liver[61]. In reproductive tissues, mancozeb exposure led to severe histological disruptions in the testes of Zebrafish, including degeneration of spermatogenic cells, disorganisation of seminiferous tubules, fibrosis, haemorrhage, vacuolization, and oedema[65].

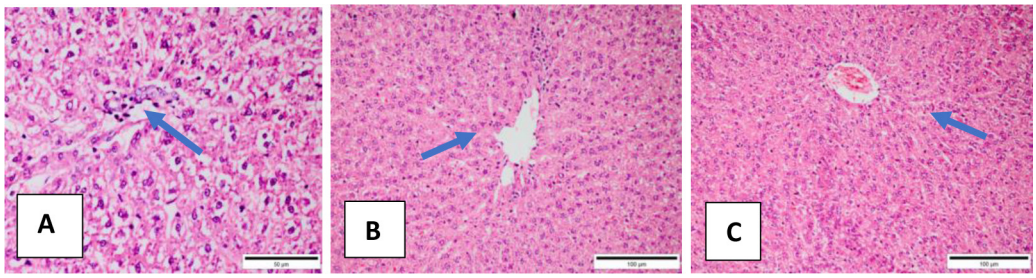


Figure 1. Representative photomicrographs of histopathological sections of liver **(A)** Group II: Necrosis and degeneration with cytoplasmic vacuolisation and mononuclear cell infiltration. (H&E, Bar=50 µm), **(B)** Group V: Restoration of the hepatocytes towards normal. (H&E, Bar=100 µm) **(C)** Group VI: Restoration of the hepatocytes towards normal (H&E, Bar=100 µm).

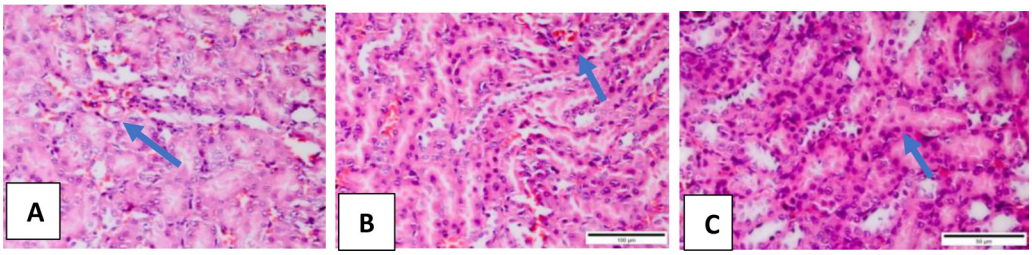


Figure 2. Representative photomicrographs of histopathological sections of kidney **(A)** Group II: Congestion and degeneration in the Proximal convoluted tubules of kidney (H&E, Bar=50 µm) **(B)** Group V: Kidney showing slight congestion in proximal convoluted tubules (H&E, Bar=100 µm **(C)** Group VI: Normal proximal convoluted tubules (H&E, Bar=100 µm).

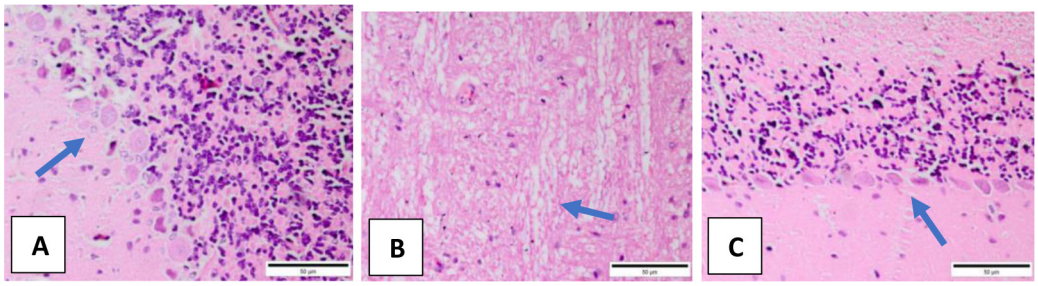


Figure 3. Representative photomicrographs of histopathological sections of brain **(A)** Group II: Cerebellum showing irregularly arranged and degenerated Purkinje cells (H&E, Bar=50 µm) **(B)** Group II: Pons showing formation of the vacuoles in the longitudinal sections of the nerve fibres. The vacuoles coalesce and form larger vacuoles. (H&E, Bar= 50 µm) **(C)** Group VI: Cerebellum showing restoration of Purkinje cells, which was observed in Group II (H&E, Bar=50 µm)

6. Conclusion

This study highlights the protective effects of *Tridax procumbens* aqueous and methanolic extracts against mancozeb-induced oxidative stress in Wistar rats. Mancozeb exposure elevated ROS levels, disrupted antioxidant defences, reduced protein and thiol content, and caused significant tissue damage. Treatment with *T. procumbens* extracts restored antioxidant enzyme activities (SOD, CAT, GPx), normalized oxidative stress markers (LPO, GSH, NO), improved protein and thiol levels, and reversed histopathological damage. These effects are attributed to the plant's rich flavonoid and phenolic content, which scavenge free radicals and support endogenous antioxidant systems. Thus, *T. procumbens* shows promise as a natural therapeutic agent against pesticide-induced toxicity.

7. References

1. Oerke EC, Dehne HW, Schönbeck F, Weber A. *Crop production and crop protection: Estimated losses in major food and cash crops***.** Amsterdam: Elsevier; 2012.
2. Oerke EC. Crop losses to pests. J Agric Sci. 2006; 144(1):31-43. https://doi.org/10.1017/S0021859605005708
3. Zadoks JC. Epidemiology and plant disease management. In: Magellona E, editor. *Proceedings of the 11th International Congress of Plant Protection***,** Manila; 1991; p. 17-19.
4. Popp J. Cost-benefit analysis of crop protection measures. J Verbr Lebensm. 2011; 6(Suppl 1):105-112. https://doi.org/10.1007/s00003-011-0677-4
5. Yahia D, El-Amir YO, Rushdi M. Mancozeb fungicide-induced genotoxic effects, metabolic alterations, and histological changes in the colon and liver of Sprague Dawley rats. Toxicol Ind Health. 2019; 35(4):265-276.
6. Van den Bosch R. The pesticide problem. Environ Sci Policy Sustain Dev. 1979; 21(4):12-42. https://doi.org/10.1080/00139157.1979.9929720
7. Chhabra RS, Eustis S, Haseman JK, Kurtz PJ, Carlton BD. Comparative carcinogenicity of ethylene thiourea with or without perinatal exposure in rats and mice. Fundam Appl Toxicol. 1992; 18(3):405-417. https://doi.org/10.1016/0272-0590(92)90139-9
8. Popp J, Pető K, Nagy J. Pesticide productivity and food security: A review. Agron Sustain Dev. 2013; 33:243-255. https://doi.org/10.1007/s13593-012-0105-x
9. Srivastava AK, Mishra S, Ali W, Shukla Y. Protective effects of lupeol against mancozeb-induced genotoxicity in cultured human lymphocytes. Phytomedicine. 2016; 23(7):714-24. https://doi.org/10.1016/j.phymed.2016.03.010

10. Belpoggi F, Soffritti M, Guarino M, Lambertini L, Cevolani D, Maltoni C. Results of long-term experimental studies on the carcinogenicity of ethylene-bis-dithiocarbamate (Mancozeb) in rats. Ann N Y Acad Sci. 2002; 982(1):123-136. https://doi.org/10.1111/j.1749-6632.2002.tb04928.x
11. Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the Agricultural Health Study. Am J Epidemiol. 2010; 171(4):455-464. https://doi.org/10.1093/aje/kwp404
12. Kackar R, Srivastava MK, Raizada RB. Induction of gonadal toxicity to male rats after chronic exposure to mancozeb. Ind Health. 1997; 35(1):104-111. https://doi.org/10.2486/indhealth.35.104
13. Manderlyophilised M. Marketing of indigenous medicinal plants in South Africa: A case study in KwaZulu-Natal. Rome: FAO; 1998. Report No.: 151.
14. Ravikumar V, Shivashangari KS, Devaki T. Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol. 2005; 101(1-3):55-60. https://doi.org/10.1016/j.jep.2005.03.019
15. Ravikumar V, Shivashangari KS, Devaki T. Effect of *Tridax procumbens* on liver antioxidant defense system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitised rats. Mol Cell Biochem. 2005; 269:131-136. https://doi.org/10.1007/s11010-005-3443-z
16. Bhagwat DA, Killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbens*. Int J Green Pharm. 2008; 2(2):126-128.
17. Sawant S, Chine V, Kalange A, Joshi P, Gawali V, Praharaj SN, *et al*. Evaluation of lyophilized extract of leaves of *Tridax procumbens* Linn. in rodent models of inflammatory and neuropathic pain. Indian J Exp Biol. 2014; 14(2):163-167. https://doi.org/10.1007/s13596-013-0143-1
18. Hitesh J. Formulation and evaluation of analgesic activity of *Tridax procumbens*. Orient Pharm Exp Med. 2006; 23(1):31-33. https://doi.org/10.4103/0973-8258.42736
19. Homayouni F, Haidari F, Hedayati M, Zakerkish M, Ahmadi K. Blood pressure lowering and anti-inflammatory effects of hesperidin in Type 2 diabetes: A randomized double-blind controlled clinical trial. Phytother Res. 2018; 32(6):1073-1079. https://doi.org/10.1002/ptr.6046
20. Benjamin MM. *Outline of veterinary clinical pathology*. 3rd ed. New Delhi: Kalyani Publishers; 1961.
21. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol. 1971; 20(1):95-111. https://doi.org/10.1111/j.1365-2141.1971.tb00790.x
22. Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr. 1974; 104(5):580-587. https://doi.org/10.1093/jn/104.5.580
23. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3):469-474.
24. Aebi HE. Methods of enzymatic analysis. Vol. III. New York: Academic Press; 1983. p. 673-684.
25. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med. 1963; 61:882-888.
26. Wang H, Pagano PJ, Du Y, Cayatte AJ, Quinn MT, Brecher P, *et al*. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. Circ Res. 1998; 82(7):810-818. https://doi.org/10.1161/01.RES.82.7.810
27. Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao G. Spectrophotometric determination of serum nitrite and nitrate by copper–cadmium alloy. Anal Biochem. 2002; 306(1):79-82. https://doi.org/10.1006/abio.2002.5676
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-275.
29. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192-205.
30. Kiernan JA. *Histological and histochemical methods: Theory and practice*. Bloxham, UK: Scion; 2008. p. 288-296.
31. Snedecor GW, Cochran WG. *Statistical methods*. 6th ed. Bombay: Allied Pacific Pvt. Ltd.; 1967. p. 180.
32. Gutteridge JM, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci. 1990; 15(4):129-135. https://doi.org/10.1016/0968-0004(90)90206-Q
33. Kelly KA, Havrilla CM, Brady TC, Abramo KH, Levin ED. Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. Environ Health Perspect. 1998; 106(7):375-384. https://doi.org/10.1289/ehp.98106375
34. Srivastava AK, Ali W, Singh R, Bhui K, Tyagi S, Al-Khedhairy AA, *et al*. Mancozeb-induced genotoxicity and apoptosis in cultured human lymphocytes. Life Sci. 2012; 90(21-22):815-824. https://doi.org/10.1016/j.lfs.2011.12.013
35. Balaji B, Rajendar B, Ramanathan M. Quercetin protected isolated human erythrocytes against mancozeb-induced oxidative stress. Toxicol Ind Health. 2014; 30(6):561-569. https://doi.org/10.1177/0748233712462465
36. Gülçin İ, Oktay M, Küfrevioğlu Öİ, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol. 2002; 79(3):325-329. https://doi.org/10.1016/S0378-8741(01)00396-8
37. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: A universal messenger in an unsolved puzzle. Immunology. 2004; 113(4):427-437. https://doi.org/10.1111/j.1365-2567.2004.01984.x

38. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants *in vivo*? Free Radic Res. 1997; 26(4):381-398. https://doi.org/10.3109/10715769709097818
39. Kim OK, Murakami A, Nakamura Y, Ohigashi H. Screening of edible Japanese plants for nitric oxide generation inhibitory activities in RAW 264.7 cells. Cancer Lett. 1998; 125(1-2):199-207. https://doi.org/10.1016/S0304-3835(97)00513-2
40. Shahidi F, Wanasundara PKJPD, Hong C. Phenolic compounds in food and their effects on health. In: *Antioxidant activity of phenolic compounds in meat model systems*. Washington, DC: ACS Publications; 1992. p. 214-222.
41. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int. 1999; 32(6):407-412. https://doi.org/10.1016/S0963-9969(99)00097-6
42. Ravikumar V, Shivashangari KS, Devaki T. Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol. 2005; 101(1-3):55-60. https://doi.org/10.1016/j.jep.2005.03.019
43. Sailaja B, Bharathi K, Prasad KVSRG. Role of *Tridax procumbens* Linn. in the management of experimentally induced urinary calculi and oxidative stress in rats. Indian J Nat Prod Resour. 2012; 3(4):535-540.
44. Banerjee BD, Seth V, Ahmed RS. Pesticide-induced oxidative stress: Perspective and trends. Rev Environ Health. 2001; 16(1):1-40. https://doi.org/10.1515/REVEH.2001.16.1.1
45. Medjdoub A, Merzouk SA, Merzouk H, Chiali FZ, Narce M. Effects of mancozeb and metribuzin on *in vitro* proliferative responses and oxidative stress of human and rat spleen lymphocytes stimulated by mitogens. Pestic Biochem Physiol. 2011; 101(1):27-33. https://doi.org/10.1016/j.pestbp.2011.06.002
46. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett. 1999; 107(1-3):33-47. https://doi.org/10.1016/S0378-4274(99)00029-6
47. Debnath D, Mandal TK. Study of quinalphos (an environmental oestrogenic insecticide) formulation (Ekalux 25 EC)-induced damage of the testicular tissues and antioxidant defence systems in Sprague-Dawley albino rats. J Appl Toxicol. 2000; 20(3):197-204. https://doi.org/10.1002/(SICI)1099-1263(200005/06)20:3<197::AID-JAT634>3.0.CO;2-7
48. Fetoui H, Makni M, Garoui EM, Zeghal N. Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. Exp Toxicol Pathol. 2010; 62(6):593-599. https://doi.org/10.1016/j.etp.2009.08.004
49. Jhariya S, Rai G, Yadav AK, Jain AP, Lodhi S. Protective effects of *Tridax procumbens* Linn. leaves on experimentally induced gastric ulcers in rats. J Herbs Spices Med Plants. 2015; 21(3):308-320. https://doi.org/10.1080/10496475.2014.973083
50. Tizard IR. *Veterinary immunology*. 9th ed. St. Louis (MO): Elsevier; 2013.
51. Teimouri F, Amirkabirian N, Esmaily H, Mohammadirad A, Aliahmadi A, Abdollahi M. Alteration of hepatic cells glucose metabolism as a non-cholinergic detoxication mechanism in counteracting diazinon-induced oxidative stress. Hum Exp Toxicol. 2006; 25(12):697-703. https://doi.org/10.1177/0960327106075064
52. Sharma A, Sharma V, Kansal L. Therapeutic effects of *Allium sativum* on lead-induced biochemical changes in soft tissues of Swiss albino mice. Pharmacogn Mag. 2009; 5(20):364.
53. Kundi H, Ates I, Kiziltunc E, Cetin M, Cicekcioglu H, Neselioglu S, Ornek E. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med. 2015; 33(11):1567-1571.
54. Mohammadi-Sardoo M, Mandegary A, Nabiuni M, Nematollahi-Mahani SN, Amirheidari B. Mancozeb induces testicular dysfunction through oxidative stress and apoptosis: Protective role of N-acetylcysteine antioxidant. Toxicol Ind Health. 2018; 34(11):798-811. https://doi.org/10.1177/0748233718778397
55. Khan SM, Sobti RC, Kataria L. Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clin Chim Acta. 2005; 358(1-2):131-138. https://doi.org/10.1016/j.cccn.2005.02.015
56. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther. 1991; 51(2):155-194. https://doi.org/10.1016/0163-7258(91)90076-X
57. Anderson ME, Luo JL. Glutathione therapy: From prodrugs to genes. Semin Liver Dis. 1998; 18(4):415-424. https://doi.org/10.1055/s-2007-1007174
58. Steenvoorden DP, Van Henegouwen GMB. The use of endogenous antioxidants to improve photoprotection. J Photochem Photobiol B. 1997; 41(1-2):1-10. https://doi.org/10.1016/S1011-1344(97)00081-X
59. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol. 2003; 66(8):1499-1503. https://doi.org/10.1016/S0006-2952(03)00504-5
60. Iorio R, Castellucci A, Rossi G, Cinque B, Cifone MG, Macchiarelli G, Cecconi S. Mancozeb affects mitochondrial activity, redox status and ATP production in mouse granulosa cells. Toxicol *In Vitro*. 2015; 30(1):438-445. https://doi.org/10.1016/j.tiv.2015.09.018

61. Yahia E, Mohamed AA, Amel C, Mohamed SB. Biochemical and haematological changes following long term exposure to mancozeb. Adv Bioresearch. 2015; 6(2):83-86. https://doi.org/10.15515/abr.0976-4585.6.2.8386
62. Girish BP, Reddy PS. Forskolin ameliorates mancozeb-induced testicular and epididymal toxicity in Wistar rats by reducing oxidative toxicity and by stimulating steroidogenesis. J Biochem Mol Toxicol. 2018; 32(2):e22026. https://doi.org/10.1002/jbt.22026
63. Wagh SS, Shinde GB. Protective effect of *Tridax procumbens* Linn against isoniazid induced hepatic damage. J Pharm Res. 2011; 4(10):3612-3614.
64. Kackar R, Shrivastava MK, Raizada RB. Assessment of toxicological effects of mancozeb in male rats after chronic exposure. Indian J Exp Biol. 1999; 37:553-559.
65. Gürol MA, Arman S, Yön ND. Effects of mancozeb on the testicular histology of the zebrafish. Int J Limnol. 2020; 56:10. https://doi.org/10.1051/limn/2020009