The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1): 461-473, 2026 www.thebioscan.com

Immunohistochemistry study of the effect of *Oxalis corniculata* extract on 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced in mice

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DOI: 10.63001/tbs.2026.v21.i01.pp461-473

Keywords
Cresyl violet staining, Crude extract, Histology, Herbal drug, Neuroprotective, Parkinson's disease.

Received on:
16-11-2025

Accepted on:
10-12-2025

Published on:
22-01-2026

ABSTRACT

Growth Immunohistochemistry (IHC) is a laboratory technique used to visualize and study the distribution of specific proteins or antigens within cells and tissues. Neuroinflammation is a hallmark of various neurological disorders. IHC enables researchers to study the distribution and activation of immune cells within the CNS, providing valuable insights into the mechanisms of neuroinflammation. Immune cells play a crucial role in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. The present study was performed to verify the protective effect of the herb O. corniculata extract on brain, liver and kidney tissues in MPTP treated mice immunohistochemically. Sohxlet extraction method was used to obtain O. corniculata ethanol extract. Histopathological changes and immunohistochemical expression in the peripheral sciatic nerve and cerebral cortex were evaluated. Six groups (I – VI) of mice were used with six mice per group. MPTP treatment elevated the stress indicators, pro-inflammatory cytokines, and apoptotic proteins.

1. Introduction

Parkinsonism describes a syndrome of Parkinson's disease (PD) it is a chronic neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons of substantia nigra pars compacta in the ventral midbrain[1,2]. Three neurotoxins, 6-hydroxydopamine (6-OHDA), 1-methyl- 4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and rotenone, are the most successful agents so far to mimic Parkinson disease *in vitro* and *in vivo*[3]. MPTP is converted by monoaminoxidase B into MPP+ and then taken up by the dopamine transporter and can be

accumulated by mitochondria, leading to complex I inhibition and the generation of free radicals[4]. Many medicinal plants from India have been shown to have activity by the traditional methods of psychoneuropharmacology. Recent studies have indicated that a part of active compounds extracted from herbal medicines, herbal extracts and herbal formulations have effects on Parkinson disease models *in vitro* and *in vivo*[5]. One plant that has been used in mental conditions and illnesses is *Oxalis corniculata* L. It is commonly known as Indian sorrel (Puliiyarai in Tamil), and belongs to the family Oxalidaceae. The present study was performed to verify the protective effect of *O. corniculata* extract on brain, liver and kidney tissues in MPTP treated mice immunohistochemically.

2. Methodology

2.1 Preparation of ethanolic extract of *Oxalis corniculata*

The medicinal plant *Oxalis corniculata* was collected from Tirunelveli District, Tamil Nadu, India. The specimens were identified referring to the Flora of Presidency of Madras[6] and Flora of Tamil Nadu Carnatic[7]. A weighed quantity of powder was subjected to continuous hot percolation in soxhlet apparatus with ethanol at 65-70^{o}C. The extracts were evaporated under reduced pressure using rota flash evaporator until all the solvent had been removed. The yield of the extract was 10% w/w. when compared to the dried starting material.

2.2 MPTP Preparation

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) was used to create an animal model of Parkinsonism. Solutions were prepared by adding sterile saline to a sealed vial of MPTP powder and then diluting this to a stock solution of 20 mg/ml[8].

2.3 Administration of *Oxalis corniculata* extract and/or MPTP

Fill a 10-µL Hamilton syringe with 5 µL *O. corniculata* extract and/or MPTP solution. Inject the MPTP solution at a rate of 1 µL/minute[9].

2.4 Experimental protocol

MPTP was dissolved in 0.9 % saline and administered i.p. Intraperitonial injection of MPTP was given to Groups II,III, IV and V. Oral dosage of Carbidopa + Levadopa (Standard drug for Parkinson's disease treatment) was given to Group III. The animals were divided into six groups, each consisting of six mice.

1. Group I served as vehicle control (Distilled water)
2. Group II received MPTP (20 mg/kg, i.p) (Sigma- Aldrich, Bangalore, India) four consecutive days,
3. Group III received MPTP + carbidopa + levodopa (100 mg/kg, p.o)
4. Group IV received MPTP + crude extract (250 mg/kg, p.o)
5. Group V received MPTP + crude extract (500 mg/kg, p.o)
6. Group VI received only crude extract (500 mg/kg, p.o)

The treatment was given on the initial day, 30 min. prior to first injection of MPTP and once a day for another six days of the experimental period. At the end of experimental period (after 7 days of treatment) the animals were fasted overnight and sacrificed by cervical decapitation before stunning. The brains were excised immediately; the striatal region was identified using the stereotaxic atlas of an albino mice brain[10]. The fresh brain was serially sectioned and the striatum was separated. The tissue was homogenised in ice cold 0.1M tris-HCl buffer solution and used for further analysis. 24 h following MPTP injection mice were perfused for immunohistochemistry as described previously[11]. Tissues are placed in the freshly prepared 0.2 M sodium phosphate buffer, pH adjusted to 7.4 and stored at 4°c. For complete fixation, tissues are refixed in the freshly prepared 4% paraformaldehyde (PFA). Fixing of tissue was confirmed by checking for hardening of the limbs. The brain was then carefully removed and posts fixed in 4% PFA for 48h and processed. 40 micron thick coronal sections were taken through entire SN (approximately -2.54 t0 -4.04 bregma)[12] on a standard vibratoma (Leica, Wetzler, Germany). For cresyl violet staining all sections were mounted on 2% gelatin coated slides and allowed to dry completely.

3. Results

3.1 Brain

Histological evaluation of brain section i.e. Striatum and Substantia Nigra were performed in all the groups of the mouse. Cresyl violet stains both neurons and glia and also used for the detection of nissl body in the cytoplasm of neurons on paraffin sections of brain[13].

The section (Plate: 1a) studied from the brain of control group shows normal morphology in the cerebral cortex. The number and morphology of neurons are normal. Surrounding

parenchyma as well molecular purkinjee layer shows normal histology. There is no evidence of neuronal degeneration or inflammations. The section studied (Plate: 1b) from the MPTP treated group shows focal neuronal injury and also shows mononuclear inflammatory infiltration in the brain parenchyma with mild alter in the morphology and number of neurons. The section studied (Plate: 1c) from the III Group (MPTP + Standard) shows normal neurons with mild reduction in number of neurons. It also shows mild lymphocytic infiltration in the parenchyma. The section studied (Plate: 1d) from the IV group, MPTP with 250 mg of *Oxalis* shows normal neurons with decrease in number in the brain cerebrum. Also it shows focal lymphocytic infiltration in the parenchyma. The molecular layer shows normal purkinjee cells. There is no perivascular infiltrates or microcystic formations. The same result has come out when the group is treated with MPTP and 500 mg of extract of *Oxalis* (Plate: 1e). But here there is no reduction of neurons in number. The group treated with only extract (Plate: 1f) shows normal morphology of brain. So as a prophylatic drug, the *Oxalis corniculata* can be used in Parkinson's disease.

3.2 Histopathology studies on Liver

The histology of liver from the control group shows (Plate: 2a) normal lobular architecture. Individual hepatocytes are normal. The central vein and sinusoids shows unremarkable. The portal tract shows normal appearance. There is no evidence of necrosis or cytoplasmic vacuolation or periportal inflammation. The animals which are treated with only MPTP shows normal (Plate: 2b) lobular architecture in their liver. But, Individual hypatocytes shows mild cytoplasmic vacuolation. The central vein and sinusoids show dilation. The portal tract shows mild inflammation with bile duct hyperplasia. The animals, treated with MPTP and standard drugs (Plate: 2c) shows normal lobular architecture in the liver. The sinusoids show unremarkable. But the central vein shows dilation. Individual hepatocytes shows interface hypatitis. The portal tract shows mild periportal inflammation. The animals treated with MPTP and 250 mg of *Oxalis* extract shows (Plate: 2d) centrilobular necrosis in the liver. Individual hepatocytes show cytoplasmic vacuolations. The central vein and sinusoids show unremarkable. The portal tract shows normal. The group treated with MPTP and 500 mg of *Oxalis* shows (Plate: 2e) normal lobular architecture. Individual hepatocytes shows interface hepatitis and kupffer cell hyperplasia. The central vein and sinusoids shows unremarkable. The portal tract

shows mild lymphocytic infiltration and bile duct hyperplasia. Comparatively, the drug of *Oxalis* 500 mg is more effective than that of 250 mg. The group which was treated with only the extract of *Oxalis* shows (Plate: 2f) normal lobular architecture. Individual hepatocytes shows mild cytoplasmic vacuolation and kupffer cell hyperplasia. The central vein shows dilatation, sinusoids shows unremarkable. The portal tract shows mild inflammation.

3.3 Histopathology studies on Kidney

The animals in the control group shows normal cortex and medulla in their kidney (Plate 3a). The glomeruli show normal morphology. Both the tubules show no significant pathology. Blood vessels show unremarkable. After induction of Parkinsons with MPTP also, the kidney shows (Plate 3b) normal morphology without any significant pathology. The group treated with standard drug shows (Plate 3c) normal cortex and medulla in their kidney. The interstitium shows dense lymphocytic infiltration, that is interstitial nephritis. Blood vessels show unremarkable. The group treated with MPTP and 250 mg of *Oxalis* extract shows (Plate 3d) normal cortex and medulla in kidney. Glomeruli show increased masangeal matrix and endocapillary masangeal proliferative hypercellularity. Both the tubules show no significant pathology. The interstitium shows normal morphology. Blood vessels show unremarkable. The group treated with MPTP and 500 mg of extract of *Oxalis* shows (Plate 3e) normal cortex and medulla. The glomeruli show normal morphology. Both tubules show no significant pathology. The interstitium shows normal morphology Blood vessels show unremarkable. The group treated only with 500 mg of *Oxalis* extract also shows (Plate 3f) the normal histology of kidney. Comparatively the extract of *Oxalis* of 500 mg is more effective. So this can be used as a prophylatic drug for treating Parkinsonism.

4. Discussion

In present study, histological findings in brain tissue indicates that MPTP treated groups showed moderate decrease in neurons, with moderate cellular hypertrophy and Karyorrhexis when compared to control group. Pre-treatment of *O. corniculata* ethanolic extract particularly 250 and 500 mg/kg significantly prevented these neuronal changes from occurring there by confirming its neuroprotective effect. *O. corniculata* is an important medicinal plant that plays a significant role in protection from oxidative stress. A number of studies have shown that *O. corniculata* has

significant antioxidant properties[14]. It has been hypothesized that antioxidants may be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals[15]. Inquiries into the role of neuro-inflammation in Parkinson's disease have coincided with increasing interests in determining whether anti-inflammatory medications may be helpful in preventing PD. Experimental evidence and animal models in particular support a preventative role for nonsteroidal anti-inflammatory drugs (NSAIDs) in Pakinson's disease. For example, studies have demonstrated that anti-inflammatory drugs such as acetylsalicylic acid are protective against MPTP-induced striatal dopamine depletion in mice[16]. Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson's disease[17]. It is widely accepted that inflammation and oxidative stress are interrelated. Oxidative stress can increase inflammatory activity and conversely, inflammation is known to cause oxidative stress[18]. Several studies have also emphasized the flavonoids and related polyphenols present in the *Oxalis corniculata* extract may be responsible for the anti-inflammatory activity[19]. Previous studies show that *Oxalis corniculata* extract was found to have anti-inflammatory property. Further studies are needed to prove whether anti-inflammatory and anti-oxidant properties of flavonoids responsible for the antiparkinson effect or whether the synergy of a number of components viz. minerals, tannic acid, etc., is responsible for the observed effects. It can be proposed that apart from the known effects of *O. corniculata*, it also has neuro-protective and anti-oxidant properties.

Histopathological examination of liver sections of the normal group showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. The hepatocytes are polygonal cells with well preserved cytoplasm, nucleus with prominent nuclei. On the other hand, in the hepatotoxic positive group, histological examination showed loss of architecture, inflammation, and congestion with cytoplasmic vacuolation, fatty change, sinusoidal dilatation, centrilobular necrosis, and displayed bundles of collagen surrounding the lobules, which resulted in huge fibrous septa and distorted tissue architecture. Such cytoplasmic vacuolations are said to occur when the cytoplasm becomes pale and swollen due to accumulation of fluid or lipids as the result of disturbance in lipid inclusions and fat metabolism[20]. It could also occur when there is disturbance to the functions of ribosomes,

uncoupling of lipid from protein metabolism or cytoplasmic alterations produced to collect the injured substances in the cell[20]. In *O. corniculata* treated animals, liver sections showed mild inflammation and mild necrosis of hepatocytes with mild cytoplasmic vacuolation, and mostly no visible changes observed. Histopathological examination also showed good recovery of MPTP-induced necrosis by ethanolic extracts as compared to standard drug. Animals treated with the low dose showed regeneration of hepatocytes surrounded by septa of fibrous tissue with a significant increase in bile ductules, fat storing cells, and Kupffer cells. Animals treated with the higher dose of plant extract showed remarkable histological regeneration compared to those of the low dose group. They showed nearly ordinary patterns with an increase normal hepatocytes parenchyma and a reduced development of fibrous septa and lymphocyte infiltration.

Possible mechanism of all of the extracts including presenting herb as hepatoprotective may be due to their anti-oxidant effects or inhibition of cytochrome P450. This might be due to the high contents of polyphenols present in the *Oxalis* extract which could have reduced the production and/or accumulation of toxic derived metabolites[21,22,23]. Histopathological examination of the liver section of the mouse treated with MPTP showed intense centrilobular necrosis and vacuolization. The mouse treated with the plant extract and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis. The hepatoprotective effect of the extract may be explained depending on the fact that it contains polyphenolic compounds which may scavenge free radicals offering hepatic protection and its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the oxidative stress induced damage.

In Parkinson's disease 58 percent of patients are experiencing urinary problems. The majority of affected Parkinson's patients have difficulty with the storage of urine more so that voiding and symptoms appear later in Parkinson's disease than they do in multiple system atrophy (MSA). Long-term damage, dysfunction and failure of the kidneys are a major complication of Parkinsonism. Disorders of the kidneys are serious secondary consequence of Parkinsonism and have been known to cause renal failure thus leading to mortality and morbidity. However the histological and histomorphometric evaluation of the present study shows atrophy rather than hypertrophy in the glomeruli of Parkinsonism animals which is

validated by significant decrease in its density and shrinkage. These observations were also characterized by diminished cellular proliferation, decreased cellular volume and ischemia. The primary function of the glomeruli is to assist in the production of ultrafiltrate of the plasma such as Na+, water and urea for further processing by the renal tubules thus playing a vital role in the maintenance of fluid and electrolyte homeostasis. Administration of the *O. corniculata* extracts improves the histoarchitecture of the kidney and by extension restores its functionality.

Based on the results on kidney tissues, the observations seem to suggest that selected medicinal plant might have renal protective ability to prevent kidney dysfunction by accelerating regeneration. Possible beneficial effects of herbs are diuresis, protection of the kidney from nephrotoxic agents, prevention or amelioration of renal lithiasis, and amelioration of kidney failure[24].

5. Conclusion

From the above results, it's clear that, indigenous medicinal plant, *O corniculata* has the potential to prevent Parkinsonism. The histologic changes induced by MPTP are also positively modulated by *O. corniculata*, so as to decrease the oxidative damage to neurons. The neuro-modulatory effect of *O. corniculata* on behavioral, oxidative stress, histological changes may be due its neuroprotective, antioxidant properties. The estimated parameters were closely relevant to clinical Parkinsonism and the drug treatment protected the diseased brain of mice. Further studies with different extracts and their fractions are encouraged to identify the chemical constituents responsible for Anti-Parkinson's activity. Also clinical studies to prove this effect is also needed for its applicability in humans for treatment of Parkinson's disease.

6. Acknowledgement

Authors acknowledge the facilities and support provided by the Rajas Dental College, Kavalkinaru, Tirunelveli, Tamil Nadu, India.

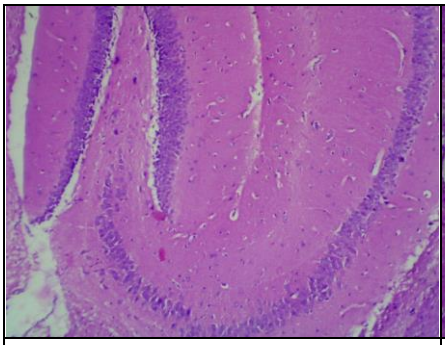
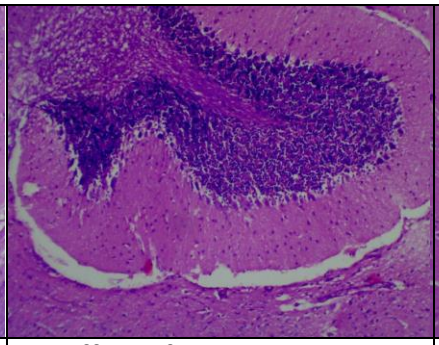
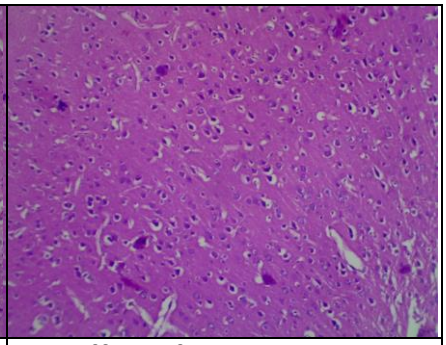
7. Funding/Conflicts of interests if any

The authors declare no conflict of interest.

8. Author contribution to the Manuscript

K. Aruna and P. Devi Rajarajeswari - Experimental work - writing—review and editing. All authors have read and agreed to the published version of the manuscript**.**

Fig: 1 Photomicrographic view of histopathology studies on male mice brain tissue (400X)

		
a) Normal mice brain - cerebral cortex	b) Effect of MPTP on cortical region	c) Effect of MPTP + Standard on cortical region

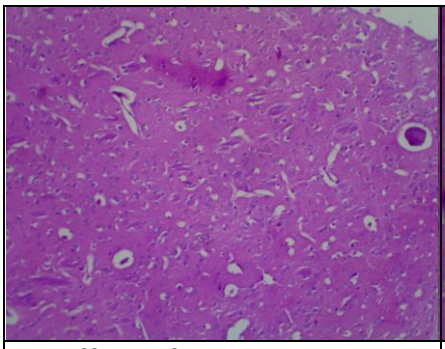
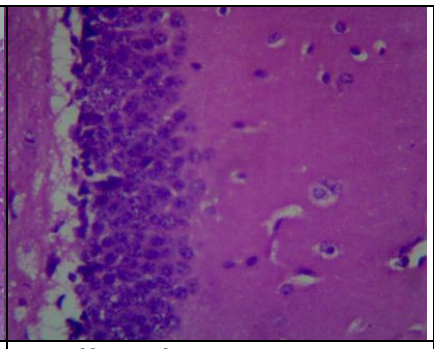
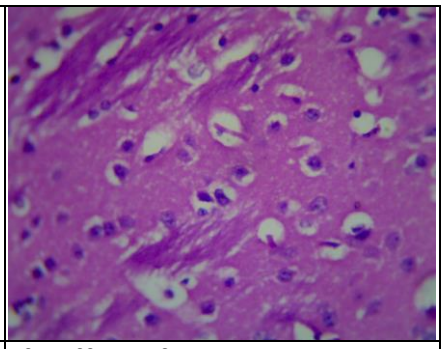
		
d) effect of MPTP with 250 mg of *Oxalis* on cortical region	e) Effect of MPTP and 500 mg of extract of *Oxalis* on cortical region	f) Effect of only *Oxalis* extract on cortical region

Fig: 2 Photomicrographs of histopathology studies on male mice liver tissue

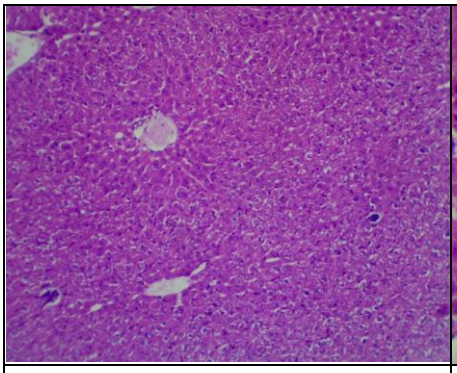
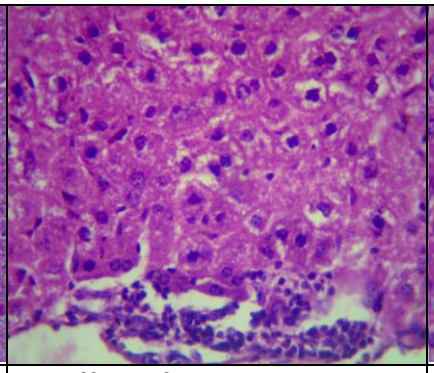
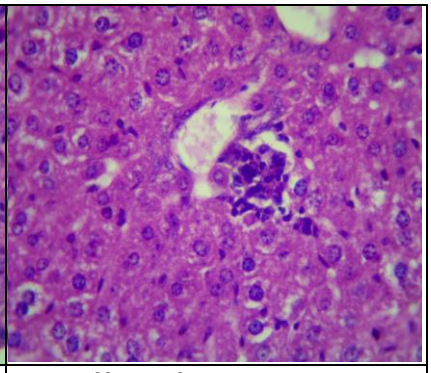
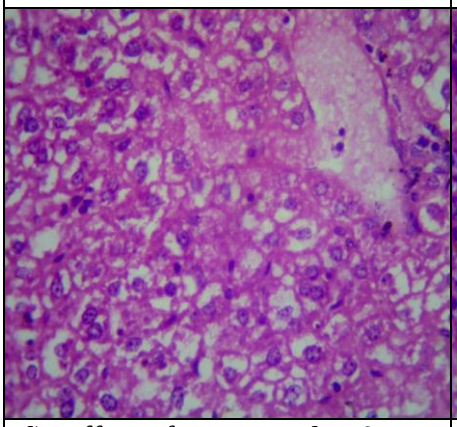
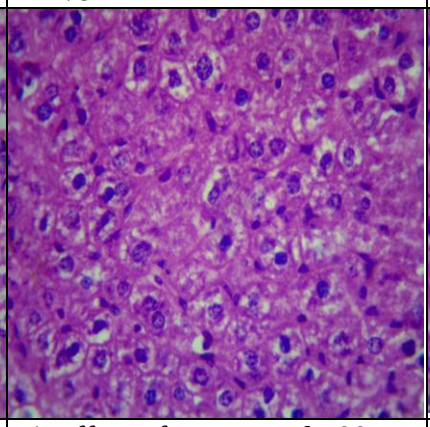
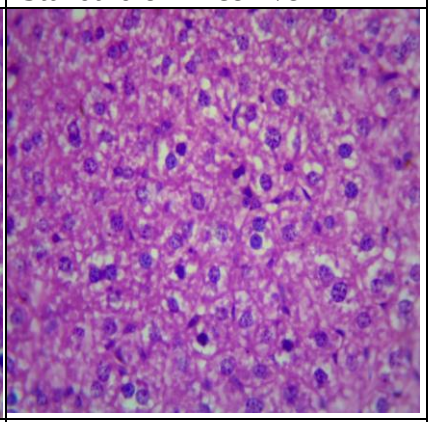
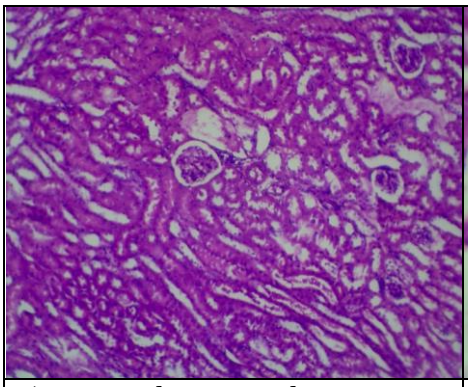
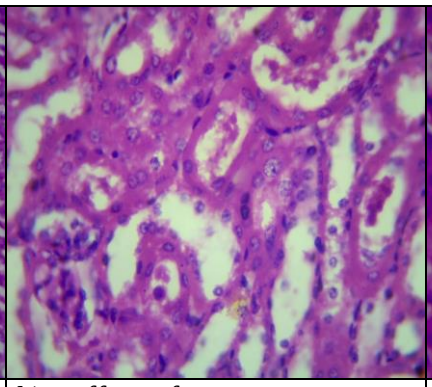
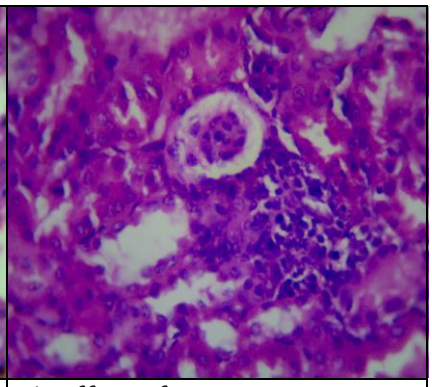
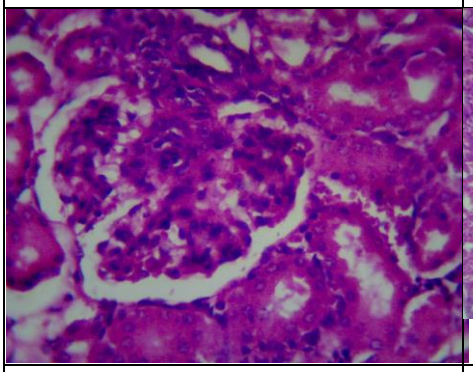
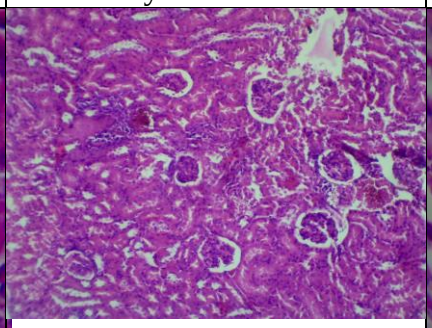
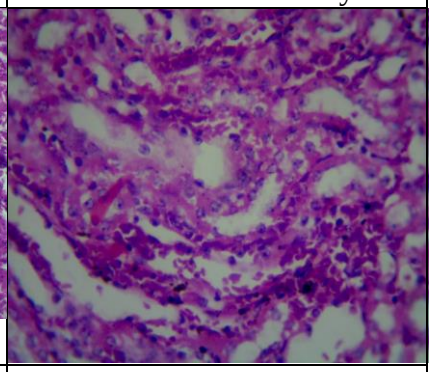
		
a) Normal mice liver	b) Effect of MPTP on mice liver	C) Effect of MPTP + Standard on mice liver
		
d) Effect of MPTP and 250mg of extract of *Oxalis* on mice liver	e) Effect of MPTP and 500mg of extract of *Oxalis* on mice liver	f) Effect of only *Oxalis* extract on mice liver

Fig: 3 Photomicrographs of histopathology studies on kidney tissue

		
a) Normal mice Kidney	b) Effect of MPTP on mice Kidney	c) Effect of MPTP + Standard on mice Kidney
		
d) Effect of MPTP and 250mg of extract of *Oxalis* on mice Kidney	e) Effect of MPTP and 500 mg of extract of *Oxalis* on mice Kidney	f) Effect of only *Oxalis* extract on mice Kidney

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