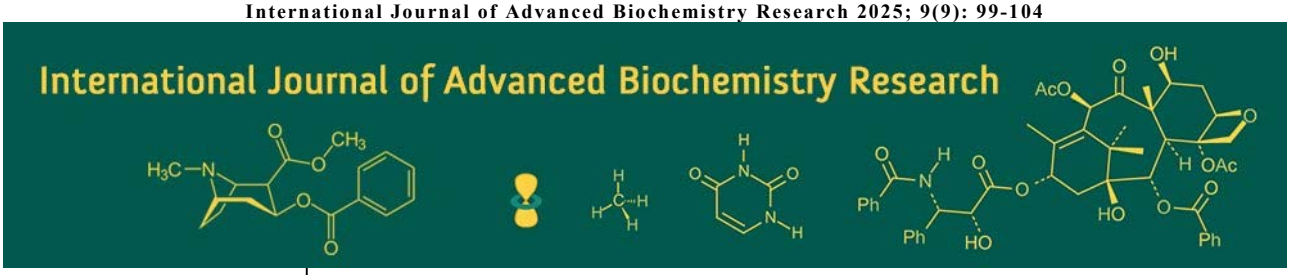

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The effect of *Tanacetum parthenium* in orthopaedic mini-drill induced

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Abstract
The present study is aimed to develop spinal cord injury by employing orthopaedic mini-drill and investigate neuroprotective effects of the herb *Tanacetum parthenium* after injury by the means of histopathological and immunohistochemical observation. A total of 48 male Wistar rats were divided into positive control (To), spinal cord injury group (T-ve group) and spinal cord injury with treatment of *Tanacetum parthenium* (T). Histopatholgical examinations were performed to understand the various changes at tissue and cellular level in multiple organs and spinal cord. Results showed axonal disruption, astrocytosis, and cavitation. Neuronal repair was noticed. There was progressive reduction in the inflammation in organs and spinal cord indicating anti-inflammatory effects of the herb. Regeneration was absent, indicating no potential effect of the herb on nerve regeneration. All the rats exhibited inability to void urine voluntarily. These findings suggest that orthopaedic-mini drill induces complete paraplegia and is an adaptable and reproducible technique. However, no neural protective effects were exhibited by the herb *Tanacetum parthenium*.

Keywords: Spinal cord injury, orthopaedic mini-drill, *Tanacetum parthenium*, rat model of spinal cord injury, fever few

Introduction
Spinal cord Injury is a devastating injury and has severe physical, psychological and economic effect on the individual and his family. Spinal Cord Injury is defined as damage to spinal cord that results in either temporary or permanent change in it sensory, motor or autonomic functions. SCI can occur from trauma or disease and can result in wide range of neurological deficits and disabilities (Boraiah and AS 2022) [1]. Paraplegia results from lower thoracic injuries while cervical injuries can lead to quadriplegia. Globally road accidents, classified under traumatic injuries, are leading cause of spinal injuries. The worldwide prevalence of acute spinal cord injuries ranges from 200 to 1, 000 cases per million (Burns *et al*., 2012) [2]. In India, the prevalence of SCI is estimated at 15, 000 to 20, 000 new cases annually, with an incidence rate of approximately 20 cases per million per year. Falls from height are the most common cause of injury, followed by road traffic accidents (Srivastava *et al*., 2015) [3]. The pathophysiological events after acute SCI can be divided into primary and secondary pathological changes. Various animal models including rats, rabbits and primates like monkeys have been experimented on to study these pathophysiologic events, closely resembling those of human spinal cord injury. Rats have been preferred widely among these animals as their neuronal responses closely resemble those of humans (Kjell and Olson 2016; Akhtar *et al*., 2008) [4, 5].

The primary clinical treatments for SCI currently include early surgical decompression and stabilization, enhanced spinal cord perfusion, high-dose intravenous corticosteroids, anti-inflammatory medications during the acute phase and neurological rehabilitation training in the chronic phase. Methylprednisolone is considered as gold standard drug in the treatment of SCI. However high doses of the drug are required to achieve neuroprotective effects, which raises concern about its potential side effects (Fehlings *et al*., 2014; Sayer *et al*., 2006) [6, 7].

The preference of alternative medicine has been on the raise in recent times as people are seeking natural and holistic approach.

Many herbs such as *Tamarindus indica*, *Plumeria rubra*, Aloe vera, *Withiania somnifera* are being tested for their therapeutic effects on various manifestations of paralysis (Montross *et al*., 2017) [8].

Tanacetum parthenium (fever few) is one such herb with anti-inflammatory and anti-nociceptive properities, that plays an important role in traditional medicine of different nations and tribes. It has been widely used in folk medicine for fever, inflammation, asthama, rheumatism and anti-nociceptive properities. The main active compound is sesquiterpene lactones. They have been reported to have anti-inflammatory and antioxidant effects. This herb inhibits platelet aggregation, release of histamine from mast cells and release of prostaglandins. Thus, helping in controlling inflammation and pain (Chavez ML and Chavez PI 1999; Jain Nk and Kulkarini SK 1999) [9, 10].

Materials and Methods

The present study was undertaken at the Department of Veterinary Pathology, Mumbai Veterinary College, Parel, Mumbai from July 2024 to February 2025. Experimental work to induce the spinal cord injury was conducted in the Laparoscopy Hall of Department of Veterinary Surgery and Radiology. Housing of the animals and experimental work to monitor the effect of *Tanacetum parthenium* leaf powder was conducted in the Central Laboratory Animal Facility of Mumbai Veterinary College, Mumbai.

Experiment animals

The experimental protocol was approved by Institutional Animal Ethical Committee. According to the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA). Ministry of Social Justice and Empowerment, Government of India, the present study was conducted on 6-8 weeks old male Wistar rats. Animals were procured from Central Laboratory Animal House Facility of Mumbai Veterinary College, Mumbai. Animals were housed in polypropylene cages with stainless steel grill tops having the facilities for the feed and water bottles and the cages were kept on stainless steel racks, in experimental room. Sterilized rice husk was used as bedding material in cages, which was changed twice a week**.** The rats were kept in an environmentally controlled room with 22±3ºC temperature and 30-70 percent relative humidity. Floor was swept and mopped with a disinfectant solution every day in the morning. After acclimatization to the environmental conditions for seven days, all the 48 rats were randomly assigned to control and treatment groups with approximately the same average weights within the different groups under the study.

Experimental Design

A total of 48 rats were used of which 08 were placed in the positive control group, 8 were in negative control group and 32 rats in the treatment group. Regular intervals of slaughter of rats were performed with ethical guidelines, at the intervals of 7th, 15th, 30th & 60th day. After necropsy all the organs were collected and preserved in 10% neutered buffered formalin.

Group	Positive Control (To)	Negative Control (T-ve)	Treatment (T)			
Days	0th day	0th day	07th day (T_1)	15th day (T_2)	30th day (T_3)	60th day (T_4)
No. of animals	08	08	08	08	08	08

Group I (Positive Control): Consisted 8 healthy rats, spinal cord was intact.

Group II (Negative Control): In this group, spinal cord was injured but no treatment was given.

Group III (Treatment group): Spinal cord injury was done and the group were treated with *Tanacetum parthenium*. The group was further divided into four groups based on the day on which they were sacrificed.

The animals from group III were treated with dried leaf powder of fever few mixed in distilled water @ dose rate of 700 mg/kg/b.wt daily for 21 days.

Development of spinal cord injury model

The orthopaedic mini drill induced spinal cord model was developed for the induction of spinal cord injury.

Induction of spinal cord injury

Pre-operative care

- The following instruments were sterilized: 2-3 pairs of forceps, 2 pairs of micro scissors, suture, needle holder, skin clips. The orthopaedic drill with inserted bit was also sterilized.
- Rats were anaesthetized using an intraperitoneal mixture of xylazine and ketamine at the dose rate of 5mg/kg and 50 mg/kg bwt respectively. Hair was shaved over the targeted area with a sterilized shaving blade.
- Skin was prepped with povidine-iodine solution and 70% alcohol.
- The animal was moved on to the surgical table that was warmed with heating pad and the animal was covered with a surgical drape.
- When the animal lost the paw pinch reflex, vertebral process was palpated, and a stab incision was given at the level of lumbar 1 vertebra.

Induction of spinal cord injury by orthopaedic mini-drill

- A drill containing a bit sized 0.5mm length and 0.5mm width was chosen.
- L1 and L2 vertebrae were palpated and drill was inserted in between the vertebrae through the spinous process and it was drilled for 30 seconds. Paraplegia was be confirmed by absence of pedal reflexes. Incised wound was sutured in the routine manner. Post-operative care was taken with analgesics and antibiotics.
- An x-ray was taken after to see the extent of damage to the vertebrae. Care was taken as to minimize the damage to the vertebrae and vertebral disc is not damaged. Paraplegia was observed immediately after the recovery from anaesthesia.

Post-injury animal care

Following the drilling, saline was administered subcutaneously to maintain adequate hydration for the rat.

- Water and food were provided ad libitum.

- Antibiotic and analgesics were given for three days following surgery.
- Bladder was emptied daily twice, once in the morning and evening post-surgery due to bladder incontinency.

Treatment with the herb *Tanacetum parthenium*

After the spinal cord injury was induced treatment with the herb *Tanacetum parthenium* was started and continued for a span of 21 days. Rats were dosed at the rate of 700mg/kg/bwt (Dharmalingam S and Natesan G 2017) [11].

The finely ground leaf powder was mixed with distilled water and was given to the rats in the treatment group by oral gavage technique**.**

Observation and monitoring

All the rats were observed daily for clinical signs, behavioural changes and mortality, if any, throughout the experimental study.

Necropsy examination

At intervals 7th, 15th, 30th, 60th day the animals were sacrificed by cervical dislocation method. Detailed necropsies were carried out on all the animals to score the lesions and observe the pathological changes exhibited by different groups of study animals. Tissues collected during the necropsy procedure were presented in 10% neutral buffered formalin.

Histopathological examinations

After necropsy examinations, the tissue from visceral organs (lung, liver, heart, kidney, bladder and spinal cord) were collected and preserved in 10% neutral buffered formalin and were processed for routine histology techniques as per the standard procedures for detailed microscopic examination. (Culling, 1974) [12]

Results and Discussion

The rats from both the negative control group and treatment groups, were sacrificed, histopathology of organs was performed and any gross and histopathological changes were notice. The important observations noted from the organs after the gross and histopathological examination are mentioned below.

Gross examination

Upon gross examination, major abnormalities showed by lungs in both the negative control and treatment were emphysema, diffuse congestion, red hepatization.

Prominent lesions on heart were congestion of blood vessels and few blood clots were present in the heart. Liver showed diffuse congestion. Congestion of both cortex and medulla was noticed on kidneys. Urinary bladder showed few haemorrhagic foci and thickening of bladder.

Histopathological examination from organs

a) Histopathological changes in Lungs

Negative control group showed multifocal areas of moderate pulmonary edema, congestion and haemorrhages. Mild to moderate compensatory emphysema and focal areas of MNC infiltration was evident (Plate 1). Moderate thickening of alveolar wall was noticed.T_1 group exhibited diffuse moderate pulmonary edema and multi focal areas of congestion. Focal areas of MNC infiltration were observed. The lungs of T_2 group of rats exhibited multifocal areas of mild pulmonary edema with congestion of vessels. Focal areas of haemorrhages were noticed. The T_3 group showed mild compensatory emphysema and focal areas of haemorrhages were observed. MNC infiltration was reduced as compared with the other groups. The severity of lesions was less in T_3 and T_4 groups (later stages) as compared with T_1 and T_2. These changes occur as SCI triggers systemic inflammatory response i.e., outside the cord, increasing inflammatory cells number in these organs resulting in the organ damage. (Antequera *et al*., 2022, Gris *et al*., 2008) [13, 14]. Atollahi *et al*., (2022) [15] discusses the anti-inflammatory properties of *Tanacetum parthenium* and its role in the treatment of various anti-inflammatory diseases suggesting the possibility of gradual reduction in the anti-inflammatory treatment groups.

b) Histopathological changes in Heart

Rats from negative control (To) showed focal areas of vascular congestion and haemorrhages. Focal areas of myocardial degeneration were noticed in certain rats. Multifocal areas of pericardial congestion were observed along with mild haemorrhages in the group T_1. No significant changes were found in animals of T_2 group. No major changes were found in T_3 and T_4 group. These changes are in accordance with Sun *et al*., (2016) [16].

c) Histopathological changes in Liver

In the negative control group and T_1 multi focal areas of perivascular and periportal congestion were observed (Plate 2). Moderate hepatic degeneration of the cells was found along with mild sinusoidal congestion. In the group T_2 multifocal areas of mild hepatic degeneration was noted. Focal areas of mild perivascular congestion were observed. Accumulation of eosinophilic fluid was detected. Mild infiltration of MNC was noticed in the liver. Mild multifocal areas of hepatic degeneration were shown by T_3 group. Multifocal areas of congestion were observed.T_4 group showed liver lobules with multifocal areas of perivascular congestion and mild hepatic degeneration. Infiltration with MNC was noticed. These findings are in agreement with study conducted by Mohammed *et al*., (2020) [17] wherein, congestion in blood vessels was found in liver. Research suggests clinically significant changes occur after SCI that results in systemic inflammation that ultimately leads to liver damage (Saurbeck *et al*., 2014) [18].

d) Histopathological changes noticed in the kidney:

The negative control group showed mild to moderate vascular changes along with the dilated capillaries. Multi focal areas of moderate tubular degeneration along with swelling of tubular cells was observed. Mild tubular changes along with changes in the glomeruli were noticed. In the animals of T_1 group mild coagulative necrosis was observed.T_2 group showed multi focal areas of moderate tubular degeneration. The above findings are in accordance with Parvin *et al*., (2021) [19] who discusses about the huge systemic inflammatory response that gets generated after SCI that impacts peripheral organs such as kidney, which ultimately leads to kidney dysfunction.

e) Histopathological changes in Urinary Bladder

The group negative control presented with multi focal areas of moderate necrosis of urothelium with MNC infiltration. In the group T_1 mild to moderate degeneration of mucosal

epithelium with moderate infiltration of MNC was observed (Plate 3). The group T_2 showed mild to moderate hypertrophy of epithelium with mild MNC infiltration. In the groups T_3 andT_4 moderate exfoliation of urothelium was observed. Ferreira *et al*., (2022) [20] observed neurogenic Detrusor sphincter dyssynergia (DSD) which leads to urine inconsistency and histopathological changes include increase in thickness of bladder epithelium in spinal cord injury.

Histopathological changes observed in spinal cord

The major histopathological changes observed in spinal cord are haemorrhages, mild neuronal necrosis and vacuolar degeneration. Infiltration of astrocytes, mild hypertrophy of chondrocytes moderate collagen synthesis and angiogenesis were observed as mentioned in Table 1.

In the T_1 and T_2 groups moderate haemorrhages and neuronal necrosis were found. Mild vacuolar degeneration was observed. Synthesis of collagen and angiogenesis were also noted. In the group T_3 hypertrophy of chondrocytes and hyperplasia was observed (Plate 4).

T_4 group showed moderate infiltration of astrocytes, presence of cavitation and hyperplasia and hypertrophy of chondrocytes (Plate 5). Abundant collagen synthesis and angiogenesis were found indicating neuronal repair. These findings are in coordination with Ramadan *et al*., (2017) and Fedorova *et al*., (2022) [21, 22] in which haemorrhages, necrosis and degenerative changes were found in neurons.

Immunohistochemical changes observed in the spinal cord

Expression of GFAP is the major indicator for astrocyte activation and glial scar formation. The major immunohistochemical changes observed in the spinal cord are listed in the table 2. In the group T_1 numerous astrocytes were observed, which gradually increased until 15th day (Plate 6). There was peak production at the interval of 30th day where increased astrocyte production and scar formation were noticed. Interestingly, elevated GFAP levels were noticed proximal to the injury site suggesting increase astrocytic reaction. However, GFAP activation was noticed even distal to the injury. Similar to the T_3 group elevated GFAP levels were noticed even in T_4 group. The abovementioned findings are in coordination with Baldwin *et al*., (1988), Farooque *et al*., (1995) and Li *et al*., (2004) [23, 24, 25] where in elevated levels of GFAP were noticed in the spinal cord injury in rats.

Table 1: Histopathological changes observed in spinal cord

Pathological changes observed	To	T-ve	T_1	T_2	T_3	T_4
Hemorrhages	-	+	++	++	++	++
Infiltration of astrocyte	-	+	+	+	++	++
Neuronal Degeneration	-	+	++	+	+	+
Cavitation	-	++	-	+	++	++
Hyperplasia/Hypertrophy of chondrocytes	-	++	-	++	++	++
Collagen synthesis / CT	-	++	+	+	++	+++
Angiogenesis	-	++	+	+	++	++

Table 2: Immunohistochemistry changes observed in spinal cord

IHC changes	To	T-ve	T_1	T_2	T_3	T_4	Inference
Astrocytes	Nil	++	++	+	-	-	Tanacetum parthenium reduces inflammation and astrocyte activation
Elevated GFAP levels	Nil	++	++	-	-	-	Elevated GFAP levels indicate increased astrocytes

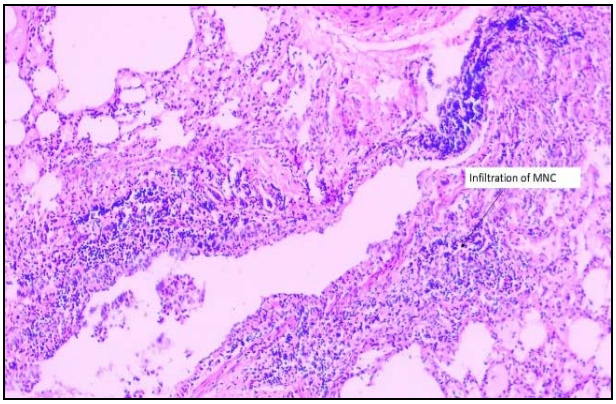

Plate 1: Representative photomicrograph showing MNC infiltration in lung in Negative control group (H&E, 40X)

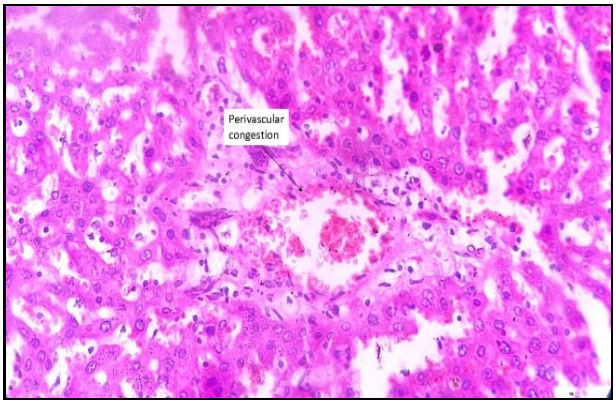

Plate 2: Liver showing perivascular and periportal congestion in T1 group (H & E 40X

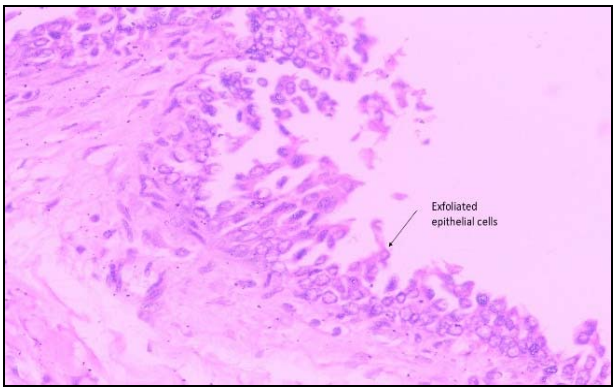

Plate 3: Exfoliation of epithelial cells in urinary bladder in group T_1 group (H & E, 40 X)

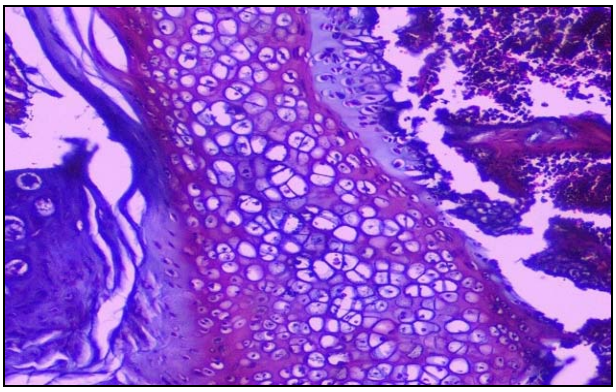

Plate 4 : Hypertrophy of chondrocytes in spinal cord in T_3 Group (LFA, 100X)

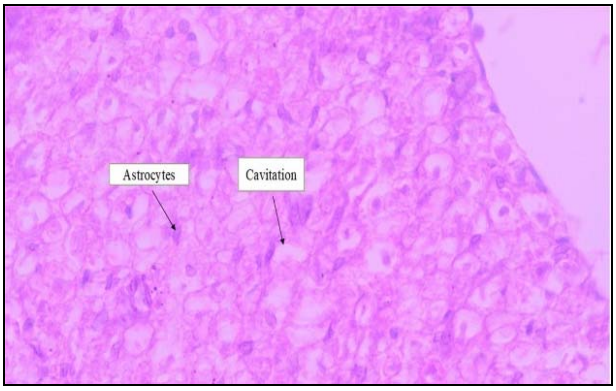

Plate 5 : Presence of cavitation and infiltration by astrocytes in T_4 group (H & E, 40 X)

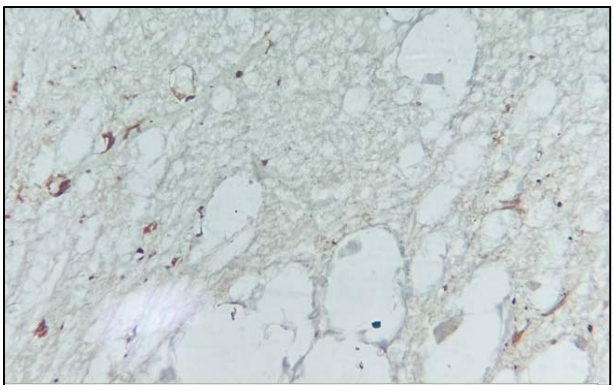

Plate 6: T_1 group showing presence of astrocytes (IHC, 40 X)

Conclusion

The present study demonstrates that orthopaedic mini-drill successfully induces paraplegia in rats. This method is easily reproducible and is suited for students at university level where adopting costly equipment such as NYU-MASCIS (New York University-Multicenter Animal Spinal Cord Injury Study) impactor is not feasible. This method is cost effective and induces complete paralysis. The histopathology of the organs reveals that apart from spinal cord lesions are also found in the organs. This happens as SCI disturbs the autonomic nervous system which in turn induces dysfunction or failure in multiple organs indicating the critical role of spinal cord in coordinating bodily functions. The major histopathological changes noticed in spinal cord are haemorrhages, infiltration of astrocytes and neuronal degeneration. Cavitation and FCT were noticed in groups of 30^{th} week and 60^{th} week in both negative control and treatment groups. IHC revealed presence of astrocytes but no glial cells. Axonal regrowth and no new glial cells were not observed indicating no regeneration. There was no improvement even by the end of 60^{th} week in hindlimb condition concluding that *Tanacetum parthenium* exhibited no regenerative properties.

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Ethical approval

The study was conducted following the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India.Approval was obtained from the Institutional Animal Ethics Committee (IAEC) of Mumbai Veterinary College, Mumbai, Maharastra.

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