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## Clinical and Antigen Serotype O Confirmation of Foot and Mouth Disease (FMD) Virus Outbreak in Punjab Spillover from Domestic Livestock

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### Abstract

Foot and Mouth Disease (FMD) is one of the most highly contagious viral diseases affecting cloven-hoofed domestic and wild animals. This study described clinical investigations of FMD outbreaks (n=13) with serotype O confirmation in seven districts of Punjab from October 2023 to April 2024. Samples from sick animals and post-mortem examinations were collected from FMD suspected animals for laboratory diagnosis. The investigation included clinical signs and lesions, blood profiles, presence of other infectious pathogens, differential diagnosis, and the cause of death in the area. A total of 13 outbreaks in farm ruminants were confirmed, detecting FMDV serotype O. Outbreaks were reported in Ludhiana, Barnala, Gurdaspur, Bathinda, Faridkot, Mansa, and Amritsar districts. Animals were diagnosed to be positive for FMDV antigen using Sandwich ELISA. A total of 692 animals, including both cattle and buffalo, were affected in these districts, with a mortality rate of 24.42% (169/692) in young animals and heifers. The most severely affected districts were Bathinda, Barnala, and Faridkot, that experienced high mortality rates, leading to significant economic losses in these areas. Histopathologically samples revealed muscle degeneration and diffuse lymphocytic infiltration, and lymphocytic myocarditis in the heart, which is characteristic of the cardiac form of FMD. Serotype O FMDV, primarily circulating in the study area, was identified as the cause of the outbreaks in farm ruminants under investigation. The local animal health authorities should take steps to minimize the spread of the disease by being prepared for risk factors, particularly animal movement and human activities linked to an outbreak and the need for improved vaccination coverage and enhanced biosecurity measures to control the spread of FMD in the region.

**Keywords:** Serotype O, Punjab, outbreak, cloven-hoofed, cardinal sign, lameness

### 1. Introduction

Punjab is one of the most progressive state of Northern region of India. The economically dependent to livestock growths that contribute to the livelihood of farmer of the state. Punjab accounts 2.2% of poultry and 2.5% of cattle and buffalo population in India while its contributions to milk, egg, and meat production are 7%, 5%, and 3.4%, respectively [1]. Livestock

supplies 16% of the revenue to small farm households [2]. The most economic loss of small farm owners of bovine due to outbreaks of infectious diseases in the farm. Among infectious disease of bovine FMD is one of the most contagious diseases affecting cattle and buffalo. Create a severe economic impact in the nations that report the disease to the World Organization for Animal Health (WOAH), with an estimated global economic

## Research Article

loss of USD 6.5 and 21 billion [3, 4]. To control FMD, several strategies have been put into place, such as vaccination, diagnostics, culling and surveillance [5]. In India the National Animal Disease Control Programme for FMD is to control FMD by 2025 with vaccination and its eventual eradication by 2030 [6]. Nationwide efforts to control FMD have adopted a comprehensive strategy that includes biannual vaccinations, sero-monitoring, and sero-surveillance. However, the elevated viral mutation rate, transient immunity to vaccination, susceptibility to different hosts, and intricate socioecological processes have presented significant obstacles to disease control [7]. In India the virus mainly spreads due to unrestricted animal movement [10]. In carrier animals, virus persists in the soft palate, pharynx, and tonsils for 2 years [8, 9]. Virus enters mainly by the respiratory route, oral route or through conjunctiva, genital route and virus initially multiplies in the epithelium [11]. Viremia sets elevated body temperature and blister formation, mainly on the mouth, tongue. Sick animals the clinical presentation involved feet hydropic degeneration and necrosis of the infected cells are hallmarks of FMD's. Blisters can cause lameness, appetite loss, and weight loss [12]. In young animals the virus tends to localized in the heart muscles and produces severe myocarditis [13]. FMD virus are seven serotypes O (Oise valley in Franch), A (Allemagne in France), C (Germany) Asia-1(Pakistan) and SAT1, SAT2 & SAT3 (South African Territories) and serotype O is also termed as Pan Asia strain [14]. All the serotypes of FMD virus are antigenically distinct and no cross protection occur and each serotype has many subtypes [15]. FMD virus is sensitive to heat, acid and alkali [16]. Currently, disease outbreaks in farm animals in India are caused by three serotypes (O, A, and Asia-1) and result in significant economic losses as well as trade restrictions [10]. The experts from Animal Disease Research Centre (ADRC) along with Scientist from Veterinary Medicine of University recently conducted disease investigations on suspected outbreaks of FMD and septicemia with respiratory involvement in various districts of Punjab Ludhiana, Barnala, Gurdaspur, Bhatinda,

Faridkot, Mansa and Amritsar. The outbreak notice in these areas was due to change climate that favor to viral transmission. In Mansa and Gurdaspur districts outbreak was reported due to introduction of newly purchased animals in the farm from affected area. Scientists examined the animals, recorded history of disease, mortality, morbidity as per information received from Veterinary Officers and owners of affected animals through direct interview. The aim of present study to investigate the recent FMD outbreaks in Punjab, viral etiology, and identify the serotype of virus circulating strain in this area. The present study was reported that potential causative agents of the disease outbreaks and need to prevent the further disease transmission of circulating strain in Punjab.

## 2. Materials and Methods

### 2.1. Disease History and outbreak investigation

In October 2023 the telephonic call received from village Badawal, District Ludhiana, through owner. The University expert team visited village farm and collected data through direct interview and observation of clinical sign and lesions of the disease in sick animals. Likewise, other outbreak data was collected and observation recoded. The predominant clinical findings included oral vesicular lesions, lameness, respiratory distress, fever, and decreased feed intake. The outbreak investigation period was from October 2023 to April 2024 and the peak period was in the month of February 2024. Higher mortality rate was observed among calves and heifers. The study encompassed 13 outbreaks involving cattle and buffalo on organized farms across seven districts in Punjab. Higher morbidity and mortality rates were noted in farms and villages without a recent history of vaccination against FMD. Out of a total population of 3,810 animals in the investigation area, 692 animals were affected or suspected of having FMD based on observed clinical signs (Figure 1) and lesions.

## Research Article



**Figure 1.** Typical clinical observation of FMD lesion (a-d) during outbreak investigation field visit in bovine species.

### 2.2. Sample collection and processing

Vesicular epithelium samples were collected in 50% Phosphate Buffer Glycerin (PBG) and submitted to the Northern Regional Disease Diagnostic Laboratory (NRDDL) in Jalandhar for confirmation of the FMD antigen using a Sandwich-Enzyme Linked Immunosorbent Assay, which identified the circulating virus serotype [17]. The traditional polyclonal antibody-based sandwich ELISA was carried out as described [17]. Briefly, 96 well ELISA plates were coated with anti- FMDV/O specific rabbit polyclonal antibody and incubated for overnight at 4 °C. After washing test antigen was added and plates were incubated for 1 hour at 37 °C. After washing, 50 µl of guinea pig anti-FMDV/O polyclonal antibody diluted (1:10,000) in blocking buffer were added and plates were incubated at 37 °C for 1 hour. Plates were washed, anti-guinea pig HRP conjugate (DAKO) diluted (1:3000) in blocking buffer were added @50 µl per well

and incubated at 37 °C for 1 hour. After final washing, 50 µl of substrate solution containing orthophenylenediamine dihydrochloride (Sigma)/hydrogen peroxide was added and allowed to stand at 37 °C for 15 min for color development. The reaction was then stopped using 1 M H<sub>2</sub>SO<sub>4</sub>. The optical density (OD) values were measured at 492 nm using an ELISA plate reader. Samples showing OD values of 0.1 or more were judged as FMD positive in ELISA. The polyclonal antibody-based sandwich ELISA has been recommended by WOAHP as the preferred procedure for detection of FMD viral antigen and identification of viral serotypes.

Additionally, various other samples were collected from randomly selected sick animals, including blood samples and nasal swabs from live animals, as well as heart blood, lung tissue, and tissue samples from dead animals preserved in 10% formalin. Random fodder samples from the outbreak regions

## Research Article

were also collected for qualitative and quantitative nitrate/nitrite estimation to rule out other potential causes of mortality in the animals.

### 2.3. Post mortem examination

The post-mortem examination was carried out on animals in day light accordance with established procedure. Samples were collected in 10% neutral buffer formalin from affected organs. A team of experts from the Department of Pathology, Microbiology, and Medicine of the University along with, a veterinary officer from State Government's Animal Husbandry Department, examined the animal carcasses in detail to determine the cause of death

### 2.4. Laboratory examination

Representative blood samples from animals across different regions were collected for complete blood count (CBC) and parasitological examination to detect the presence of hemoparasites. Blood smears were prepared for this purpose, and pathogen detection was conducted through microscopic examination of these smears.

### 2.5. Histopathological examination

After thorough gross examination, small representative pieces (approximately 0.5 cm thickness) of various organs (lungs, heart, liver, spleen, intestine etc) were collected from animals and fixed in 10% neutral buffered formalin. After proper fixation for 3-4 days, tissues were cut into thinner sections (1-2 mm thick). The tissues were washed in running water for 7- 8 h, dehydrated in ascending grades of ethyl alcohol, cleared in benzene and embedded with paraffin wax. The paraffin blocks were prepared and the sections were cut at 4-5  $\mu$  thickness with a hand-operated microtome. The paraffin embedded sections were then passed through sequential steps of deparaffinisation in xylene, and rehydration through descending grades of ethyl alcohol to running water. Sections were stained by routine

hematoxylin and eosin stain [18]. Significant and consistent lesions in different organs were noticed.

## 3. Result

### 3.1. Hematological examination

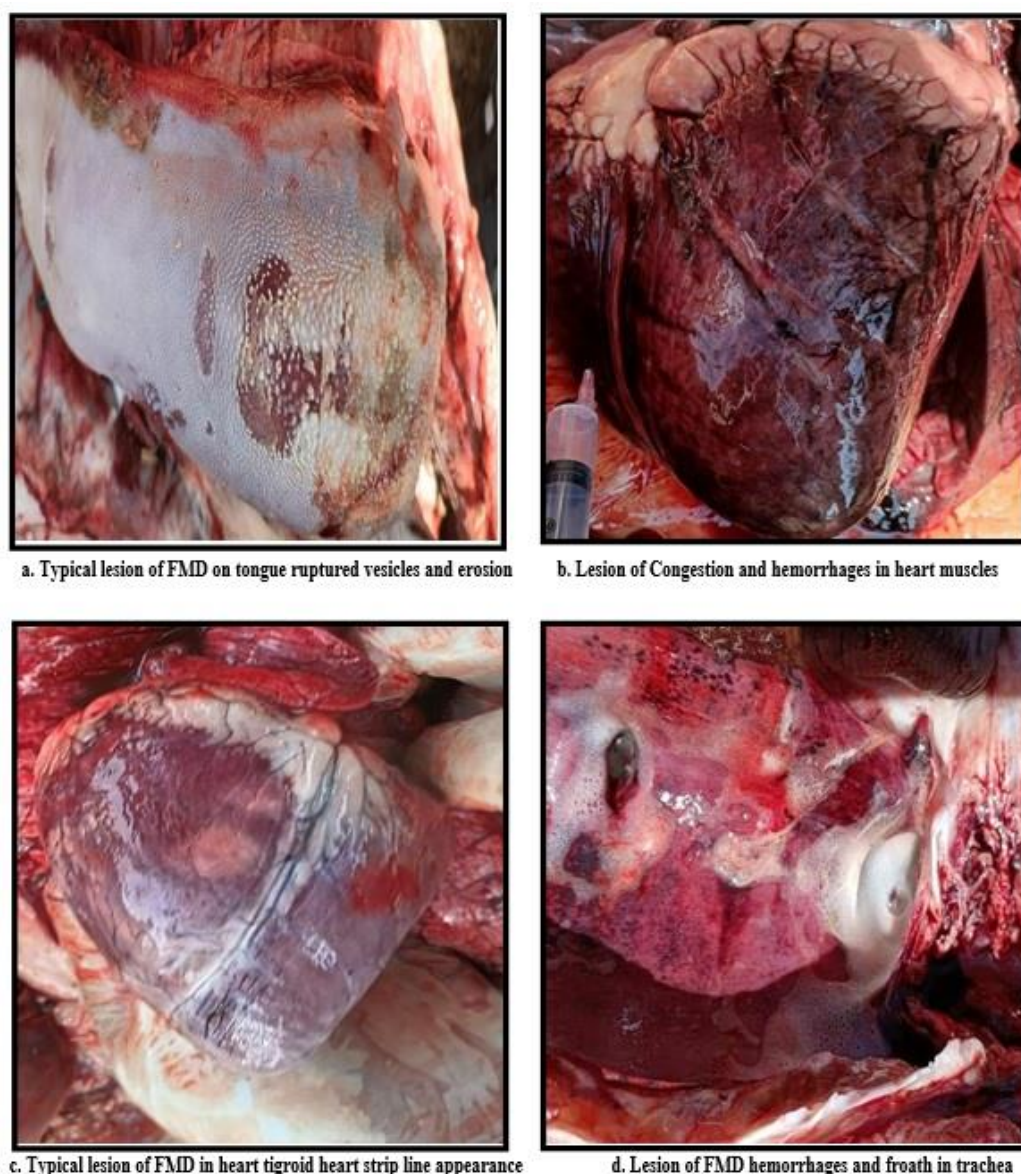
Hematological examination revealed absolute neutrophilia/leukocytosis with a mild to moderate left shift in 20 animals. Parasitological examination of blood smears showed the absence of hemoparasites. Blood smears, lack of enlarged lymph nodes, absence of anemia, and the absence of typical post-mortem findings of punched necrotic ulcers in the abomasum indicated that theileriosis, a tick-transmitted disease mainly affecting crossbred cattle, was not present. Theileriosis outbreaks usually occur during favorable conditions for the survival of the tick vector (*Hyalomma anatolicum*), such as summer and post-rainy hot humid conditions. However, the present outbreaks were reported during the extreme winter period.

### 3.2. Postmortem examination

Post-mortem examinations revealed specific findings consistent with FMD, such as white streaks on the heart (referred to as Tigroid Heart, characteristic of the cardiac form of FMD) mainly in young animals (Figure 2). The ventricular myocardium showing necrotic grey to white streaks of variable size and shape giving 'tigroid heart' appearance. In necropsy findings the heart muscles have tiger striped appearance (tigroid heart appearance) and severe hyaline degeneration and necrosis. This finding varied between outbreaks. As mentioned in the manuscript tigroid heart lesions were seen in young animals at necropsy. Additionally, there were epicardial hemorrhages, petechial hemorrhages on the spleen, hepatomegaly with rounded borders, hemorrhagic enteritis, and the presence of foamy froth in the trachea. The lungs were hemorrhagic and edematous with thickened interalveolar septa, indicative of concurrent septicemia with respiratory involvement.



## Research Article



**Figure 2.** Typical Post- mortem observation of FMD lesion (A-E) during outbreak investigation field visit in bovine species.

### 3.3. Feed and Fodder Analysis

Analysis of fodder samples for nitrate estimation revealed nitrate levels in all tested samples ranging from 522.864 to 956.88 ppm, which is well below the toxic limits (2500 ppm).

### 3.4. Laboratory Examination

The NRDDL Jalandhar confirmed the presence of FMD serotype O in all districts investigated (Barnala, Bathinda, Gurdaspur, Faridkot, and Mansa) using the Sandwich ELISA test, revealing the circulating FMD virus as serotype O. A total of 692 animals, including cattle and buffalo, were affected in these

districts, with a mortality rate of 24.42% (169/692) among young animals and heifers (Figure 3). The most severely affected districts were Bathinda, Barnala, and Faridkot, which experienced high mortality rates, leading to significant economic losses in these areas.

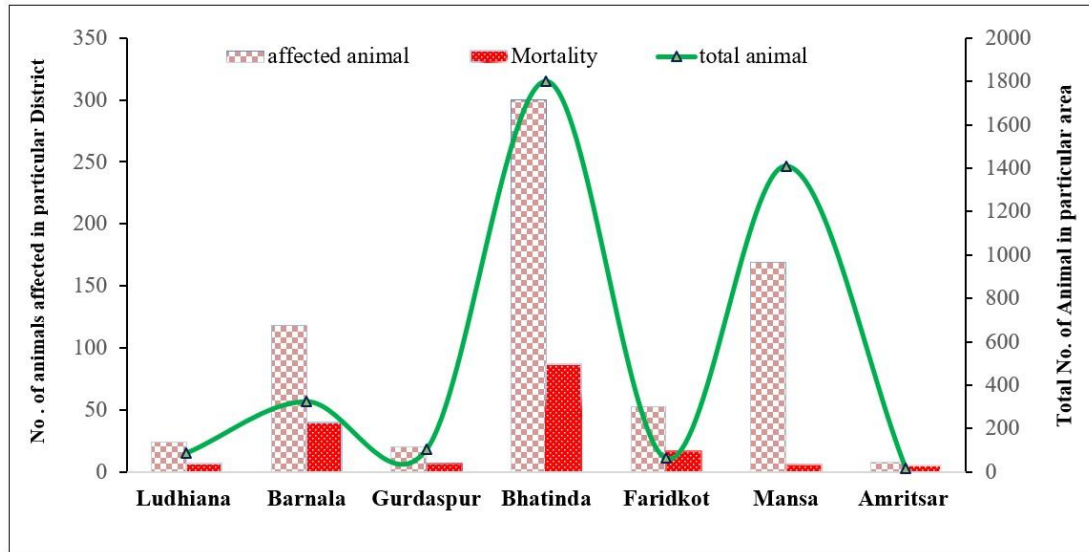
### 3.5. Histopathological Examination

Histopathological examination of tissue samples revealed muscle degeneration and diffuse lymphocytic infiltration, and lymphocytic myocarditis in the heart, which is characteristic of the cardiac form of FMD. The lungs showed hemorrhages,

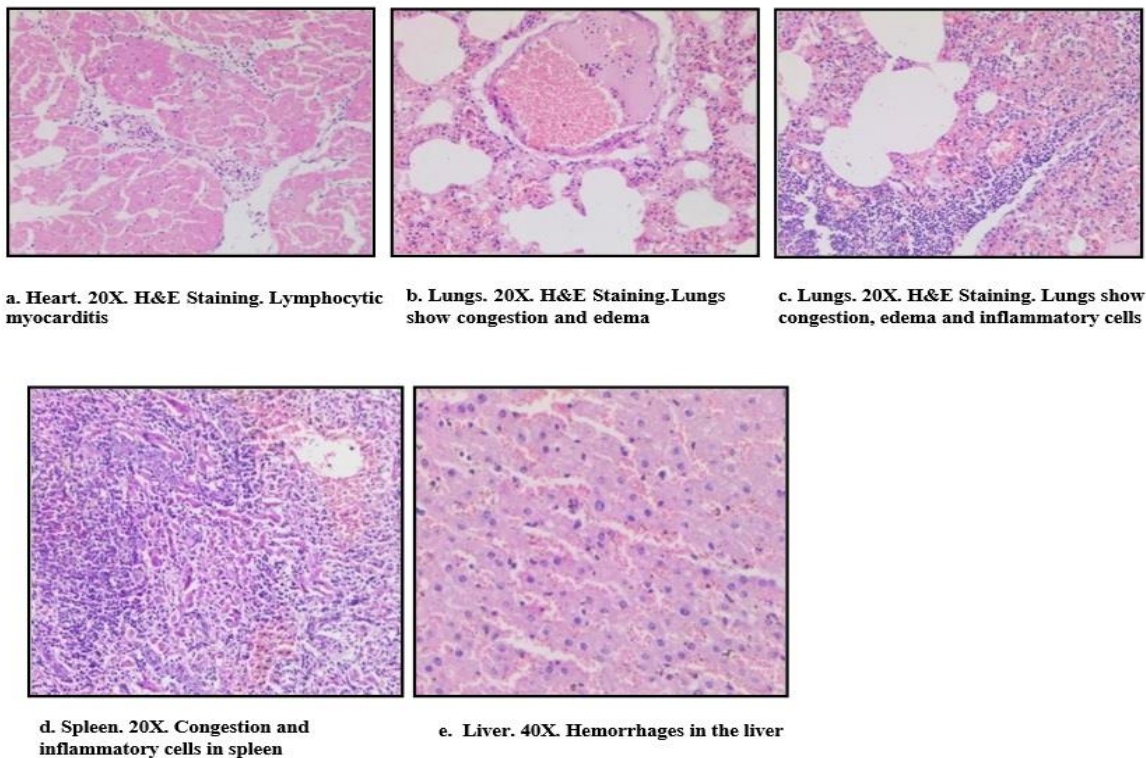
## Research Article

edema, congestion, emphysema, and thickening of interalveolar septa with mixed infiltration of neutrophils and lymphocytes (Figure 4). There was lymphoid tissue depletion in the spleen,

loss of epithelium, and focal lymphocytic infiltration in the intestine. The tongue showed loss of superficial epithelium and lymphocytic infiltration, along with sarcocysts in muscle tissue.



**Figure 3.** District wise case representation of FMD outbreak investigation in Punjab.



**Figure 4.** H&E stained section heart a, Lung b& c, spleen d, and Liver e, from FMD infected P.M. tissue of anima. Typical histopathological observation of FMD lesion (a-e) during outbreak investigation field visit.

## Research Article

### 4. Discussion

The foot and mouth disease (FMD) epidemic reduces the output of cattle, meat, wool, and other products [19]. The disease spillover due to interstate movement and trade of affected animals within the country and inadequate vaccination. The results of clinical research and study of the virulence of viruses will help with disease epidemiology and the adoption of effective methods of control. Diseased animals excrete large amount of virus through saliva, urine, nasal discharge, faeces, milk direct contact with diseased animal the virus may spread wind flow facilitates the spread of the virus to distant place, Mechanical spread of the virus can occur through farm equipments, utensils and workers [20]. This study examined FMD outbreaks in 2023–2024, focusing on clinical findings and the antigenicity of the serotype O virus. The reported FMD outbreaks led to reduced milk production and high mortality rates due to uncontrolled animal movement and inadequate vaccination. Laboratory diagnosis in this study confirmed serotype O FMDV in 13 outbreaks across affected districts. Serotype O is widely prevalent in India [20, 21]. The FMD outbreaks between 2011 and 2020 in northern India (Uttar Pradesh, Punjab, Haryana, Himachal Pradesh, Uttarakhand, Jammu & Kashmir, Ladakh, Delhi, and Chandigarh), primarily affected cattle and buffalo due to their overt clinical signs. FMDV serotype O was overwhelmingly dominant, detected in 97.5% of the positive samples, while serotype A accounted for only 2.5%, and serotype Asia1 was completely absent [4]. Further, the results of a two-dimensional viral neutralization test revealed that all field isolates were antigenically matched [22] to the currently used Indian vaccine strain O INDR2/1975. These results suggest that the serotype O vaccine strain can protect against outbreaks caused by all three circulating lineages.

The high morbidity and mortality observed in the current outbreaks, particularly in the Bathinda district of Malwa region were primarily due to the lack of recent vaccinations and the increased stress on animals caused by harsh winter conditions. Typically, there is higher mortality among calves during FMD

outbreaks due to the cardiac form of FMD [23, 24, 25], which was diagnosed in this investigation. However, in the present outbreaks, mortality was also observed in heifers and adults due to concurrent respiratory septicemic disease.

The results of this study will aid in understanding the disease's epidemiology and adopting effective control methods. In this investigation, ruminants such as cattle, buffalo, and calves exhibited FMD lesions and clinical symptoms [26]. The severity of the disease varied depending on host species, age, immunization status, and virus exposure level. In regions where FMD is under control, herd immunity has developed, leading to a significant decline in cases. The Department of Animal Husbandry and Dairying, part of the Ministry of Fisheries, Animal Husbandry, and Dairying of the Government of India, is responsible for implementing FMD control and other related programs [27]. Upgrading diagnostic labs, improving quarantine facilities, enhancing risk analysis processes, prioritizing research, creating databases, and adhering to WHO/FAO/WOAH standard operating procedures are crucial for effective implementation. Restricting animal movement and human activities during outbreaks will help minimize FMD spread. Limitation of study focus on tropism and circulating strain of FMD and limited to the geographic focus on only seven districts of Punjab. And molecular detection of FMD could not be carried out in present study.

### 5. Conclusion

A study conducted on an outbreak FMD in ruminant farms across seven districts of Punjab found that the outbreak was caused by the serotype O FMD virus. The clinical investigation from this study will be valuable for field veterinarians, helping them track and diagnose the disease promptly. This timely diagnosis is crucial for effective management, control, and biosecurity measures by the local animal health authorities. These measures aim to reduce the spread of the disease and protect animal health in the region.



## Research Article

### Animal Ethics

The study has been conducted under University animal ethical Reg. No.497/GO/Res/ReRcL/02/CPCSEA. No experimental work or the treatment has been carried out by the authors on the live experimental animals.

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### Conflict of Interest

All the authors declare no conflict of interests.

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## Research Article

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**Research Article**

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