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PRELIMILARY PHYTOCHEMICAL INVESTIGATION AND FREE RADICAL SCAVENGING OF POLYGONUM AVICULARE L. ETHANOLIC LEAVES EXTRACT

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ABSTRACT
In the present study the preliminary phytochemical content and anti-oxidant activity of *Polygonum aviculare L.* extract was determined. Phytochemical investigation and anti-oxidant activity of *P. aviculare* ethanolic plant leaves extract extract were carried out from the plant. The *P. aviculare* belong to the family *Polygonacea.* This plant is used in traditional medicine for a number of ailments. *P. aviculare* is a persistent plant with juicy, thick, and stout stem; they are pale or dark green to dark yellow. Leaves are small and cluster like. Rhizomes are formed under the soil; sends up shoots near the parent plant. The preliminary phytochemical screening of ethanolic plant leaves extract extract of *P. aviculare* have alkaloids, flavonoids, terpenoids, tannins and phenolic compound, ethanolic plant leaves extract extract of *P. aviculare* plant and exhibited antioxidant activity when subjected to the radical scavenging tests. The results (IC_{50}) for DPPH free radical scavenging assay was found to be (224.26µg/ml).

KEYWORDS: *Polygonum aviculare,* DPPH, Preliminary Phytochemical Investigation, Ascorbic acid, Anti-oxidants.

INTRODUCTION
General herbal medicine has been used since ancient time. All the living beings have the power to combat the disease, as the diseases are appeared and so the survival of diseases goes hand in hand with the appearance of the diseases. The plants and herbs have been extensively used as the remedy for the diseases by the human beings from decades. Due to its superior intelligence men have augmented the quest for the cure of their diseases and the oldest times human being mainly depends on the plant. They depend on plants for the need of food, clothing, shelter and traditional medicine to cure many types of diseases. Early humans recognized their dependence on nature for both health and wellness. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years.[1-2] The use of natural products as medicines must, of course, have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants, which led to vomiting, diarrhea, coma, or other toxic reactions-perhaps even death. However, in this way, early humans were able to develop knowledge about edible materials and natural medicines.[3] The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus.[4] In the twentieth century, much of the pharmacopeia of scientific medicines was derived from the traditional herbal knowledge of native people. People know about the knowledge of plant-based drugs development gradually and were passed on, thus laying the foundation for many systems of traditional medicine all over the world. Plants have been in continuous use in one way or the other for the treatment of various ailments as per the research though literature. Through many surveys a report had revealed that 50% of top prescription drugs in the USA are based on natural products and the raw materials are locked up in the topical world interiors of Africa, Asia, and Latin America. The local uses of medicinal plants are common particularly in those areas, which have little or no access to modern health services.

The WHO (World Health Organization) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. In India about 2000 drugs of natural origin are used and almost all of which are derived from different traditional systems (WHO, 2002). India is a unique country and proud to possess a well-documented and well established system of medicine which has been practised for centuries, flourishing from 2500 to 500 B.C. The Ayurvedic system of medicine has written Vedic texts dating back to 3,000

B.C. that deal extensively with the medicinal properties of plants. Two other systems of medicine, Siddha (developed and established in South India) and Unani (introduced by Mughals) are used in the country.[5] Through an estimate the world market for herbal medicine including herbal products and raw materials has been increased annually with a growth rate between 5-15%. Total global herbal drug market is estimated as US $ 62 billion and is expected to grow US $ 1 to 5 trillion by the year 2050. India is a great diversity of traditional knowledge and wisdom. Indian Ayurveda system contributes Rs 3500 crores (US $813 million) annually to the international market. Approximately about 7-15% growth rate were observed in Indian medicinal plants-based industry annually.

The value of medicinal plants-related trade in India is estimated about Rs. 5000 crores per annum. Global trend leading to increased demands of medicinal plants for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other products is an opportunity sector for Indian trade and commerce.[6]

Plant Drug Profile

Plant name: *Polygonum aviculare L.*

Figure 1- Polygonum aviculare.

Polygonal aviculare or common knotgrass is a plant related to buckwheat and dock. It is also called prostrate knotweed, bird weed, pigweed and low grass.

It is an annual found in fields and wasteland, with white flowers from June to October. It is widespread across many countries in temperate regions, apparently native to Eurasia and North America, naturalized in temperate parts of the Southern Hemisphere.[7-8]

Botanical Name: *Polygonum aviculare*

Family: *Polygonaceae* (Knotweed family); Synonyms: *Polygonum aviculare L.*

Medicinal uses: The whole flowering plant is used to make medicine. Knotweed is used for bronchitis, cough, gum disease (gingivitis), and sore mouth and throat. It is also used for lung diseases, skin disorders, and fluid retention. Some people use it to reduce sweating associated with tuberculosis and to stop bleeding.[9]

Research Methodology

Plant Selection and Collection of plant material: The Plant leave (*P. aviculare L)* were selected of the exhaustive literature survey and collected from nearby area Sonepat, (Haryana), India in the month of March 2021.

Authentication: The plant leaves of *Polygonum aviculare L.* leaves was authenticated by a senior Botanist Head of Department of Botany, Singhania University, Pacheri Bari, Jhunjhunu (Raj.), India.

Preparation of plant material for study: The plant leaves of *Polygonum aviculare L.* leaves was authenticated by a senior Botanist Head of Department of Botany, Singhania University, Pacheri Bari, Jhunjhunu (Raj.), India.

Plant *P. aviculare L.* was selected for the study. The ethanolic plant leaves extract were collected, washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were ground using the electronic grinder. The powder was observed for its colour, odour, taste and texture**.** Dried plant material was filled in an air tight container till further use.

Preparation of Extraction: The Plant leaves of *Polygonum aviculare L.* was collected reduced to small size after dried in shade for one month or till dry and crushed to form coarse powder. The powdered drug (250 gm) was subjected to continuous hot extraction with the help of soxhlet apparatus using a solvent ethanol. The plant material was dry in hot air oven at 50^0C for an hour. After the effective extraction, the solvent will distilled off, the extract will then concentrated on water bath to become dried. The obtained extract was weighing and stored in an air tight container. [Lin et al.,].

Phytochemical Investigation[10-11]

A solution of an extract of *P. aviculare L.* were prepared separately in ethanolic and subjected to various qualitative tests for identification in plant constituents.

Test for Carbohydrates

- **Molish Test:** 2 ml of extract was taken, treated with two drops of alcoholic α-naphthol solution in a test tube and then 1 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction might indicate the presence of carbohydrates.
- **Benedict's test:** Equal volume of Benedict's reagent and *P. aviculare L.* extract were mixed in a test tube and heated on the water bath for 5-10 minutes. Solution appeared green, yellow or red which might indicate the presence of reducing sugars.
- **Fehling's Test:** To 1 ml of *P. aviculare L.* extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated on water bath for 10 minutes. Formation of red precipitate might indicate the presence of reducing sugar.
- **Barfoad Test:** 1 ml of *P. aviculare L.* extract and 1 ml barfoad reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to the formation of cupric oxide might indicate the presence of monosaccharide.

Test for Proteins and Amino acids

- **Biuret's Test:** The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour might indicate the presence of proteins.
- **Million's test:** 3ml of extract was mixed with 5ml of million's reagent. White precipitate formed which on heating turned brick red, might be indicating the presence of proteins.
- **Ninhydrin test:** Take 3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour might indicate the presence of amino acids.

Tests for Glycosides

- **Borntrager's Test:** To 3ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red colour in ammonical layer might indicate the presence of anthraquinone glycosides.
- **Legal's test:** 1ml of test solution was dissolved in pyridine. 1ml of nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour might indicate the presence of cardiac glycosides.
- **Keller-Killiani test:** To 2ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5ml of concentrated sulphuric acid was added carefully by the side of the test tube. Formation of blue colour in the acetic acid layer might indicate the presence of cardiac glycosides.

Test for Alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, following tests were performed.

- **Mayer's test:** To 2-3 ml of filtrate, few drops of Mayer's reagent were added along the side of the test tube. Formation of white or creamy precipate might indicate the presence of alkaloids.
- **Wagner's test:** To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate might indicate the presence of alkaloids.
- **Dragendroff's test:** To 1-2ml of filtrate, few drops of Dragendroff's reagent were added in a test tube. Formation of red precipitate might indicate the presence of alkaloids.
- **Hager's Test:** To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate might indicate the presence of alkaloids.

Test for Flavonoids

- **Lead acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate might indicate the presence of flavonoids.
- **Shinoda test:** To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink colour might indicate the presence of flavonoids.
- **Alkaline Reagent Test:** The extract was treated with few drops of sodium hydroxide separately in a test tube. Formations of intense yellow colour, which become colour lesson addition of few drops of dilute acid, might indicate the presence of flavonoids.

Tests for Tannin and Phenolic compounds

- **Ferric chloride test:** Some amount of extract was dissolved in distilled water. To this solution 2ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour might indicate the presence of phenolic compounds.
- **Lead Acetate Test:** Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate might indicate the presence of phenolic compounds.
- **Iodine Test:** To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color might indicate the presence of phenolic compounds.

- **Gelatin Test:** Some quantity of extract was dissolved in distilled water. To this solution 2ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate might indicate the presence of phenolic compounds.

In-Vitro Antioxidant Evaluation

DPPH free Radical Scavenging Assay: The DPPH assay of ethanolic plant leaves extract was determined by according **(Pin Der Duh *et.* At., 1995)**[12-16]

Preparation of Standard Ascorbic acid solutions: Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100 µg/ml). 200 µM solution of DPPH (in methanol) was prepared and 1.5ml of this solution was added to 1.5 ml of a methanolic ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

Preparation of Test solution: Various solutions of leaves extract were prepared in 90% methanol to obtain different concentrations (10-100 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5 ml of an ethanolic plant leaves extract solution of different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

Preparation of Control solution: For control, 1.5 ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. The absorbance of the control was taken after 30min against methanol (as blank) at 517 nm.

The antioxidant activity of ethanolic plant leaves extract and ascorbic acid were calculated by using the following formula in terms of % inhibition:

$$\% \text{ Inhibition} = \frac{\text{Ac } 230 - \text{At } 230\text{nm}}{\text{Ac } 230\text{nm}} \times 100$$

Where
Ac = Absorbance of control,
At =Absorbance of ascorbic acid / leaves extract.

Statistical Analysis: The data of results obtained were subjected to statistical analysis and expressed as regression curve and % Inhibition curve value with help of EXCEL. The data were statically analyzed by Graph pad prism Software version (7.1).

RESULT

The phytochemical screening of ethanolic plant leaves extract exhibited that the main components i.e. alkaloids, flavonoids, terpenoids, tannins and phenolic compound, were present in the extract **(Table 1).**

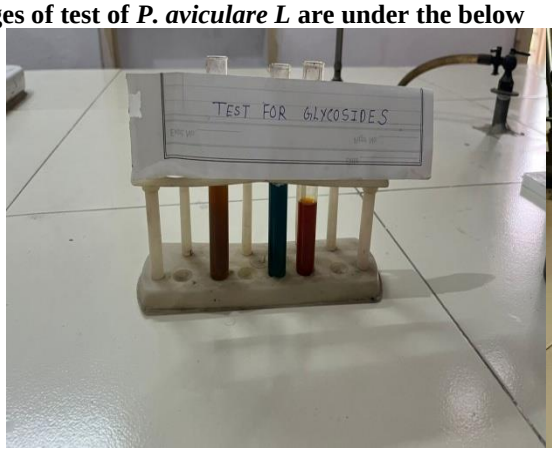
Table 1: Phytochemical screening of ethanolic plant leaves extract P. aviculare L.

S.No	Phytochemical	Test	*P. aviculare L.*
1.	Alkaloids	Mayer's test	+
		Wagner's	+
		Hager's	-
		Dragendroff' s	+
2.	Flavonoids	Lead acetate test	+
		Alkaline reagent test	-
		Shinoda test	-
3.	Glycosides	Killer killians test	+
		Legal's	+
		Bortrager's test	-
4.	Tannins & phenolic compounds	Ferric chloride test	+
		Lead acetate test	-
		Dilute Iodine test	+
		Gelatine test	-
5.	Carbohydrate test	Molish test	+
		Benedict's test	+
		Fehling's reagent test	+
6.	Amino acid proteins	Biuret' s test	-
		Ninhydrin test	+

"+" indicating presence of compound, "–" indicating absence of compounds

Phytochemical screening of crude extract of *P. aviculare L.* ethanolic plant leaves extract was found that amino protein, carbohydrate, tannin, glycosides, flavonoids, alkaloids.

All images of test of *P. aviculare L* are under the below



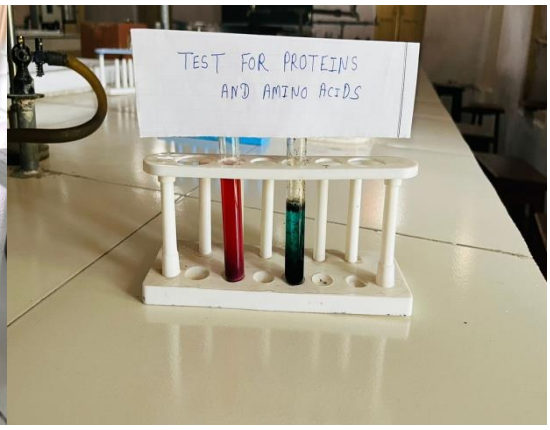
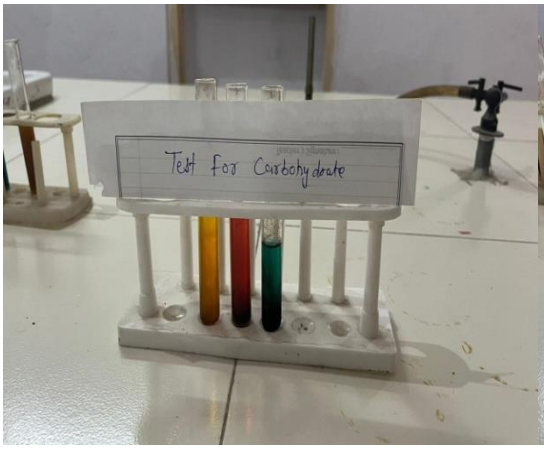


Figure- 2 Tests for glycosides Figure -3 Tests for proteins and amino acids.



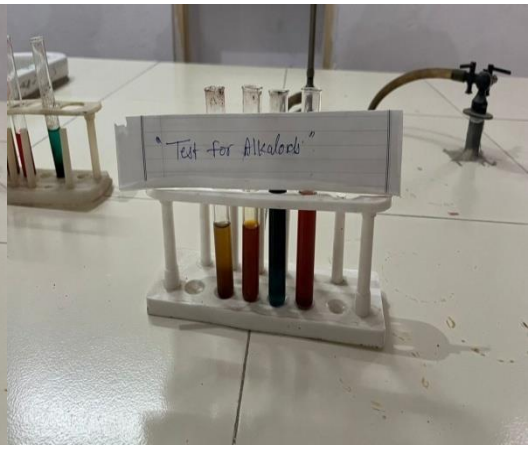


Figure -4 Tests for carbohydrates Figure -5 Tests for alkaloids.

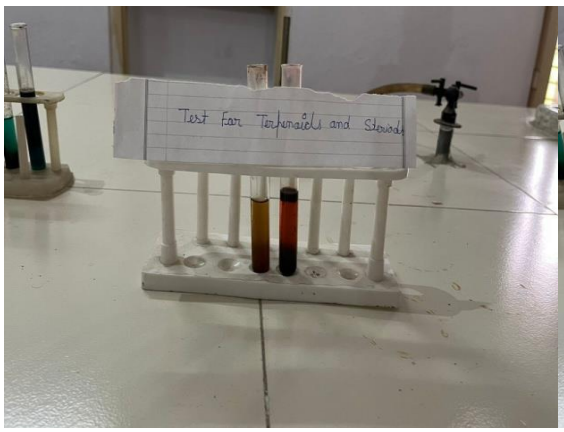


Figure -6 Tests for flavonoids Figure -7 Tests for terpenoids and steroids

In-Vitro Antioxidant Assay: Antioxidant activity was assessed on the basis of one respective assay methods i.e. DPPH radical scavenging, scavenging activity (Table 2, 3) and (Table 4).

DPPH Free Radical Scavenging Assay: DPPH (1, 1 diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activity in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule. DPPH assay for ethanolic plant leaves extract extract of *P. aviculare* was performed by using ascorbic acid solution as standard. The absorbance data were recorded against the selected concentrations (20-100 µg/ml) for ascorbic acid and (20- 100µg/ml) for ethanolic plant leaves extract of *P. aviculare* at 517nm. The percentage (%) inhibition curves for DPPH free radical scavenging assay of ascorbic acid and ethanolic plant leaves extract were plotted from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and ethanolic plant leaves extract were calculated using regression equation.

Table 2: Percentage (%) inhibition data for DPPH assay of ascorbic acid.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.780	0.634	18.71
2	40		0.601	22.94
3	60		0.579	25.76
4	80		0.556	28.71
5	100		0.539	30.89

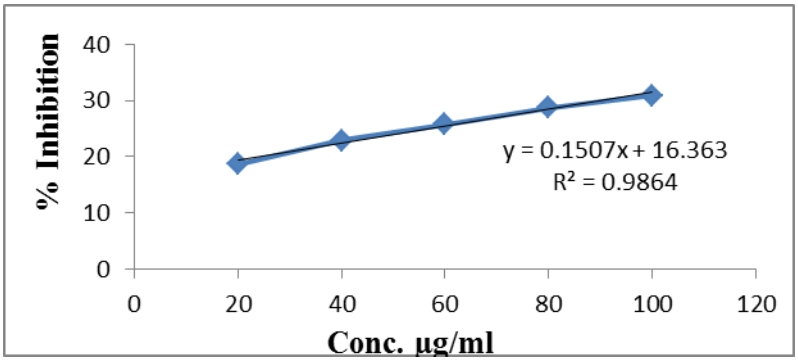


Figure 8: % inhibition data for DPPH assay curve of ascorbic acid.

Table 3: Percentage (%) inhibition data for DPPH assay of *Polygonum aviculare L* ethanolic plant leaves extract.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.780	0.705	09.61
2	40		0.670	14.10
3	60		0.651	16.53
4	80		0.634	18.71
5	100		0.610	21.79

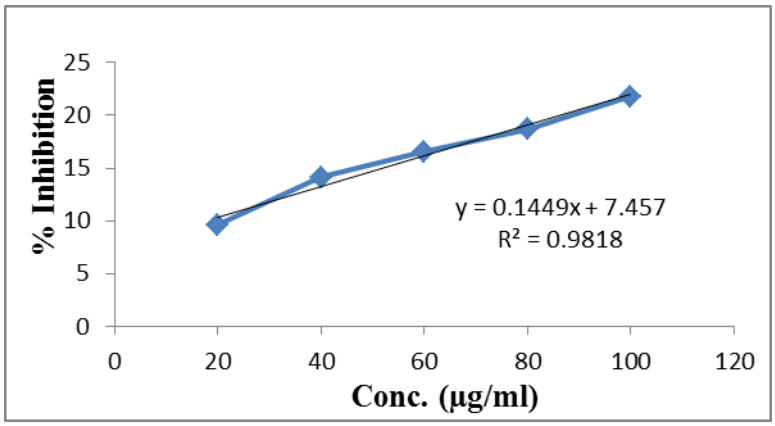


Figure 9: % Inhibition data for DPPH assay curve of *Polygonum aviculare L*. ethanolic plant leaves extract.

IC_{50} value was calculated by using straight-line equations. In DPPH scavenging assay, it was observed that extract served as a good scavenger of DPPH in the concentration range of 20-100µg/ml. IC_{50} for ascorbic acid was found to be 224.26 µg/ml while that for it was found to plant leaves extract of *P. aviculare* be 295.43 µg/ml.

Table 4: IC_{50} value and Statistical analysis of ascorbic acid and plant leaves extract of *P. aviculare L.*

Sample	IC_{50} Value	Equation	R^2 value	F value	Dfn and Dfd	P value
Ascorbic acid	224.26	Y=0.150x+16.36	R^2=0.986	758.2	1,3	<0.0001
Plant leaves extract	295.43	Y=0.144x+7.457	R^2=0.981	1055.	1,3	<0.0001

DISCUSSION

Preliminary phytochemical screening showed the presence of amino protein, carbohydrate, tannin, glycosides, flavonoids, alkaloids compounds. Moreover, recent studies suggests that DPPH is a strong oxidizing agent and can inactive few enzymes directly, usually by oxidation of essential thiol (-SH) groups. DPPH can cross cell membrane rapidly. Once entered the cell,

DPPH can react with Fe+2/Cu2+ to form hydroxyl radical leading to the origin of many of its toxic effects. The study concluded that plant leaves extract of *P. aviculare* exerted higher potential even better than ascorbic towards the DPPH radical scavenging activity.

CONCLUSION

The present was concluded that Primilary phytochemical investigation of *P. aviculare L* ethanolic plant leave extract. We hypothesized that plant leaves extract may help in investigation of Primilary phytochemical & antioxidant potential.

The study concluded that the presence of flavonoids, carbohydrates, triterpenoids & steroids, tannin & phenols, glycosides and alkaloids in *P. aviculare* and also conluded that better antioxidant potential.

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AUTHOR CONTRIBUTION STATEMENT

Mr. Avinash Joriya conceptualized and gathered the data with regard to this work. Dr. Anand Singh analyzed these data and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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