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THERAPEUTIC EFFECT OF *TAMARINDUS INDICA* EXTRACTS ON THE PATHOGENESIS OF *ENTAMOEBA HISTOLYTICA IN-VIVO*

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Keywords:

Entamoeba histolytica, *Tamarindus indica*, Histopathology, Toxicity, *In-vivo*

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ABSTRACT: *Entamoeba histolytic* is a protozoan parasite which causes amoebiasis, about 50 million people are affected yearly, and it caused more than 100,000 deaths per year. The study investigates the therapeutic effect of ethanolic and aqueous *Tamarindus indica* leaves extracts on infected rats with *Entamoeba histolytica*. Twenty-one *Rattus norvegicus* rats were infected with *Entamoeba histolytica* and divided into seven groups (three rats in each group). Groups (1, 2, and 3) were treated with aqueous *Tamarindus indica* extracts at doses 125, 250, and 500 mg/kg respectively for ten days. Groups (4, 5 and 6) were treated with ethanolic extract of *Tamarindus indica* at doses 125, 250, and 500 mg/kg respectively for ten days. Group (7) was left untreated as a positive control group. The results showed a significant decrease at $p \leq 0.05$ in the numbers of parasite cell from the second day of treatment in all the groups treated with aqueous as well as ethanolic extract. This decrease gradually continued during the days of treatment until it reached zero on the last day of treatment for the dose of 500 mg/kg for both aqueous and ethanol extract. Histopathological study showed that *Entamoeba histolytica* causes mucosal damage, inflammation and necrosis for colon, while ethanolic extract of *Tamarindus indica* at dose 500 mg/kg repairs the parasite damage in comparison with a range of positive and negative control groups. Extracts of *Tamarindus indica* leaves indicated neither any toxic effect nor mortality up to the 1000 mg/kg.

INTRODUCTION: Approximately 50 million of the world populations are infected by *E. histolytica* and the reach a prevalence of 50% of the general population and it causes more than 100,000 deaths annually [1]. Metronidazole is used for the treatment of *E. histolytica* infection; this drug is resisted by drug resistance from *E. histolytica*, [2] however, this drug has some unwanted side effects such as headaches, metallic taste, nausea, vomiting as well as neurotoxicity, this makes it unpleasant to be taken by patients [3].

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E. histolytic has registered some levels of resistance to most of the medicines rendering them ineffective [4]. In addition to that, the effective and safer antiprotozoal agents are urgently required [3]. In developing countries, plants are considered as popular because their products are safe and widely available at low cost [5]. This necessitates a search for a safer and effective alternative against *E. histolytica*. This study was performed to evaluate the efficacy of *T. indica* extracts against *E. histolytica* in rats and the study of histological changes after 10 days of treatment.

MATERIALS AND METHODS:

Collection and Identification of Plant: The leaves of *T. indica* were collected from the campus of Dr. Rafiq Zakaria College for Women. This plant was authenticated by herbarium of the Botany Department, Azad College, University of Dr.

Babasaheb Ambedkar Marathwada, Aurangabad. The collected plant leaves were washed thoroughly with water to remove the adhering soil, dust, and debris. The leaves were dried in the shade at room temperature, then ground into powder. The powder was stored in an airtight container to protect the powder from light and air.

Preparation of Extracts:

Preparation of Ethanolic Extract: Harborne method [6] was used to prepare ethanol extract by weighing 40 grams of dry plant powder, and 400 ml of ethanol were placed in extraction thimbles which were placed in a chamber of the Soxhlet apparatus at a temperature of 50-55 °C and extraction was continuous and carried out until the color disappeared. Then, the extract was transformed into Rotary evaporator. It was evaporated in the oven at 60 °C until it was, and the extract was weighted.

Preparation of Aqueous Extract: *T. indica* leaves were blended with water at a ratio of 1:10 (*T. indica* leaves: distilled water). The mixture was put on a magnetic stirrer for 24 h. It was first filtered with four layers of gauze cloth through a Buchner funnel, and then centrifuged at 3500 rpm for 10 min, the supernatant was put in the oven at 60 °C for drying. The dried mass was weighted.

Determination of Lethal Dose (LD_{50}): LD_{50} of the plant extract was determined by the method [7] using thirty-three rats (*Rattus norvegicus*) weighing between 200-220g. The first phase, 15 rats were divided into five groups (each group contains three rats) treated with aqueous extract at doses of 125, 250, 500, 750 and 1000 mg/kg body weight. At the second phase, 15 rats were divided into five groups each group was treated with ethanol extract at doses of 125, 250, 500, 750, and 1000 mg/kg body weight. Also, three rats are kept as a negative control group. To determine the value LD_{50} of aqueous and ethanolic extracts of plant *T. indica* orally and was observed during 24 h and mortality, according to law following:

$$LD_{50} = \text{Highest dosage} - \sum (a \times b) / n$$

Highest lethal dosage = Lethal dose causing the 100% death of all test rats

a = Difference between two successive doses of the administered extract.

b = Average number of dead rats in two successive doses.

n = Total number of rats in groups.

Stool Samples: Samples were collected from patients infected with *E. histolytica* from public Hospitals.

Stool Examination:

Macroscopic Examination: The stool samples were examined with naked eyes before the microscopical examination for, color, consistent and bloody mucus.

Microscopic Examination: All stool samples were examined microscopically by direct wet mount method with normal saline 0.85% and Lugol's Iodine stain under high power (10x) and (40x) for detection of the trophozoite and cyst stage of *E. histolytica* [8].

Experimental Animals: Twenty-four albino rats (*Rattus norvegicus*) of both sexes male and female (3-3.5 months old) weighing (200-220g), were used for the experiment. Their stool was examined before the beginning of the experiment to make sure that the rats were free from any intestinal parasites. The rats were maintained under proper environmental conditions, *i.e*., temperature 25 ± 2 ºC and humidity 50 ± 5% with a 12 h light and dark period. They were housed in polypropylene shoebox type cages with stainless steel grill top, bedded without saw dust. The rats were provided with pelleted diet and water. Twenty-one of them were infected by oral administration (17×10^3 cell/ml) of *E. histolytica* obtained from the stool. Three rats were kept at the same environmental conditions as the negative control.

After 7-10 days, the feces of each rat was examined. All the infected rats kept in a separate cage and divided into seven groups (three rats in each group). Groups (1, 2 and 3) were administered with aqueous *T. indica* extract at doses 125, 250 and 500 mg/kg respectively for ten days, while groups (4, 5 and 6) were administered with ethanolic *T. indica* extract at doses 125, 250 and 500 mg/kg respectively for ten days. The group (7) was infected with the parasite, untreated with plant extracts, and considered as the positive control, in addition to that negative control group which was non-infected with the parasite and untreated with

plant extract. The feces of all rats from the first day to last day of treatment were collected and examined daily by light microscopy. After the screening of the slides, the number of *E. histolytica* was counted by using hemocytometer. The effect of ethanolic and aqueous *T. indica* extract was determined through parasites account number (by eosin stain) to obtain the number of parasite per gram of feces. It was calculated according to the following formula: [9]

$$N = S / (Vol \times Wt)$$

N: Number of the parasite in 1g of feces
S: Counted number of the parasite in a hemocytometer
Vol: Used volume of quantity (0.01ml)
Wt: Used weight of stool sample (1g)

At the end of the experiment, rats were sacrificed, and the colon was removed from each rat, fixed in formalin 10% for histopathological study [10].

Statistical Analysis: The results of the present study were analyzed by GenStat 5.2 by using general treatment structure (no blocking), factorial experiment, with 3 replications. The data were expressed as the mean deviation. Least significant different test (LSD) was used to test the difference between means (groups) at P≤0.05 and was considered significant.

RESULTS:

Effect of Ethanolic and Aqueous Extracts of *T. indica* on *E. histolytica in-vivo*: Effect of ethanolic and aqueous extracts of *T. indica* in the infected rats shown in **Table 1**. At the onset of infection, it was found that the rates of parasite number in feces of infected groups were close to each other, which reached between 2100 to 2250 parasite/g feces. The control group has increased the rates of parasite number within eight days, and then the rate of parasite numbers started to decrease from the 8th day.

A significant decrease is observed at p<0.05 in the numbers of parasite cell from the second day of treatment in all treated groups for both ethanolic and aqueous of *T. indica* extract, this decrease continued during the days of treatment until it reached zero on the last day of treatment at the dose of 500 mg/kg for both ethanolic and aqueous of *T. indica* extract. While at the last day of treatment the number of *E. histolytica* was reduced in the rats treated with the doses 250 and 125 mg/kg of both ethanolic and aqueous extract comparing to the very less number at the dose of 500 mg/kg.

The obtained results of the treated groups shown significant decreases in the number of the *E. histolytica* in days during the treatment period at the P-value of p≤0.05. While at P-value of p>0.05, the results have shown no significant differences were observed. The interaction between time and doses has a significant effect (P≤0.05) on the decreased of *E. histolytica* number *in-vivo*. At the dose 500 mg/kg of body weight, the number of *E. histolytica* in the feces was decreased down to zero at the end of the treatment. So, it was observed that the higher dose of the extract decreased the number of the *E. histolytica* more than the lower doses of both ethanolic and aqueous extract.

TABLE 1: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACT OF *T. INDICA* PLANT ON *E. HISTOLYTICA IN-VIVO*

Extract	Dose	Experimental Period (Days) (Number of *E. Histolytica* in 1 g × 10^3)										
		1	2	3	4	5	6	7	8	9	10	Means
Ethanolic	Control	2.100	2.100	2.200	2.390	2.777	2.877	3.577	3.600	2.800	2.590	2.701
	500 mg/kg	2.217	1.800	1.800	1.033	0.417	0.400	0.267	0.100	0.133	0.00	0.780
	250 mg/kg	2.317	2.083	2.083	1.683	1.533	1.207	1.167	1.100	0.833	0.517	1.434
	125 mg/kg	2.200	2.000	2.000	1.567	1.433	1.260	1.283	1.267	1.133	1.083	1.506
Means		2.208	1.996	1.842	1.668	1.540	1.436	1.573	1.517	1.225	1.047	1.605
Aqueous	Control	2.100	2.100	2.200	2.390	2.777	2.877	3.577	3.600	2.800	2.590	2.701
	500 mg/kg	2.117	1.800	1.633	0.967	0.550	0.467	0.433	0.217	0.217	0.00	0.840
	250 mg/kg	2.300	2.183	1.817	1.433	1.133	1.207	0.900	0.950	0.900	0.850	1.367
	125 mg/kg	2.117	2.167	1.933	1.733	1.400	1.367	1.200	1.033	1.050	1.117	1.512
Means		2.158	2.063	1.896	1.631	1.465	1.479	1.527	1.450	1.242	1.139	1.605
Average in habitation capacity of extracts	Control	2.100	2.100	2.200	2.390	2.777	2.877	3.577	3.600	2.800	2.590	2.701
	500 mg/kg	2.167	1.800	1.533	1.000	0.483	0.433	0.350	0.158	0.175	0.00	0.810
	250 mg/kg	2.308	2.133	1.858	1.558	1.333	1.333	1.033	1.025	0.867	0.683	1.401
	125 mg/kg	2.158	2.033	1.883	1.650	1.417	1.417	1.242	1.150	1.092	1.100	1.506
Means of Days		2.183	2.029	1.869	1.650	1.502	1.457	1.550	1.483	1.233	1.093	
LSD 5%		Extracts (E) = 0.0555, Dose (D) = 0.0785, Days (d) = 0.1242, D × d = 0.2483										

Acute Toxicity Study (LD_{50}): Administration of the *T. indica* extract in both treated groups showed no physical changes in their appearances and signs of toxicity at 125, 250, 500, 750 and 1000 mg/kg body weight of both ethanolic and aqueous extracts of *T. indica***.**

Histopathological Examination: The histological study showed that the *T. indica* extracts were able to repair the structure of colon tissue of the infected rats with *E. histolytica* when it was treated with extract compared with negative control and positive control. In the negative control rats, the colon tissues showed within the normal limit, no villus atrophy, no necrosis, nohyperplasia, and no inflammatory cells **Fig. 1**. In contrast, the colon tissue of untreated rats (positive control) shown shortening, atrophy, and villi fusion and desquamation of most villi with necrosis of some enterocytes in the colon tissue **Fig. 2**.

FIG. 1: HISTOPATHOLOGICAL SECTION OF COLON IN THE RAT OF NEGATIVE CONTROL

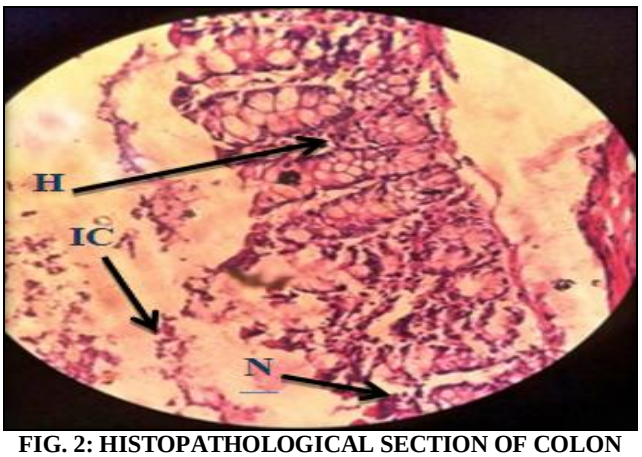
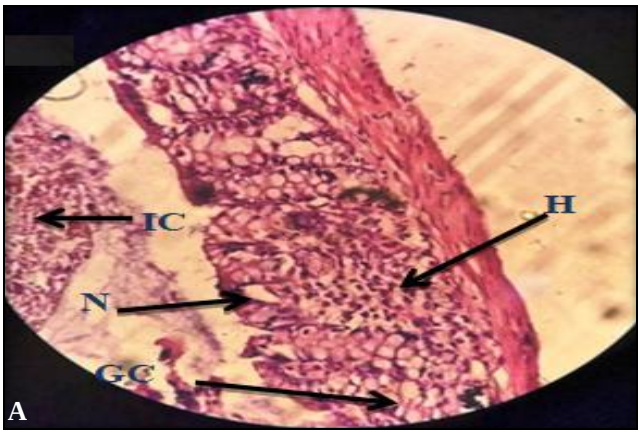
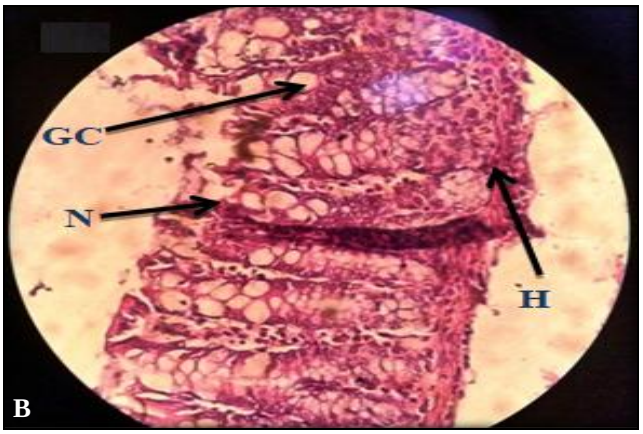


FIG. 2: HISTOPATHOLOGICAL SECTION OF COLON IN THE RAT OF POSITIVE CONTROL

The colon of rats treated with ethanol extract at a dose of 125 mg kg-day showed necrosis and sloughing of the upper portion of the villi and inflammatory cellular infiltration in the mucosa and hyperplasia **Fig. 3a**. However, rats administered for 10 days with ethanol extract at a dose of 250 mg kg-day had less intestinal damage or mildly inflamed villi after treatment **Fig. 3b**, while treated rats with ethanol extract of *T. indica* at a dose of 500 mg/kg-day had colon structures identical for the negative controls, with normal villous and decrease in inflammatory cells **Fig. 3c**.

In rats administered for 10 days with aqueous extract at a dose of 125 mg/kg-day demonstrated show degeneration and shortening of intestinal villi, goblet cell, and necrosis and hyperplasia **Fig. 3d**, in rats treated with aqueous extract for 10 days at a dose of 250 mg/kg-day showed necrosis in the mucosa, hyperplasia of goblet cells and glandular hyperplasia **Fig. 3e**, while the rats of treated by aqueous extract for the same period at a dose of 500 mg/kg-day show still necrosis in the mucosa, showing inflammatory cells inside the villi and increase in the thickening of the mucosa **Fig. 3f**.





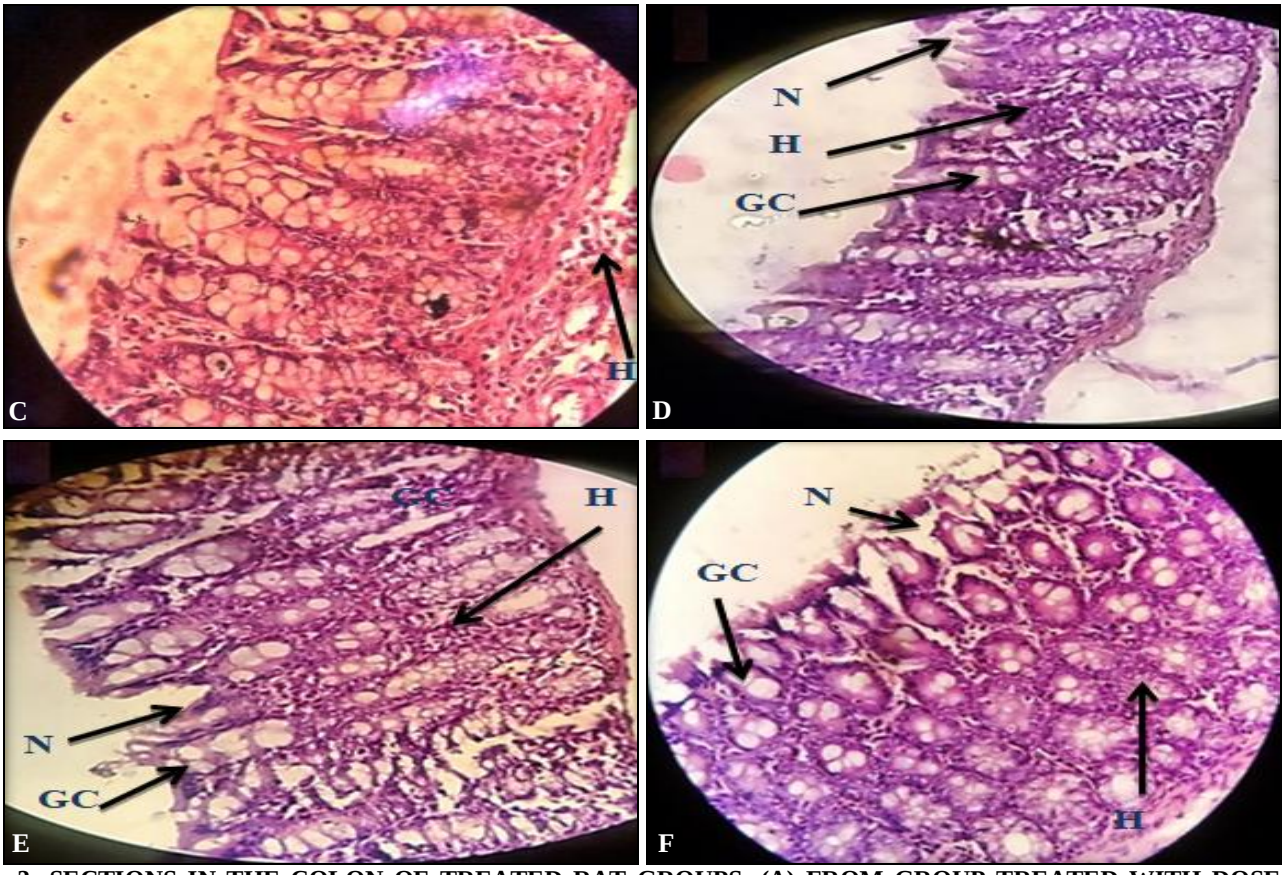


FIG. 3: SECTIONS IN THE COLON OF TREATED RAT GROUPS, (A) FROM GROUP TREATED WITH DOSE 125 mg/kg OF ETHANOL EXTRACT, (B) FROM GROUP TREATED WITH DOSE 250 mg/kg OF ETHANOL EXTRACT, (C) FROM GROUP TREATED WITH DOSE 500 mg/kg OF ETHANOLIC EXTRACT, (D) FROM GROUP TREATED WITH DOSE 125 mg/kg OF AQUEOUS EXTRACT, (E) FROM GROUP TREATED WITH DOSE 250 mg/kg OF AQUEOUS EXTRACT AND (F) FROM GROUP TREATED WITH DOSE 500 mg/kg OF AQUEOUS EXTRACT
Whereas; N=necrosis, H=hyperplasia, IC=inflammatory cells, GC=goblet cells

DISCUSSION: Due to the resistance of *E. histolytica* strain against the metronidazole, there is a great need for the development of new, effective, and safe medication for the treatment of amoebiasis. It was clear from this study that the extract of *T. indica* had reduced the numbers of *E. histolytica* increased proportionally with the increase of the doses, it was the highest when dose 500 mg/kg used **Table 1**. In contrast, the infected control rats increase in *E. histolytica* numbers. The inhibition activity by ethanolic and aqueous *T. indica* extract against *E. histolytica* could be a result of the phytochemicals present in *T. indica. T. indica* contains active constituents such as alkaloids, phenols, tannins, flavonoids, glycosides and resins [11].

These compounds eliminate *E. histolytica* parasite inside the large intestine and therefore these substances inhibiting *E. histolytica* parasite and preventing it from dividing and multiplying within the large intestine cavities. Thus, active constituent present in the *T. indica* stops parasite from causing pathogenicity and severe histological changes in the large intestine cavities, especially in the colon. Where alkaloids break down the cell membrane of *E. histolytica* which causes to the exodus of the cell contents (proteins and fats), as well as interfere with DNA of *E. histolytica* and lead to the death of *E. histolyttica* [12]. While tannins are inhibiting the enzymes and transport proteins that are located on the cell membrane [13]. Also, tannins and phenols precipitate proteins through the formation of hydrogen bonds between the groups of hydroxyl phenols, nitrogen compounds, and proteins, leading to inhibiting of enzymes in the parasite. The *T. indica* extract contains high levels of oxalic acid, ascorbic acid and, particularly, a tartaric acid, [14] which creates an unsuitable environment for the growth of *E. histolytica*.

T. indica had pronounced effect on other parasites, fungi and bacteria. Also, the results were consistent with the study effect of *T. indica* extract on

tapeworm (*Taenia solium*), [15] also, study the effect of *T. indica* extract as antimalarial (*Plasmodium*) [16]. It was showed *T. indica* extracts have antidiarrheal activity, especially diarrhea caused by rotavirus [17]. The report of *T. indica* extract has an effect on many fungi [18]. The earlier work of showed the inhibitory effect of *T. indica* in the growth of some bacteria species causing diarrhea [19]. The literate survey showed that *T. indica* extract has activity against negative and positive gram bacteria strains, [20] ethanolic, and methanolic extracts were effective against *S. paratyphi* and *P. aeruginosa* [21].

The histopathological results for effect *E. histolytica* parasite on the colon mucosa of the positive control group agree with several reports [22]. The interaction between *E. histolytica* parasite and the colon epithelium was responsible for the villus alterations, and necrosis and inflammatory cells are due to toxins of *E. histolytica* that damage of colon mucosa [23].

Histopathological examination of the colon revealed a moderate increase in the number of goblet cell and thickening of the mucosa of the colon when administered with *T. indica* extracts. The increased number of goblet cells after treatment with *T. indica* extracts, these cells indicated that the immunity increased in the mucosa of the colon, anti-microbial antibodies production [24]. Ethanolic extract at the high dose in this study especially in a dose of 500 mg/kg-day was more effective than low dose, not only in decreasing number of *E. histolytica* in fecal but also in repairing the histopathological changes of the mucosa of the colon.

Moreover, *T. indica* possesses flavonoids, which protect tissues against from cellular damage of *E. histolytica* toxins [25]. *T. indica extract* indicated neither any toxic effect nor mortality in white albino rats (*Rattus norvegicus*) up to the 1000 mg/kg agree with report [17].

CONCLUSION: The reduce in the number of *E. histolytica in-vivo* and the repair the intestine tissue after using extracts of *T. indica* and compared with the control that considers as a good indicator of the possibility of using extracts of *T. indica* as a new natural drug to cure or mitigate the symptoms for amoebiasis.

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CONFLICT OF INTEREST: Nil

ETHICS APPROVAL: Institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were by the ethical standards of the institution or practice at which the study was conducted date 16/08/2018.

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