Original Article

Developmental and Innervation Changes in the Circumvallate Papilla of Turmeric Treated diabetic Rats' Offspring Versus Untreated (A Randomized Animal Controlled Trial)

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ABSTRACT

Background: Diabetes mellitus has a deleterious effect on the offspring including a high tendency for abnormalities and a defected immune system. The current study was performed to investigate the effect of maternal diabetes on the development and the innervation of circumvallate tongue papillae in their offspring and the possible beneficial effect of turmeric administration to the diabetic mother in preventing the defective development.

Materials and Methods: The study was carried out on 60 pups from diabetic mother rats that were classified into 2 main groups: group I which included untreated diabetic mother rats and group II which included diabetic mother rats treated with 30 mg/kg turmeric by oral gavage. The pups were sacrificed after 3, 10 and 60 days. The circumvallate papillae were dissected and examined histologically, immunohistochemically for S100, histomorphometrically and by quantitative reverse transcriptase-polymerase chain reaction for cytokeratin 8.

Results: The histopathological results revealed a deformed outline of the papillae in the untreated rats' offspring with a decreased number of well-formed taste buds, degenerative effects in Remak's ganglion and decreased innervation while treated rats' offspring revealed nearly normal histological structure for the circumvallate papilla and the underlying structures. Immunohistochemical results showed significant differences between untreated and turmeric treated groups in terms of S100 expression. CK8 gene expression results revealed a significant increase in the turmeric treated diabetic subgroups as compared to diabetic untreated subgroups at the same age.

Conclusion: Maternal diabetes has a degenerative effect on the taste system of the rat offspring including fewer taste buds with a deformed outline and defective innervation. Treating diabetic mothers with turmeric markedly improved the degenerative effect of diabetes on the offsprings' circumvallate papillae.

BACKGROUND

Diabetes is a very common disease with a high prevalence rate among people. According to the World Health Organization, the worldwide prevalence of diabetes for adults was estimated to be 8.8% in 2017 and will be 9.9% in 2045. The total number of people with diabetes in the Middle East and North Africa region is expected to double from year 2017 to 2045, from 38.7 million to 82 million. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men[1].

Congenital malformations are more frequent in offspring of diabetic mothers. The most common anomalies associated with pre-existing diabetes involve the cardiovascular system, the central nervous system, the face and the extremities[2] as well as histological and morphometric changes in the submandibular glands[3] besides, enamel hypoplasia in primary teeth of children born to diabetic mothers[4].

The risk of major malformations is markedly increased in infants of diabetic mothers, ranging from 4% to 10%, which is 2- to 3-folds higher than in the general population, with even higher absolute and relative risks for particular malformations, such as neural tube defects (1% risk)[2]. Impaired gene expression in the embryo, resulting from oxidative stress and consequent apoptosis or disturbed organogenesis, could be the mechanism that explain diabetic embryopathy[5].

Oxidative stress is defined as excessive production of reactive oxygen species (ROS) in the presence of diminished anti-oxidant substance. It has been shown that oxidative stress has an adverse effect on glucose metabolism[6].

It was proven that congenital malformations caused by experimental diabetes can be prevented by in *vivo* anti-oxidants treatment. Anti-oxidants alleviate the imbalance in the metabolism of free oxygen radicals which is involved in the embryonic mal-development in diabetic pregnancy[7].

Turmeric which is present in the roots of the Curcuma longa plant possesses anti-oxidant, anti-tumorigenic and anti-inflammatory properties, its supplements could reduce the negative effects of diabetes in the embryo. This occurs via blocking cellular stress and activation of endogenous anti-oxidants[8].

The current research focuses on diabetes related histological and structural changes in diabetic Albino rats' offspring's' circumvallate tongue papillae and the possible beneficial effect of turmeric supplementation to overcome these changes.

MATERIALS AND METHODS

Experimental Procedure

The inbred Wistar albino rats recruited in this study were obtained from the animal house, Faculty of Medicine, Cairo University according to the recommendations and approval of the Institutional Animal Care and Use Committee (IACUC), Cairo University. The rats were housed into sterile, controlled environment (temperature $25 \pm 2°C$, relative humidity 30-70% and 12 hr dark/light cycles) and fed with standard pellets diet and tap water ad libitum. All rats were kept under the same housing and feeding conditions. Twenty female rats were housed for mating in 4 cages each containing five virgin females and one male. When pregnancy occurred (determined by pregnancy test), each pregnant female was caged separately and given a medication according to its group. At 5th day of pregnancy the twenty female rats were given a single intra-peritoneal injection of Streptozotocin (STZ) (35-40 mg/kg body weight in 0.01 M citrate buffer at PH 4.5)[9]. Three days after STZ administration, peripheral blood was harvested from vena caudalis to evaluate the blood glucose level. Animals were considered diabetic if they had peripheral blood glucose concentration of 16.7 mmol/L or greater.

The twenty female diabetic mother rats were randomly distributed into two groups each of them consisted of ten rats; ten as untreated diabetic group (I) and ten as turmeric treated diabetic group (II) that were fed daily by oral gavage from the first day of pregnancy distilled water and turmeric 30 mg/kg in distilled water, respectively[10]. After delivery, the offsprings were sacrificed after 3, 10 and 60 days (10 from each group at each day interval). For descriptive and comparative purposes, 30 pups born to non-diabetic mother rats were sacrificed at the same intervals and served as controls. All pups were sacrificed with intraperitoneal injection of ketamine at 100 mg/kg. Their tongues were dissected and prepared for histological and qRT-PCR examination.

Tissue Samples

The dissected tongues were washed in saline solution, fixed in 10% neutral buffered formaline, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Paraffin blocks were sectioned at a 5um thickness and mounted on glass slides for staining.

Histological Examination

To assess the histopathological changes and perform the histomorphometric analysis, a set of sections was stained with haematoxylin and eosin solutions, dehydrated, mounted and examined by a Leica light microscope equipped with digital camera and image analysis software.

Immunohistochemical Examination

Five micron sections were mounted on positively charged microscopic slides. The steps of the immunohistochemical staining were done according to Abbass *et al*.[11] whereas in a Dako Autostainer under the same conditions.

Histomorphometric Analysis

Histomorphometric analysis was carried out to calculate the area percentage of the positive S100 nerve fibers and taste cells in the immunohistochemistry stained slides and to count the number of taste buds in H&E stained sections using magnification (40x). The data were obtained using Leica Qwin 500 image analyzer computer system for histomorphometric measuring. The image analyzer was calibrated automatically to convert the measurement units (pixels) produced by the image analysis program into actual micrometer units.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) for CK8

CK8 is an intermediate filament protein expressed in simple epithelium and taste bud forming cells. Quantitative real time PCR for CK8 was carried out to obtain a relative measure to the amount of normal taste cells in the whole papilla. It was carried out at the Biochemistry Department, Faculty of Medicine, Cairo University using StepOnePlus™ Real-Time PCR System.

Statistical Methods

The data of the histomorphometric analysis of the histological sections, quantitative assessment of immunohistochemical stain and qRT-PCR results were presented as mean and standard deviation (SD) values. Statistical analysis was performed using a commercially available software program (SPSS 18; SPSS, Chicago, IL, USA). Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), so paired (dependent) t test was used to compare area percentage of S100 immunoexpression in untreated and treated corresponding groups, while one-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was used to compare normal, untreated and treated corresponding groups regarding number of taste buds. The level of significance was set at $P < 0.05$.

RESULTS

Histological Examination Results

3 Days Age

Examining the specimens from the untreated diabetic rats' offspring at this age showed that the papilla had a deformed outline with broad base and narrow apex while in the treated rats' offspring and controls, normal papilla outline was found. Moreover, there was a slight decrease in the number of small developing taste buds in untreated diabetic rats' offspring as compared to the treated rats' offspring and controls. The underlying connective tissue showed numerous dilated capillaries, wide blood vessels engorged with RBCs and even areas of extravasated RBCs surrounded by chronic inflammatory cells infiltration. The developing von Ebner's gland (VEG) for the untreated diabetic rats' offspring lied deep in the connective tissue beneath the trough. It was consisted of extending cords and cell nests that began to differentiate into small ducts but no acini were detected while some pure serous acini were detected in the treated rats' offspring and controls. The developing von Ebner's gland for the latter appeared more well developed than both untreated and treated rats' offspring (Figure 1).

10 Days Age

Examining the specimens from the untreated diabetic rats' offspring at this age showed the papillae with a deformed outline, as it appeared narrow and elongated and surrounded by a wide trough if compared with both treated rats' offspring and controls. The gustatory epithelium showed notable decrease in the number of taste buds in comparison to the treated and controls. Atrophied taste buds with oval outline and undetected taste pore were also noticed. The von Ebner's gland appeared underdeveloped, canalized and mal-organized. The acini showed slight loss of normal architecture (amalgamation) with less defined cell boundaries. Excretory ducts were dilated with stagnant secretion and with hyperplastic epithelial lining. In the treated rats' offspring, the gland consisted of proliferating cell cords, few serous acini and some ducts were less developed than the controls. (Figure 2)

60 Days Age

Histological sections of the circumvallate papilla from two months' age offspring from diabetic untreated mother rats, showed deformity of the general outline. The covering epithelium lost its normal architecture with degenerative vacuolization of epithelial cells with pyknotic nuclei and apoptotic bodies. The whole epithelial lining was thickened. The taste buds showed decrease in number, signs of degeneration and separation of taste cells from the surrounding epithelium. Some taste cells remained attached to the basal and apical ends of the bud with condensation of darkly stained elongated nuclei at its base. Some taste buds decreased in size with degeneration of taste cells. Other taste cells showed nuclear hyperchromatism and pleomorphism. The nearby epithelium showed nuclear hyperchromatism of basal cell layer and basilar hyperplasia.

The C.T core showed marked increase in the inflammatory cells. Some blood vessels were dilated and engorged with RBCs and areas of extravasated RBCs. The Nerve bundle at the core of the papilla appeared shrunken. The ganglion cells showed signs of degeneration; it was either shrunken with dark cytoplasm and pyknotic nucleus, or swollen with cytoplasmic hyalinization and absence of nuclear details. There was massive dilatation of blood vessels with infiltration of inflammatory cells near the nerve bundle. The gland acini showed amalgamation while the excretory ducts were dilated with stagnant secretion besides vacuoles of ingested lipids in the lumen. Mucous acini were detected in the untreated rats' offspring. While, in the specimens of the treated rats' offspring, the papillae had normal outline and normal epithelial covering; however few taste buds appeared swollen. The ganglionic plexus of nerve fibers appeared normal with small fusiform Schwann cells and large rounded ganglionic cells. However, sporadic chronic inflammatory cells near the ganglion were detected, von Ebner's gland was normal. (Figure 3)

Immunohistochmical Results for S100

3 days

According to the amount and distribution of the brown color, the offspring from diabetic untreated mother rats at this age showed moderate S100 immunoreaction in the nerve plexus at the middle core of the papilla and the subgemmal and intragemmal nerve plexus. Stronger S 100 immunoreaction in the same areas in the turmeric treated rats' offspring was revealed which denoted higher innervation. The covering epithelium showed negative S100 immunoreaction in the treated rats' offspring while the untreated ones revealed moderate reaction which denoted inflammation. (Figure 4)

10 days

At this age, the treated rats' offspring showed stronger reaction in the subepithelial and intraepithelial nerve plexus than the untreated one. While in both groups, the taste buds showed moderate reaction. Strong reaction in the covering epithelium and the inflammatory cells in the connective tissue core was noticed in the untreated diabetic rats' offspring .VEG also revealed stronger reaction in the latter group. (Figure 5)

60 days

Remak's ganglion, subepithelial and intraepithelial nerve plexus showed marked strong reaction in the treated rats' offspring. While weak to moderate reaction was noticed in the untreated diabetic rats' offspring in the same areas. Instead strong reaction in the inflammatory cells, diffuse moderate reaction in the covering epithelium and VEG of the untreated diabetic rats' offspring was revealed which denoted inflammation. (Figure 6)

Histomorphometric Results

The histomorphometric analysis of the mean number of taste buds at 3 days revealed a non-significant difference between control, untreated and treated subgroups (p=0.107),

where the highest value was recorded in control, followed by the turmeric treated subgroup. At 10 and 60 days, comparing control, untreated and turmeric treated subgroups revealed a significant difference (p=0.005) and (*p*<0.001) respectively, where the highest value was recorded in control, followed by the treated subgroups. Tukey's post hoc test revealed a non-significant difference between control and treated subgroups (Table 1).

The comparison of mean area percentage of S100 immuno-expression between the untreated and then treated corresponding subgroups revealed a statistically significant increase in treated subgroups at 3, 10 and 60 days (*p*<0.001; p=0.002; p=0.025 respectively). The highest mean value was recorded in the treated group at 2 months age (24.72±4.53). (Table 2).

Quantitative Real Time Polymerase Chain Reaction Results for Cytokeratin 8

By comparing mean CK8 expression values in control, untreated and treated corresponding subgroups at 3, 10 and 60 days, an extremely significant difference (p<0.001), where the highest values were recorded in control, followed by treated subgroup. Tukey's post hoc test revealed a non-significant difference between control and treated subgroups at 3 and 10 days, while at 60 days a significant difference between each 2 subgroups was recorded (Table 3).
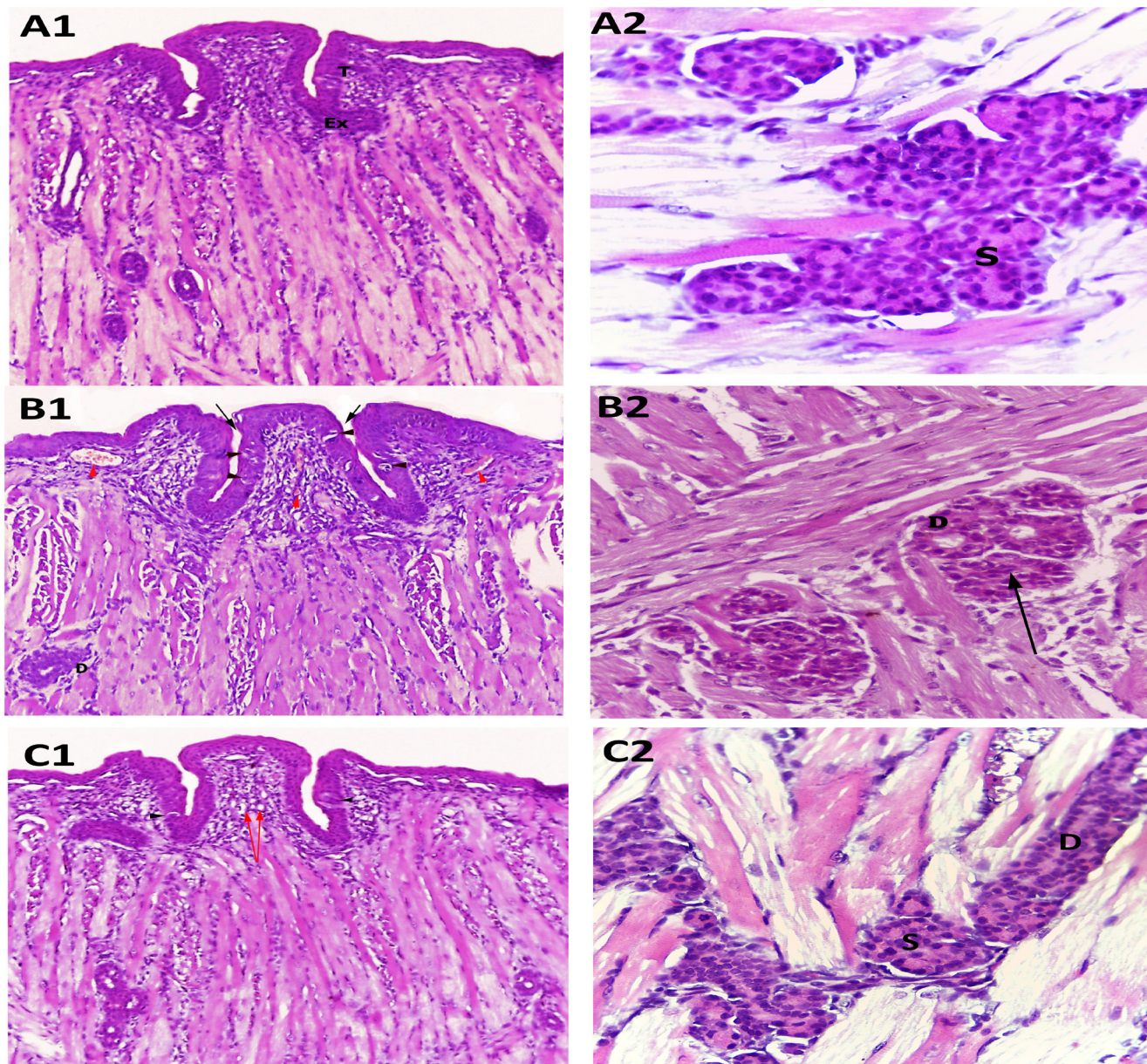

Fig. 1: A photomicrograph of circumvallate papilae at 3 days age (A) control, (B) untreated and (C) turmeric treated. A1 showing a taste bud (T). Excretory duct (Ex) of VEG opened at the depth of the trough. A2 showing small serous acini (S) of VEG. B1 showing narrowing of the apex of CVP (arrows) and widening of the base. Taste buds (black arrowheads) and widened blood vesseles (red arrowheads). B2 showing the developing VEG, small developing duct (D) and aggregates of developing secretory epithelium (arrow). C1 showing papilla with normal outline, taste buds (black arrowheads) and small blood vessels (red arrows). C2 showing VEG formed of small serous acini (S) and proliferating epithelial cords (D). (A1, B1 and C1, Orig Mag, x20; A2, B2 and C2, Orig Mag, x40)
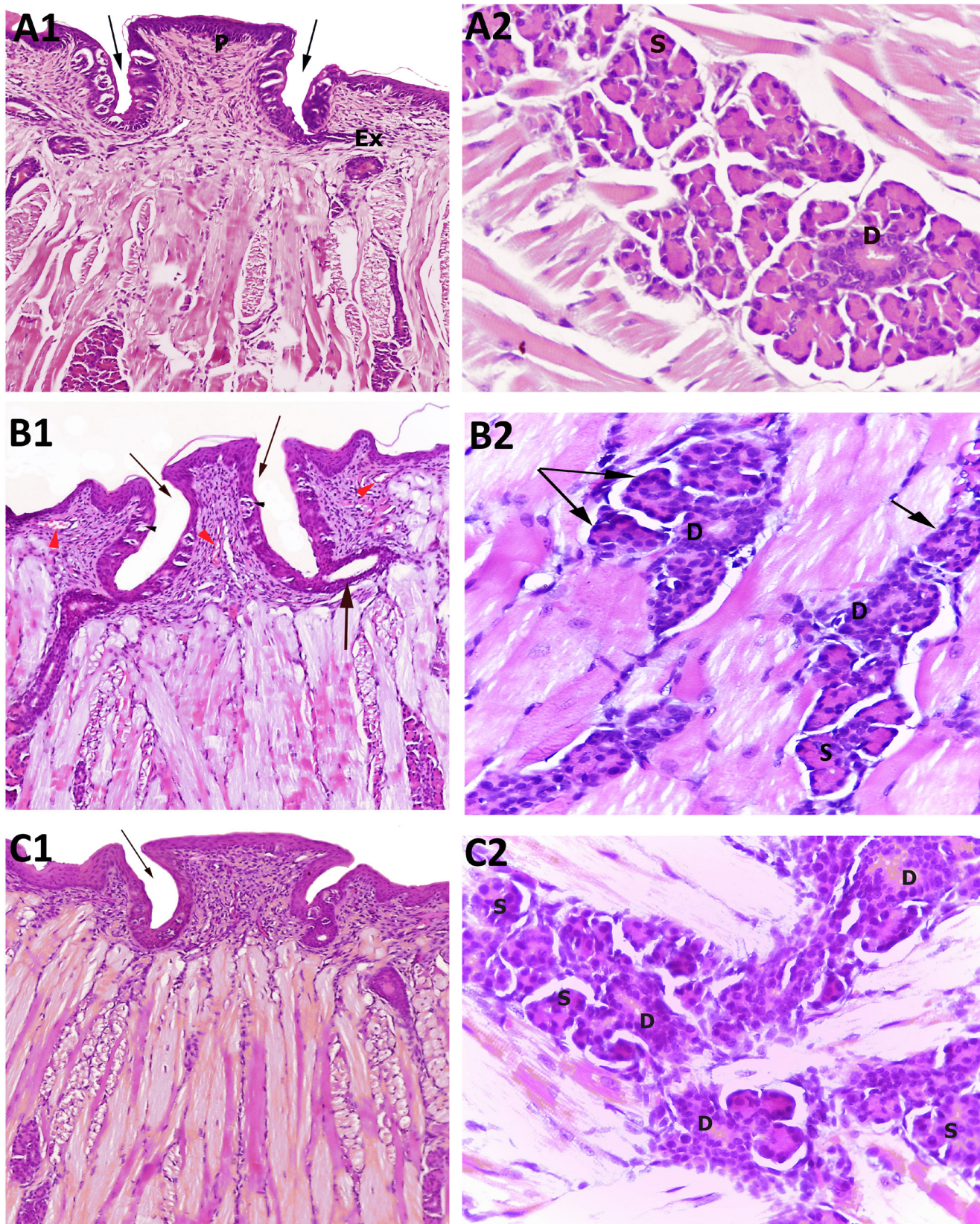
Fig. 2: A photomicrograph of circumvallate papillae at 10 days age (A) control, (B) untreated and (C) turmeric treated. A1 showing normal CVP covered with keratinized stratified squamous epithelium, narrow troughs (arrows), normal C.T, secondary C.T papilla (P) and excretory duct (Ex) of VEG. A2 showing well developed serous acini (S) and Ducts (D) of VEG. B1 showing deformity in the general outline of the CVP (arrows). Atrophy of some taste buds (arrowheads). C.T showed dilated blood vessels engorged with RBCs (red arrowheads). B2 showing underdeveloped VEG with small amalgamated serous acini (S), areas of differentiating secretory epithelium (arrows), small newly formed ducts (D). C1 showing slight widening of the trough (arrow). C2 showing VEG with small developing serous acini (S) and proliferating cell cords & Ducts (D). (A1, B1 and C1, Orig Mag, x20; A2, B2 and C2, Orig Mag, x40)
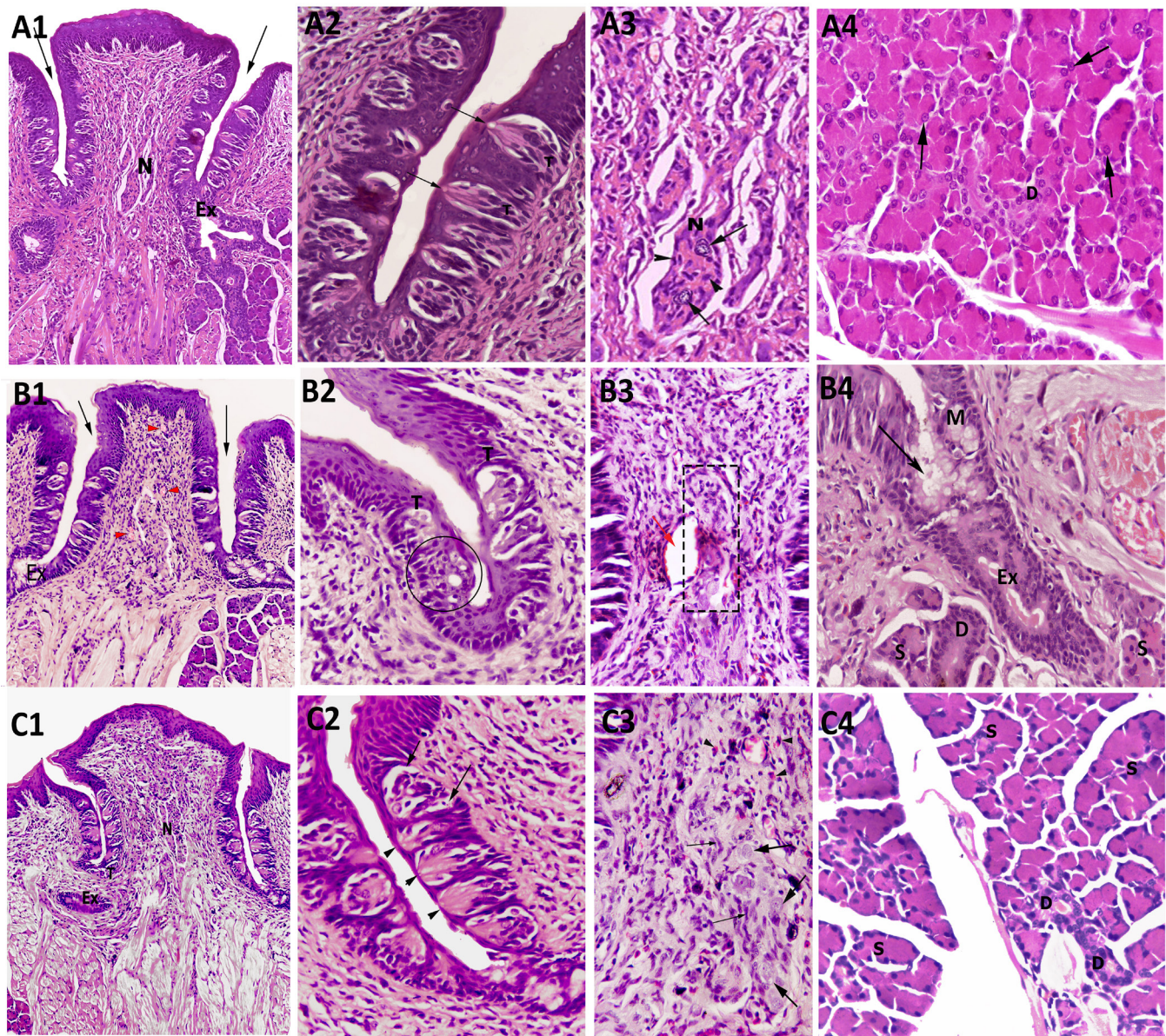
Fig. 3: A photomicrograph of circumvallate papillae at 60 days age, (A) control, (B) untreated and (C) turmeric treated. A1 showing CVP with normal outline and trough (arrows) in which the VEG excretory duct (EX) opens. Remak's ganglion (N). A2 showing numerous tastebuds (T) with taste pores (arrows). A3 magnification of Remak's ganglion (N) showing Schwann cells nuclei (arrowheads) and Ganglion cells (arrows). A4 showing VEG with normal serous acini (arrows) and ducts (D). B1 showing some deformity of the general outline (arrows). C.T showed increase of inflammatory cells and multiple dilated blood vessels (red arrowheads). Excretory duct (Ex) showed lipid vacuoles inside the lumen. B2 showing degenerated taste bud (T) within the epithelium (circle). B3 showing shrunken Remak's ganglion (rectangle) & massively dilated blood vessel with extravasated RBCs (red arrow). B4 showing; execratory duct (Ex) of VEG with mucous transformation in the lining (M) and lipid vacuols in the lumen (arrow). The ducts (D) showed Stagnant secretion and the acini (S) showed mild amalgamation. C1 showing normal papilla. New developing taste buds (T), Excretory duct (Ex) of VEG and the ganglionic nerve plexus (N) at the basal core.C2 showing numerous mature taste buds with taste pores (arrowheads) and a swollen taste bud (arrows). C3 showing the ganglionic bundle of nerve fibers which is surrounded by mild aggregation of inflammatory cells (arrowheads). Schwann cells nuclei (thin arrows) and Ganglion cells (thick arrows). C4 showing serous acini (S) of VEG with normal architecture. VEG ducts (D) with normal lining and stagnant secretion inside it. (A1, B1 and C1, Orig Mag, x20; A2, B2,C2,A3,B3,C3,A4,B4 and C4, Orig Mag, x40)
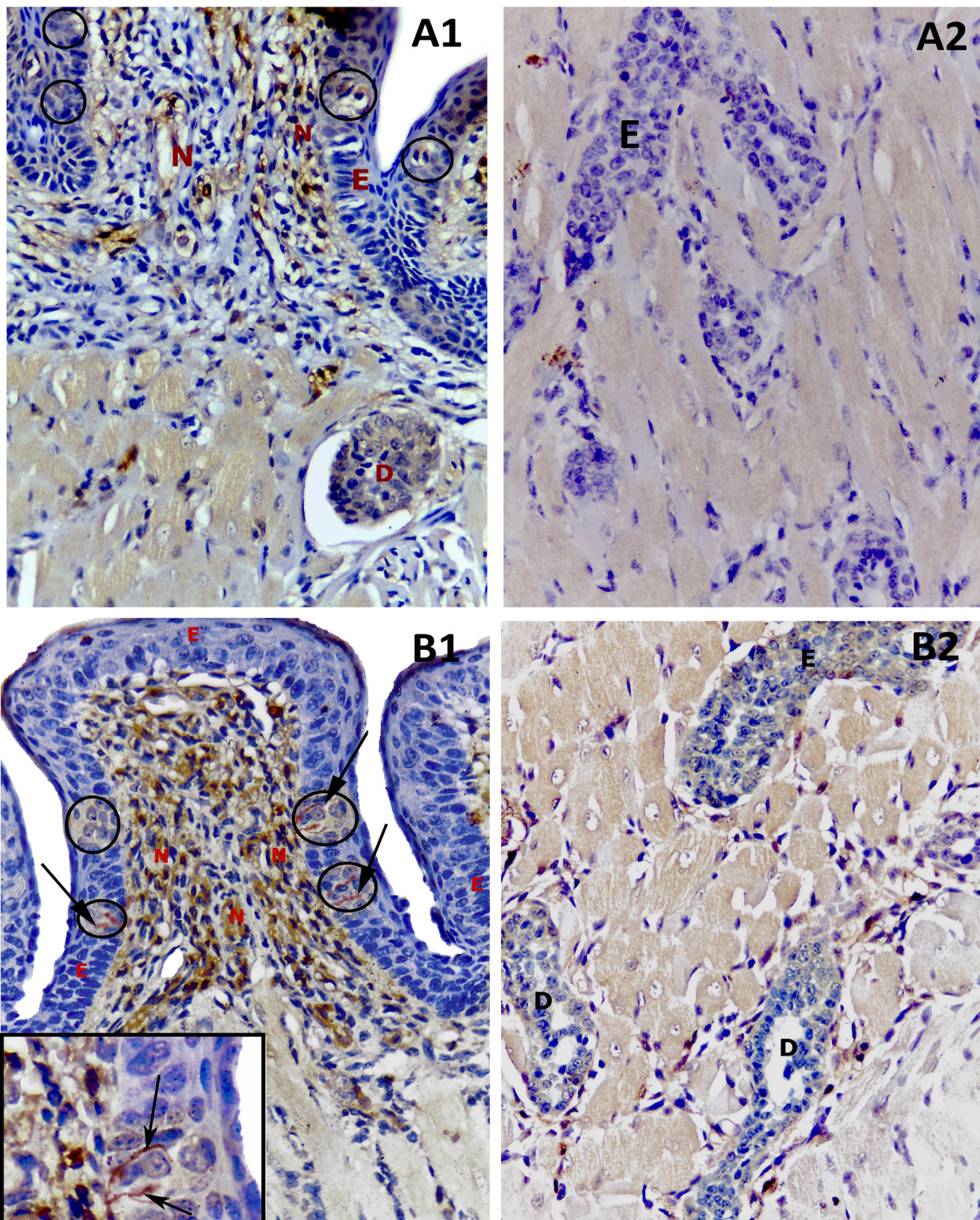
Fig. 4: A photomicrograph of circumvallate pipillae immunostained with S100 at 3 days, (A) untreated and (B) turmeric treated. A1 showing moderate positive S-100 immunoreactivity in the nerve plexus (N) located in the core of the papilla and the subepithelial nerve plexus beneath taste buds. Moderate positive immunoreactivity in taste cells (circles) and weak to moderate immunoreactivity in surrounding epithelium (E) and VEG duct (D). A2 showing negative reaction in the epithelial cords (E) of the developing VEG. B1 showing strong positive S-100 immunoreactivity in the subepithelial nerve plexus (N) and in perigemmal & intragemmal nerve fibers that reached the apical end of the taste buds (arrows), moderate immunoreactivity in some taste cells (circles) and negative immunoreactivity in surrounding epithelium (E). B2 showing moderate immunoreactivity in the duct cells (D) and epithelial cords (E) of VEG. (A1,A2,B1and B2 Orig Mag, x40; the inset, Orig Mag, x100)
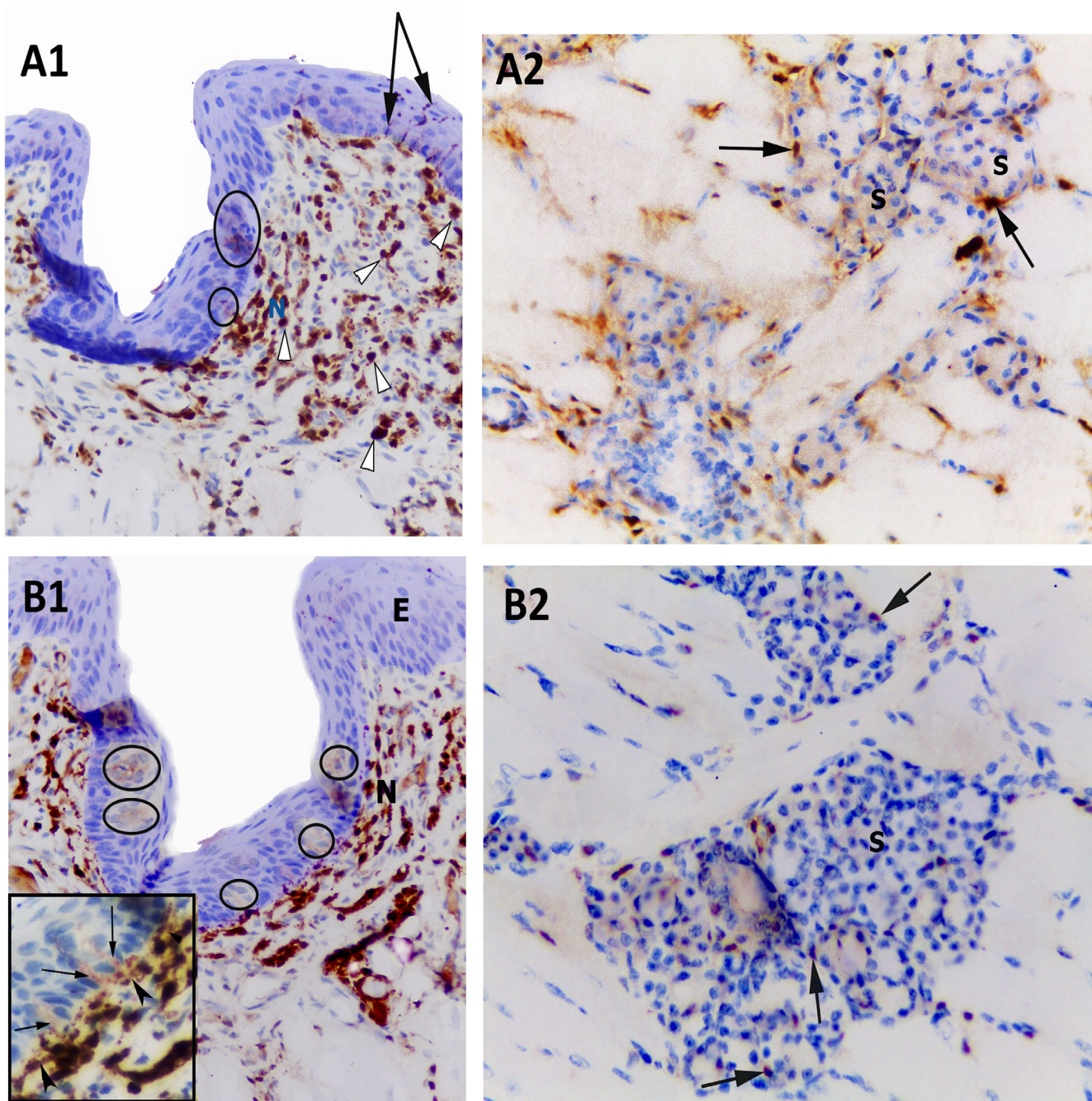
Fig. 5: A photomicrograph of circumvallate papillae immunostained with S100 at 10 days age, (A) untreated and (B) turmeric treated. A1 and B1 showing strong reactivity in the subepithelial nerve plexus (N) and moderate reactivity in the taste buds (circles). A1 showing negative reaction in the covering epithelium (arrows) and positive immunoreactivity in the inflammatory cells (white arrowheads). B1 showing negative reaction in the covering epithelieum (E). The inset shows higher magnification of the perigemmal and intragemmal nerve fibers (arrows) that entered around and inside taste buds and the dense highly reactive subepithelial nerve plexus (arrowheads). A2 showing mild to moderate reaction in VEG acini (S) and strong reaction in the myoepithelial cells (arrows) while B2 showing negative reaction in the acini (S) and both of them showing strong reaction in the myoepithelial cells (arrows). (A1,A2,B1and B2 Orig Mag, x40; the inset, Orig Mag, x100)
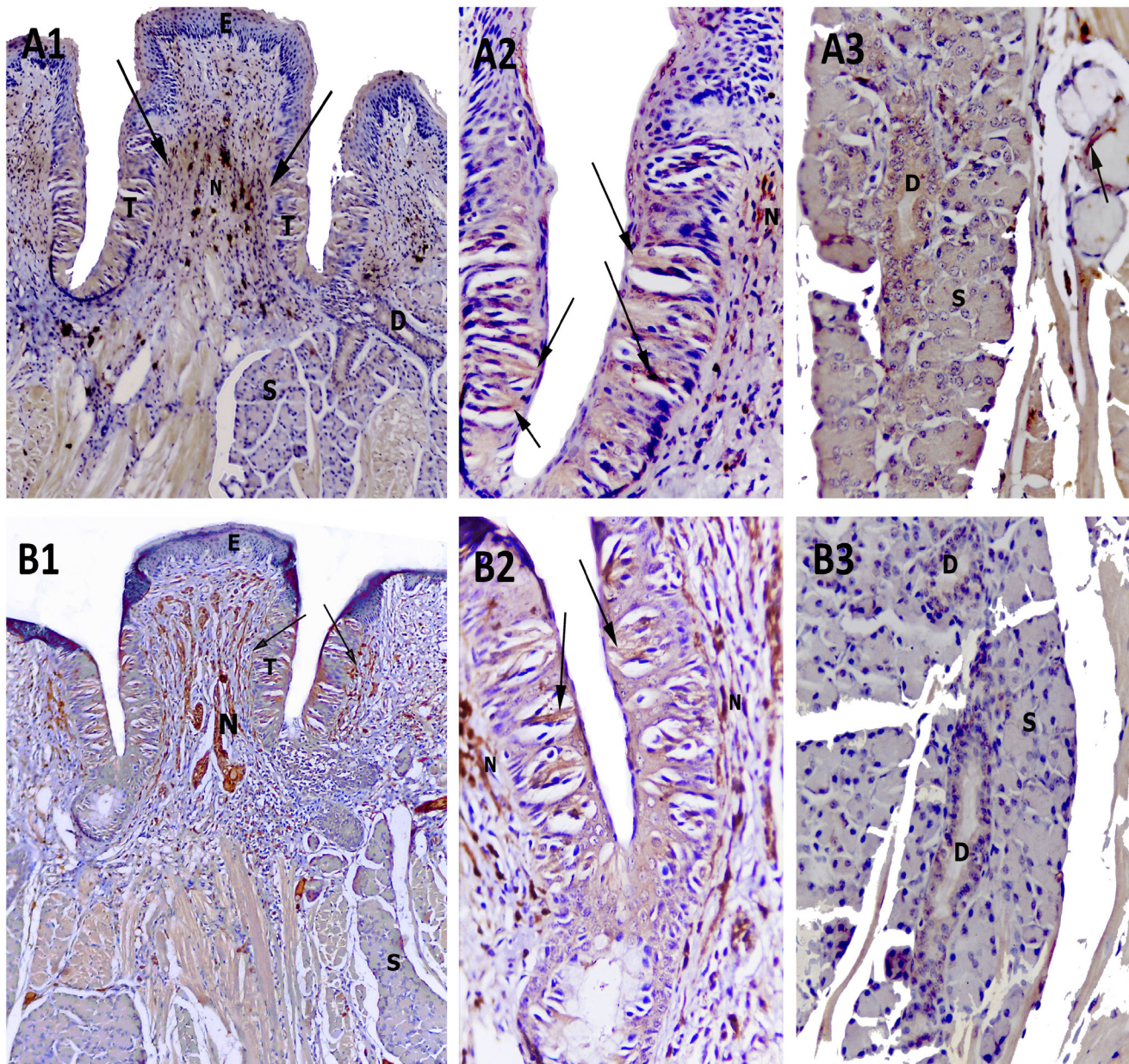
Fig 6: A photomicrograph of circumvallate papillae immunostained with S100 at 60 days age, (A) untreated and (B) turmeric treated. A1 showing moderate while B1 showing strong S100 immunoreaction in Remak's gamglion (N), subepithelial and intraepithelial nerve plexus (arrows) and taste buds (T). A1 showing moderate while B1 showing mild reaction in the covering epithelium (E) and VEG serous acini (S) and duct (D). A2 showing moderate while B2 showing strong S100 immunoreaction in the subepithelial (N) and intraepithelial nerve plexus (arrows). A3 showing moderate while B3 negative to mild S100 immunoreactivity in the VEG ducts (D) and acini (S). (A1 and B1, Orig Mag, x20; A2, B2,A3 and B3, Orig Mag, x40)

Table 1: Comparison of number of taste buds in control, untreated and treated subgroups within the same age (ANOVA test)

		Mean	SD	95% Confidence Interval for Mean		P
				Lower Bound	Upper Bound	
	Control	5.00e	0.82	4.42	5.58	0.107ns
3 days	Untreated I	3.90e	1.49	2.62	5.18	
	Treated II	4.90e	1.29	3.98	5.82	
	Control	11.40c	.97	10.71	12.09	0.005*
10 days	Untreated I	8.70d	2.21	7.12	10.28	
	Treated II	11.10c	2.02	9.65	12.55	
	Control	25.20 a	2.39	23.49	26.91	
60 days	Untreated I	15.60b	1.90	14.24	16.96	<0.001*
	Treated II	25.10a	2.77	23.12	27.08	

Significance level *P*<0.05, *significant, ns=non-significant

Table 2: Comparison of S100 protein immuno-expression area percentage in untreated and treated corresponding groups (paired t test)

		Mean	SD	95% Confidence Interval of the Difference		P
				Lower	Upper	
3 days	Untreated I	5.68	1.32	-6.84	-4.63	<0.001*
	Treated II	11.41	1.11			
10 days	Untreated I	11.85	1.04	-12.80	-5.32	0.002*
	Treated II	20.91	3.66			
60 days	Untreated I	14.68	3.86	-18.21	-1.87	0.025*
	Treated II	24.72	4.53			

Significance level *P*<0.05, *significant

Table 3: Comparison of CK8 expression values in control, untreated and treated within the same observation date (ANOVA test)

		Mean	SD	95% Confidence Interval for Mean		P
				Lower Bound	Upper Bound	
	Control	1.230a	0.128	1.138	1.322	0.001ns
3 days	Untreated I	0.600b	0.216	0.445	0.755	
	Treated II	1.203a	0.281	1.003	1.404	
	Control	2.397a	0.298	2.184	2.610	0.001*
10 days	Untreated I	.900b	0.082	.842	.958	
	Treated II	2.067a	0.170	1.945	2.188	
	Control	4.933a	0.613	4.495	5.372	
60 days	Untreated I	2.753c	0.271	2.559	2.948	<0.001*
	Treated II	4.300	0.638	3.844	4.756	

Significance level *P*<0.05, *significant

DISCUSSION

Maternal health plays a significant role in determining as well as predicting the health of the offspring later in their life. Fetal exposure to maternal diabetes in utero increases the risk of obesity/adiposity, glucose intolerance and development of type 2 diabetes for the offspring in their later life[12]. Diabetes is the most common medical complication during pregnancy, representing about 16.8% of all births[1].

Taste system is a perfect model to study nerve degeneration and regeneration because the taste system is highly plastic and the regeneration is robust. Besides, degeneration and regeneration can be easily measured since taste buds arise in discrete locations and nerves that innervate them can be accurately determined[13]. Since taste impairment in diabetes is an expression of diabetic neuropathy[14], the circumvallate papilla was the target in the present study for evaluating the histopathological changes caused by maternal diabetes.

In the current study, maternal diabetes was induced by intraperitoneal injection of 35mg/kg STZ at day 5 of gestation as maternal STZ administration before pregnancy affects fertility and impairs embryo development during preimplantation period[15]. Moreover, the induction of diabetes by STZ injection on day 5 of gestation does not lead to abortion[9].

The sacrifice dates of the pubs at 3 and 10 days in the present investigation were chosen to assess early effects of maternal diabetes on the circumvallate papilla of their offspring since these dates showed the most obvious morphological alterations during circumvallate papilla development[16], while the 2 months was chosen to assess the later effects.

In the present study, the von Ebner's gland of control rats at 3 days age appeared very primitive, there was epithelial invagination from the depth of the trough toward the connective tissue, ducts with little branches began to appear with little amount of secretory epithelium at its end. These findings are in agreement with Sbarbati *et al,*[17] who stated that at birth, the von Ebner's gland was immature and developed from the basis of the trench while the gustatory epithelium first showed solitary chemosensory cells and then taste buds. Therefore, the most complex morphogenetic events took place in the circumvallate papillavon Ebner's gland complex in parallel with the starting of breast feeding and could be triggered by gustatory inputs.

Furthermore, the absence of the circumvallate ganglion (Remak's ganglion) and the detection of subgemmal and intragemmal nerve plexus using S100 immunostaining at 3 days age in the control rats and the other two experimental subgroups (I and II) in the current investigation, supported the results of Sbarbati *et al,*[17] who reported that the majority of the neurons of the intrinsic nervous system develop in the days immediately after birth starting from isolated neuroblasts located at the bases of the circumvallate papilla, close to the position that in the adult occupied by the Remak's ganglion.

The epithelial thickening and the hypertrophy of the epithelium seen in group I in the current study, may be due to disturbance in apoptosis rate under the influence of diabetes[3]. It also may be owed to the increase in the intercellular spaces, decrease in the intercellular junctions and intercellular edema that was obvious in all off spring of untreated diabetic mother rats in our study[18]. Similar findings were reported by Popel' *et al.,*[19] who noticed thickening of the covering epithelium of the papillae in diabetic rats in comparison to healthy ones. On the other hand, another study attributed the hypertrophy to the elevated blood glucose level and increased glucose uptake by the cells. This happens in infants of moderately diabetic mothers, as their pancreas respond to hyperglycemia by hypertrophy and secretion of more insulin. Increased insulin level encourages more glucose uptake by the tissues leading to macrosomia and hypertrophy in many tissues[20].

In the current study, the deformity of the general outline of the papillae in group I is in agreement with Uemura *et al.,*[21] who reported change in the general outline of the filiform papilla of type 2 diabetic adult rat model. Popel' *et al.,*[19] reported deformity and shortening in all tongue papillae of rats with induced diabetes mellitus. This is in disagreement with the current study, as most papillae of all offspring of untreated diabetic mother rats appeared elongated. This diversity may be due to decrease in the width of the papilla and widening of the trough giving the papilla a thin elongated appearance in the current study.

A decrease in the number of taste buds and the presence of apoptotic bodies were noticed in H and E stained specimens of the offspring of untreated diabetic group and was supported with statistical results where a statistically significant decrease in the number of taste buds in the offspring of untreated diabetic group than their corresponding treated one was evident. This decrease in taste buds could be explained by Cheng *et al.,*[22] who proposed that diabetes may induce apoptosis by regulating the expression of Bcl-2 and Bax , leading to apoptosis of taste cells and degeneration of taste buds. Another explanation could be due to decreased and defective innervation which was observed in S100 immunostained sections. Taste buds are sustained by the on-going trophic influence of axons that transport putative trophic agents and they are thought to be a classic example of neurotrophically dependent receptor cells[23]. Pai *et al.,*[24] also detected a decrease in the number of taste buds in circumvallate papilla of diabetic rats and attributed this to defective innervation.

Moreover, El-sayyad *et al.,*[25] noticed the presence of apoptotic bodies and dilated capillaries within the architecture of diabetic rats' offspring liver. This is in agreement with our findings in group I, where numerous apoptotic bodies were noticed in prickle and granular layers of the covering epithelium and within the atrophic taste buds. They also reported a decrease in number and shrinkage in the pancreatic acini of the untreated diabetic rats' offspring, which is in accordance with the results of the current investigation, as we noticed underdevelopment, shrinkage

and disorganization of von Ebner's gland's acini in group I at 3 and 10 days age.

The nuclear changes detected in group I at 2 months age such as pleomorphism, hyperchromatism and increase in the nuclear cytoplasmic ratio are signs of cellular ageing and decrease in the rate of turn over. This occurs in the epithelium of diabetics as a secondary reaction to atherosclerosis which is one of diabetes complication. Inflammation and accumulation of advanced glycation end products (AGEs) also enhances these nuclear changes[26]. These findings support those of Jajarm *et al*.,[27] who found a considerable increase in N/C ratio with pleomorphism and hyperchromatism in diabetic buccal and tongue dorsum smears and also supported those of Ali *et al*.,[3] who noticed nuclear hyperchromatism and increase in mitotic activity in submandibular gland acinar cells in the offspring of diabetic rats.

In group I, Remak's ganglion appeared shrunken with swollen as well as degenerated ganglionic cells and was surrounded by severely dilated blood vessels and inflammatory cells infiltration. Moreover, subgemmal and intragemmal nerve plexus were less dense and reactive to S100 as compared to group II. These are in agreement with Hevér *et al*.,[14] who reported inflammation and degeneration of Remak's ganglion and marked decrease in the intragemmal and subgemmal nerve plexus density in the circumvallate papilla of diabetic rats. They proposed that nerve degeneration is an expression of diabetic neuropathy and this result in taste deterioration and increase in taste threshold in diabetics.

Amalgamation and loss of architecture in serous acini of von Ebner's gland noticed in all untreated diabetic rats' offspring in the present study are in agreement with El-sayyad *et al*.,[25] who reported amalgamation in the acini of the pancreatic islets in the offspring of diabetic rats.

In the present study, lumens of many ducts in group I at 10 days and 2 months age were stagnated with retained secretory material. This finding may reflect retardation in glandular development and function leading to impaired salivary flow in addition to the reduction in the secretory cells activity. This is in agreement with Ali *et al*.,[3] who reported stagnation in most of submandibular gland ducts in the diabetic rat offspring.

Some excretory ducts group I at 2 months age, showed lipid vacuoles inside the lumen. These lipid vacuoles may be ingested fats that were delayed in its digestion by the lingual lipase. Normally at this age, fat digestion depends mainly on the pancreatic lipase but in diabetic rats' offspring it seems to be underdeveloped due to a defected pancreas[25]. Other ducts appeared as proliferating nest of cells with collapsed lumen which is another expression of immaturity and delayed development.

Most of excretory ducts in group I at 2 months age, showed mucous cells in its lining. Occasionally, mucous cells could be seen in large ducts of normal rats but in this study,

it was only detected in this group and was not detected in turmeric treated diabetic group nor in the controls. This may be due to delayed rate of cells turnover in case of diabetes[26] that allowed accumulation of such occasionally appearing cells in this site and so permitted its detection.

The absence of all the previous defects associated with diabetes in the turmeric treated group may be due to the anti-hyperglycemic effect of turmeric on the maternal blood. Turmeric can elevate plasma insulin level and can increase lipoprotein lipase activity. Moreover, it is involved in activating of enzymes in liver, which are associated with glycolysis, gluconeogenic and lipid metabolic process. This creates a normo-glycemic environment to the fetuses preventing defective development[28].

The detected non-significant difference between taste buds' count in the control and turmeric treated diabetic subgroups in the current study is in agreement with CK8 PCR results. This could be attributed to the anti-inflammatory property of turmeric. The mechanism by which curcumin reduces inflammation is that it suppresses the activities of T, B-lymphocytes and macrophages by inhibiting proliferation, antibody production and lymphokine secretion. Curcumin also restores transmembrane potential and stiffs membrane fluidity, limiting the release of pro-inflammatory factors, from the endothelial and immune cells in the presence of high glucose or increased concentrations of AGEs. Alleviating inflammation prevents nerve degeneration and taste bud atrophy[29].

Innervation of circumvallate papilla and the subgemmal and intragemmal nerve plexus density in turmeric treated group was comparable to the control and was significantly higher than the untreated group. The neuroprotective effect of turmeric on these offspring may be occurred through its anti-oxidant and anti-inflammatory effect. This is in in agreement with Wu *et al*.,[8] who reported that curcumin treatment could block high glucose-induced endoplasmic reticulum stress markers in the neural tube. This could prevent neural tube defects formation in the offspring of diabetic rats.

The strong S100 reaction in Remak's ganglion at the base of the papilla and in the subgemmal and intragemmal nerve plexus in the turmeric treated group that was recorded in the current study, is in concomitant with Marettová *et al*.,[30] who studied the immunohistochemical distribution of S100 positive nerve fibers in the circumvallate papilla and its taste buds in the adult cat. Nerve fibers positive for S100 protein were observed as dense nerve plexus located in the core of the papilla, also, in the bands of fine nerve fibers that present under the lining epithelium, mainly at the base of the taste buds in which nerve fibers enter and branch out among the supporting and sensory cells. A weak reaction was displayed by the taste bud cells and the surrounding epithelial cells. The reaction of nerve plexus in the untreated diabetic group was markedly decreased and this denotes nerve fiber degeneration. This is in accordance with Hevér *et al*.,[14] who reported a decrease in the density of the subgemmal

and intragemmal nerve plexus in diabetic rats' circumvallate papilla.

A significant limitation in the current study is lack of documentation of the blood glucose level during the study course, although it was measured twice during the pregnancy; 3 days following STZ administration and at the 14th day of pregnancy. The blood glucose level data are important to prove the alleviating effect of turmeric over the diabetic condition.

CONCLUSION

In the present study, maternal diabetes strongly deteriorated the circumvallate papilla structure, development and innervation. Moreover, it significantly decreased the number of taste buds and caused a delay in the development and maturation of the von Ebner's gland. All these adverse effects didn't resolve with time and continued till the second month of age. Despite the administration of turmeric to the diabetic mother was expected to protect the pups only in their early life, but it continued up to 60 days age in the offspring of the turmeric treated mothers. This may be due to early protection from the teratogenic effect of hyperglycemia and oxidative stress on the nerve development. Proper innervation led to proper development later on.

RECOMMENDATIONS

Further clinical studies are needed to apply the findings of the present study on humans. More studies are encouraged to be carried out to investigate the effect of turmeric treatment on the offspring themselves and weather it is beneficial in the recovery of the taste system or not.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التغيرات التكوينية والعصبية لحليمة اللسان المحوطة في ذرية الفئران المصابة بالسكري والغير معالجة مقابل ذرية الفئران المصابة بالسكري والمعالجة بجذور الكركم (دراسة عشوائية في الحيوان)

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الخلفية: مرض السكري له تأثير ضار على النسل بما في ذلك الميل العالي لحدوث تشوهات الأجنة وضعف الجهاز المناعي. تم اجراء الدراسة الحالية للتحقيق في تأثير مرض السكري للأمهات على حليمة اللسان المحوطة وبراعم التذوق المرتبطة بها وغدة فون ابنر اللعابية لذريتهن. كذلك دراسة التأثير النافع المحتمل لإعطاء مستخلص الكركم للأم المصابة بالسكري.

طريقة البحث: أجريت الدراسة على ٦٠ من صغار الفئران الذين ولدوا لفئران مصابة بداء السكري واللاتي تم تقسيمهم إلى مجموعتين، المجموعة الأولى؛ الجرذان المصابة بداء السكري والغير معالجة والمجموعة الثانية؛ الفئران المصابة بداء السكري والمعالجة بمستخلص الكركم بجرعة ٣٠ ملجم / كجم عن طريق الفم. تمت التضحية بالفئران في ثلاثة أعمار مختلفة: ثلاثة أيام وعشرة أيام وشهرين. تم تشريح العينات ودراستها نسيجيا عن طريق إخضاعها لصبغة الهيماتوكسيلين والإيوسين وتم دراستها باستخدام كيمياء المناعة لـ S١٠٠. كما تم قياس التعبير الجيني لـ CK٨ باستخدام الـ RT- PCR.

النتائج: أظهرت نتائج الدراسة ان جميع الفئران المولودة لأمهات مصابات بالسكري وغير معالجات لديهم تشوه في الشكل العام للحليمة ونقص في عدد براعم التذوق و ضمورها. كما ظهر انكماش في عقدة ريماك العصبية الموجودة في قاعدة الحليمة مع تورم و اضمحلال في الخلايا العقدية وغزو خلايا الالتهاب المزمنة للعقدة العصبية. بينما اظهرت المقاطع المصبوغة للفئران المولودة لأمهات مصابات بالسكري وتناولن مستخلص الكركم تحسناً و صفات مقاربة للمجموعة الضابطة. أظهرت نتائج كيمياء المناعة اختلافات كبيرة بين المجموعات المعالجة بالكركم و الغير معالجة من حيث تعبير S١٠٠. اظهرت نتائج التعبير الجيني لـ CK٨ زيادة كبيرة في مجموعة مرضى السكري المعالجين بالكركم عن المجموعات الغير معالجة في نفس العمر.

الاستنتاج: مرض السكري لدى الأمهات يسبب تغير تكويني في براعم التذوق والجهاز العصبي الداخلي الخاص بها لدى النسل. كما ان علاج الامهات بالكركم يمنع هذا التغير السلبي في نسلهن.