Toxicology Reports 10 (2023) 633–646

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Downregulation of inflammatory erectile dysfunction by *Mantisa religiosa* egg-cake through NO-cGMP-PKG dependent NF-kB signaling cascade activated by mixture of salt intake

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ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
NaCl
Monosodium glutamate
Praying mantis egg-cake
Erectile dysfunction
Rat model

ABSTRACT

We hypothesized whether 10% praying-mantis-egg-cake (10% PMEC) can be applied against inflammatory-erectile-dysfunction and whether it could be linked to NO-cGMP-dependent PKG signaling cascade. Ninety male albino-rats were randomly distributed into nine (n = 10) groups. Group I was given distilled water. Group II and III were pre-treated with 80 mg/kg NaCl and 75 mg/kg MSG, respectively. Group IV was pre-treated with 80 mg/kg NaCl + 75 mg/kg MSG. Group V was administered with 80 mg/kg NaCl+ 3 mg/kg Amylopidin. Group VI was given 80 mg/kg NaCl + 10% PMEC. Group VII was treated with 75 mg/kg MSG + 10% PMEC. Group VIII was treated with 80 mg/kg NaCl+ 75 mg/kg MSG + 10% PMEC. Group IX was post-treated with 10% PMEC for 14 days. Penile PDE-5[1], arginase, ATP hydrolytic, cholinergic, dopaminergic (MAO-A) and adenosinergic (ADA) enzymes were hyperactive on intoxication with NaCl and MSG. The erectile dysfunction caused by inflammation was linked to alteration of NO-cGMP-dependent PKG signaling cascade via up-regulation of key cytokines and chemokine (MCP-1). These lesions were prohibited by protein-rich-cake (10% PMEC). Thus, protein-rich-cake (10% PMEC) by a factor of 4 (25%) inhibited penile cytokines/MCP-1 on exposure to mixture of salt-intake through NO-cGMP-PKG dependent-NF-$_{K}$B signaling cascade in rats

1. Introduction

Salt intake are often resulting into salt poisoning or intoxication from the excessive intake of sodium chloride [1] either in solid form or in solution (saline water). Salt poisoning is frequently found in children or infants [2] who consume excessive amounts of table salt. Also, too much salt intake in adults occur from drinking of sea water or soy sauce [2]. The major source of sodium in our diet is salt. It also comes from monosodium glutamate (MSG), used as a condiment in several parts of the world [3]. Humans are apparently exposed to salt intoxication (NaCl and/or MSG) from processed foods such as ready meals, processed meats like bacon, ham, salami, cheese, salty snack foods and instant noodles. NaCl/or MSG may also be consumed in large amount when added to food during cooking (bouillon, stock cubes) or at the table (meat and fish sauce). Short-term consumption of high amounts of salt causes water retention, mild high blood pressure, excessive thirst, hypernatremia and

Abbreviations: ACh, acetylcholine; AChE, acetylcholine esterase; ADA, adenosine deaminase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; BSA, bovine serum albumin; BuChE, butyrylcholine esterase; cAMP, cyclic guanosine monophosphate; cGMP, cyclic guanosine mono phosphate; COX-2, cyclooxygenase-2; CSIF, cytokine synthesis inhibitory factor; DAB, 3,3 diaminobenzidine; Ecs, endothelial cells; ED, erectile dysfunction; ELISA, enzyme-linked-immuno-sorbent assay; eNOS, endothelial nitric oxide synthase; GC, guanylate cyclase; HIF-1, hypoxia inducible factor-1; HOCl, hypochlorous acid; IED, inflammatory erectile dysfunction; IL-10, interleikin-10; IMED, inflammatory mediated erectile dysfunction; iNOS, inducible nitric oxide synthase; MAO-A, monoamine oxidase-A; MCP-1, monocyte Chemoattractant Protein-1; MSG, monosodium glutamate MSG; NaCl, sodium chloride; NF-kB, nuclear factor-kappa β; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NTPDase, econucleotidase; p53, tumor suppressor gene; PDE-5[1], phosphodies-terase-5[1]; PKG, protein kinase G; PMEC, protein mantis egg cake; PNPP, para nitrolphenyl phosphate; SLE, systemic lupus erythematous; TBA, thiobarbituric acid; TBARS, thiobarbituric reactive substance; TCA, trichloroacetic acid; TNF-α, tumor necrotic factor-α.

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https://doi.org/10.1016/j.toxrep.2023.05.007
Received 11 January 2023; Received in revised form 6 May 2023; Accepted 13 May 2023
Available online 20 May 2023

Table 1
Salt mixture exposed group and cake formulation for control and treated group.

Treatment	Control	NaCl	MSG	NaCl+MSG	NaCl+AML	NaCl+PMEC	MSG+PMEC	NaCl+MSG+PMEC	PMEC
Corn oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin- premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sucrose	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Corn starch 69.0	69.0	69.0	69.0	69.0	20.0	20.0	20.0	20.0	
Casein	16.0	16.0	16.0	16.0	16.0	55.0	55.0	55.0	55.0
PMEC	-	-	-	-	-	10.0	10.0	10.0	10.0
Total (g)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Protein ratio	1.0	1.0	1.0	1.0	1.0	4.0	4.0	4.0	4.0

restlessness [4]. Also, exposure to salt-rich diets for the long term increased high blood pressure, mental health problems, aging and stomach cancer [5,6]. Significantly, individuals with health conditions like heart failure, liver or kidney disease and endothelial dysfunction may experience fatal effects on exposure to salt overdose or low dose for a long-term. This forms the basis for selecting the mixture of salt (NaCl/or MSG) in the study.

The endothelial cells (ECs) of blood vessels mediates osmoregulation by balancing production of vasodilators such as nitric oxide (NO) and vasoconstrictors [7, 8, 9]. Penile erection occurs with the release of NO from vascular endothelial cells [10]. Additionally, the metabolic role of endothelium in erectile function showed that the phosphodiesterase type 5 (PDE-5[1]) inhibitors could enhance erectile function [11], while L-arginine is a powerful inhibitor of all the three types of NOS [12]. We hypothesized that low level of NO with corresponding up-regulation of PDE-5[1] enzyme may be mediated by mixture of salt intoxication to decrease penile blood flow.

Globally, erectile dysfunction (ED) is a highly prevalent disorder that affects approximately 50% of men above 40 years [13]. Specifically, epidemiological finding demonstrated that the prevalence of ED ranged from 2% to 9% in men aged 40–49 years, 20–40% in men aged 60–69 years while, it affects all the men above 70 years [14]. Recent studies reported that inflammation plays a critical role in ED and progresses into inflammatory erectile dysfunction (IED) [15, 16, 17].

Cytokines are constitutively produced by white blood cells in response to inflammatory stimuli [18]. Nuclear factor-kappa B (NF-κB) can also trigger the progression of inflammatory erectile dysfunction, hypertension and cardiovascular diseases [19,20]. Multiple dissimilar signaling cascades can cause the activation of NF-κB, including proteasome independent pathways [21]. However, we postulated that protein-rich-cake (PMEC) by a factor of 4, prepared from *Mantisa religiosa* egg may be a promising therapy for inflammatory erectile dysfunction (IED).

Mantisa religiosa egg is a secreted liquid substance by female insect known as praying mantis egg (PMEC). PMEC was used against chronic cough, pulmonary dysfunction, and prevention of congested blood vessels with evidence of clearways and absence of pulmonary emphysema in animals [22]. It has antioxidant, anti-microbial and anti-inflammatory potential [23,24]. PMEC is most effective agent for enhancing protein content and lowering the cadmium accumulation in muscular and bone tissues of African catfish [25]. It is an inhibitor of myloperoxidase activity which catalyzes the formation hypochlorous acid (HOCl), one of the major steps in biosynthesis of ROS and oxidative stress [25]. The beneficial effects of PMEC had been attributed to its constituents including phenols, carotenoids, flavonoids and micronutrients [22]. However, the potentials of PMEC may also be attributed to the other mechanisms independent of pulmonary dysfunctions. It may increase NO bio-availability and provide stability to vascularized blood flow. Therefore, we postulated that cake formulated with praying mantis egg (PMEC), with protein-rich by factor of 4 (25%), may reduce IED on exposure to NaCl/MSG in rat model. It was also examined whether PMEC could promote penile erection through NO-cGMP-PKG and NF-κB signaling pathways.

2. Materials and methods

2.1. Chemicals and reagents

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), acetylcholine iodide, adenosine, butyrylcholine iodide and para nitrolphenyl phosphate (PNPP) were obtained from Sigma-Aldrich Inc, (St Louis, Missouri, USA). Other chemicals and reagents used for the experiments were of analytical grade.

2.2. Animal treatment and experimental design

Following acclimatization for 2 weeks, ninety male Wistar rats weighing 200–250 g were purchased and housed in a well aerated plastic cages. They were all allowed to have free access to commercial feed and water as well as 12 – hour light/dark cycle. The handling of the animals followed the institutional guidelines for animal use, care and research [26], while the Biochemistry Departmental Board of Examiner of Federal University of Agriculture, Abeokuta, Nigeria approved the research protocol. The experimental approved number of the researcher is 2015/0869. However, the animals were arbitrarily grouped into 9 (n = 10), and treated as sketched below:

- Group I (control) served as the untreated control, and was fed with normal diet for 14 days.
- Group II (NaCl only) was exposed to 80 mg/kg NaCl for 14 days, and was fed with normal diet for 14 days.
- Group III (MSG) was exposed to 75 mg/kg MSG for 14 days, and was fed with normal diet for 14 days.
- Group IV (NaCl + MSG) was exposed to mixture of 80 mg/kg NaCl + 75 mg/kg MSG for 14 days and fed with normal diet for 14 days.
- Group V (NaCl + AML) was exposed to 80 mg/kg NaCl for 14 days and post-treated with 3 mg/kg Amlodipine for 14 days, and was fed with normal diet for 14 days.
- Group VI (NaCl + PMEC) was exposed to 80 mg/kg NaCl for 14 days and was post-treated with protein-rich cake (10% PMEC) by a factor of 4 for 14 days.
- Group VII (MSG + PMEC) was exposed to 75 mg/kg MSG for 14 days and post-treated with protein-rich cake (10% PMEC) by a factor of 4 for 14 days.
- Group VIII (NaCl + MSG + PMEC) was exposed to 80 mg/kg NaCl + 75 mg/kg MSG for 14 days and post-treated with protein-rich cake (10% PMEC) by a factor of 4 for 14 days.
- Group IX (PMEC only) was post-treated with protein-rich cake (10% PMEC) by a factor of 4 for 14 days.

2.2.1. Cake formulation and dose selection

We selected the dose of protein-rich cake (10% PMEC) using our previous study [22]. Additionally, we hypothesized that ingestion of protein-rich cake by a factor of 4 (25%) may be applied to manage erectile dysfunction. To realize this estimation, we formulated the normal diet with the following constituents: casein – 16 g, corn oil – 10

Table 2
Primer sequences of p53, TNF-α, IL-10, HIF-1, MCP-1 and β-actin genes for Reverse Transcription (RT) PCR.

Gene	Forward primer 5'– 3'	Reverse primer 5'– 3'	Product length
P53	CAGCTGTGGGTTGATTCCAC	CTGGGGACCCTGGGCAAC	176 bp
TNF-α	ATGAGCACTGAAAGCATGATCCGG	GCAATGATCCCAAAGTAGACCTGC	695 bp
IL-10	AAGGCAGTGGAGCAGGTGAA	CCAGCAGACTCAATACACAC	440 bp
HIF-1	TGCTTGGTGCTGATTTGTGA	GGTCAGATGATCAGAGTCCA	672 bp
MCP-1	GTGCTGACCCCAATAAGGAA	TGAGGTGGTTGTGGAAAAGA	185 bp
β-actin	GGGTCAGAAGGATTCCTATG	CTAGAAGCATTTGCGGTGGAC	1000 bp

g, sucrose – 2 g, vitamin premix – 3 g, and corn starch – 69 g (Table 1). While, the protein-rich cake (10% PMEC) by a factor of 4 contains casein – 55 g, corn oil – 10 g, sucrose – 2 g, vitamin premix – 3 g, corn starch – 20 g and PMEC – 10 g (Table 1). Basically, cake was formulated to contain 10% protein with casein, taking into cognizant the crude protein content of PMEC. For example, the quantity of casein necessary to obtain 10% protein in the cake was calculated from the following equation:

$$\frac{100 \times 10}{Y}.$$

However, the protein ratio of normal diet and 10% PMEC, respectively was considered as 15 g: 60 g (1: 4). Also, doses of 80 mg/kg NaCl [27] and 75 mg/kg MSG [28] were selected for this study. Amlodipine 3 mg/kg (vasodilator drug) was intubated using the technique of Yamanaka et al. [29].

2.3. Preparation of penile homogenate

The penile tissues were carefully excised into 10 volumes of 0.1 M phosphate buffer, pH 7.4 on ice, after sacrificed by cervical dislocation. The penile tissues were homogenized and centrifuged at 10,000 rpm for 15 min at 4^0 C by electrically driven homogenizer. Then, the collected supernatants were transported into a pre-chilled Eppendorf tube, which was kept at 4^0 C until analysis. And, we quantified the protein content by the method of Coomassie blue [30] using bovine serum albumin (BSA) as standard.

2.4. Bioassays

2.4.1. Lipid peroxidation

Penile lipid peroxidation was measured as malondialdehyde (MDA) using the method of Ohkawa et al. [31] and expressed as nmoles MDA/mg protein.

2.4.2. Assay of NO as a marker of NO synthesis

Penile NO level was assessed following the method of Miranda et al. [32] and expressed as μM/mg protein.

2.4.3. Phosphodiesterase-5' (PDE-5') activity determination

Penile phosphodiesterase-5' activity was determined by the protocol of Thompson and Appleman [33]. A 100 μl of penile tissue was measured into 800 μl of 125 mM Tris-Base Buffer (pH 7.4) and incubated at 37 °C for 10 min. Then, 100 μl of para nitrophenyl phosphate (PNPP) as substrate was added to the reacting mixture. The resulting mixture was read at optical density of 400 nm and expressed as PDE-5^1 activity/min/mg protein.

2.4.4. Determination of ATPase, ADPase and AMPase activities

The activities of ATPase and ADPase were assessed as described by Schetinger et al. [34], while the AMPase activity was determined following the method described by Heymann [35]. The reaction mixture was pre-incubated at 37 °C for 10 min. The reaction was initiated by the addition of ATP or ADP or AMP to obtain a final concentration of 1.0 mM and incubation proceed for 20 min in either case. Reaction was stopped by the addition of 200 mL of 10% trichloroacetic acid (TCA) to obtain a final concentration of 5%. The 5^1-nucleotidase (AMPase) activity was determined in a reaction medium containing 10mMMgSO_4 and 100 mM Tris-HCl buffer, pH 7.5, in a final volume of 200 μl. The released inorganic phosphate (Pi) was measured at 700 nm and enzyme activities are reported as nmol Pi released/mg of protein.

2.4.5. Adenosine deaminase activity determination (ADA)

Penile ADA activity was estimated using the protocol described by Guisti and Galanti, [36]. A 50 μl of enzyme preparation reacted with 21 mmol/l of adenosine, pH 6.5, and was incubated at 37 °C for 60 min. The results were expressed in units per mg protein (U/mg protein). One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine under standard assay conditions.

2.4.6. Determination of acetylcholine esterase (AChE) and butyrylcholine esterase (BuChE) activities

Penile AChE and BuChE activities were estimated by the method of Perry et al. [37]. Concisely, 100 μl of 3.3 mM 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB) in 0.1 M phosphate buffer pH 8.0 was mixed with 100 μl of penis supernatant, followed by addition of 500 μl of phosphate buffer (pH 8.0), and incubated for 20 min at 25 °C. Afterward, acetylthiocholine iodide (100 μl of 0.05 mM solution) was mixed as the substrate and AChE activity was quantified by measuring the changes in optical density at 412 nm. The calculated results were expressed as AChE activity/min/mg protein and BuChE activity/min/mg protein, respectively.

2.4.7. Monoamine oxidase-A (MAO-A) activity determination

Penile MAO-A activity was estimated using benzylamine as substrate [38]. Reaction mixture contained 100 mmol phosphate buffer of pH 7.4, 200 μm benzylamine and 0.4 mg/mL of homogenate. The final volume of the reaction mixture was 250 μl. Mixtures were incubated at 37 °C for 1 h and cooled on ice. 500 μl of distilled water, 250 μl of 10% $ZnSO_4$ and 50 μl of 1 mol NaOH were heated for 2 min, cooled on ice and centrifuged (1000*g* for 10 min). The supernatant was diluted (by 5×) with 1 mol NaOH, while the absorbance was read at 450 nm. The result was expressed as MAO-Aactivity/mg protein.

2.4.8. Examination of penile histopathology

The penis was placed in 4% para-formaldehyde at 4° C for 48 h. After dehydration, transparency, paraffin immersion and paraffin embedding, the penis was sliced along the median anteroposterior axes at a thickness of 6 μm. The section was stained with hematoxylin and eosin for morphological observation and defining positions. Sections were read and images were captured using microscope.

2.4.9. ELISA of penile necrotic factor Kappa-β (NF-kB)

Penile NF-kB was quantified using ELISA kit (Cusabio Biotech. Ltd, USA) and expressed as U/l.

2.5. Isolation of RNA

Total RNA was extracted from the penile homogenate using tissue TRIzol RNA extraction kit (Invitrogen Life Technologies, USA).

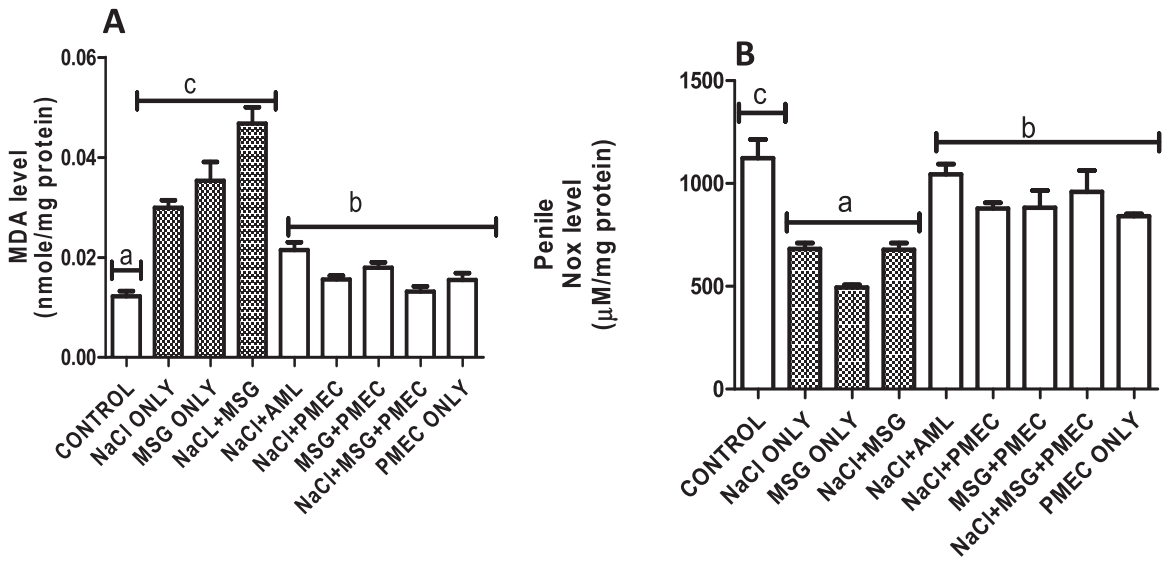

Fig. 1. Effect of PMEC on penile malonaildehyde [(MDA) (graph A)] and penile NO levels (graph B) in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at [a] $p < 0.05$, [b] $p < 0.05$, [c] $p < 0.05$.

2.5.1. Real-Time PCR analysis of penile TNF-α, p53, HIF-1 and MCP-1

The isolated RNA was subjected to RT-PCR analysis according to the manufacturer's instructions. Total RNA was reverse transcribed using RevertAid™ First strand cDNA synthesis kit (Thermo Fisher Scientific, USA) as per specific primers (Table 2) for p53, TNF-α, HIF-1, MCP-1 and β-actin (as a reference gene). Each sample was run in 25 μl reaction mixture. The 25 μl reaction mixture contained 12.5 μl SYBR Green master mix, 0.5 μl from 10 pmol working solution of gene specific forward and reverse primers, 1 μl cDNA and the volume was made up to 25 μl with Nuclease free water. The RT-PCR reaction was started with initial incubation at 94° C for 15 min followed by 40 cycles amplification with denaturation at 94° C for 30 s, annealing at 60° C for 30 s and extension at 72° C for 2 min for each cycle in a thermal cycler (Bio-rad™ MJ Minicycler). The amplified products were resolved by gel electrophoresis (Bio-radTM submarine elctrophoresis) on 1.5% agarose and visualized by ethidium bromide (0.5 μg/mL). Images of the RT-PCR ethidium bromide stained agarose gel was obtained using AlphaImager™ Gel Documentation, USA. Quantification of the results was done by measuring the optical density of the labelled bands, using the computerized Alpha View Software (USA). The intensities of the bands of the genes were normalized relative to that of β-actin bands. β-actin acted as control for sample to sample variations in reverse transcription and PCR condition. Each RT-PCR was repeated twice and representative results are shown.

2.6. Statistical analyses

Results are plotted as means ± SEM with the aid of Graph Pad Prism 5 by subjecting to one-way analysis of variance (ANOVA) followed by Tukey's test. $p < 0.05$ was taken as significant difference across the groups.

3. Results

3.1. 10% PMEC reduced penile malonaildehyde (pMDA) with corresponding up-production of NO levels in rats exposed to mixture of NaCl and MSG

To confirm that mixture of salt intake could cause impairment to cell membrane integrity of penis, we checked the level of penile MDA as shown in Fig. 1A. The penile nitric oxide (NO) level was equally

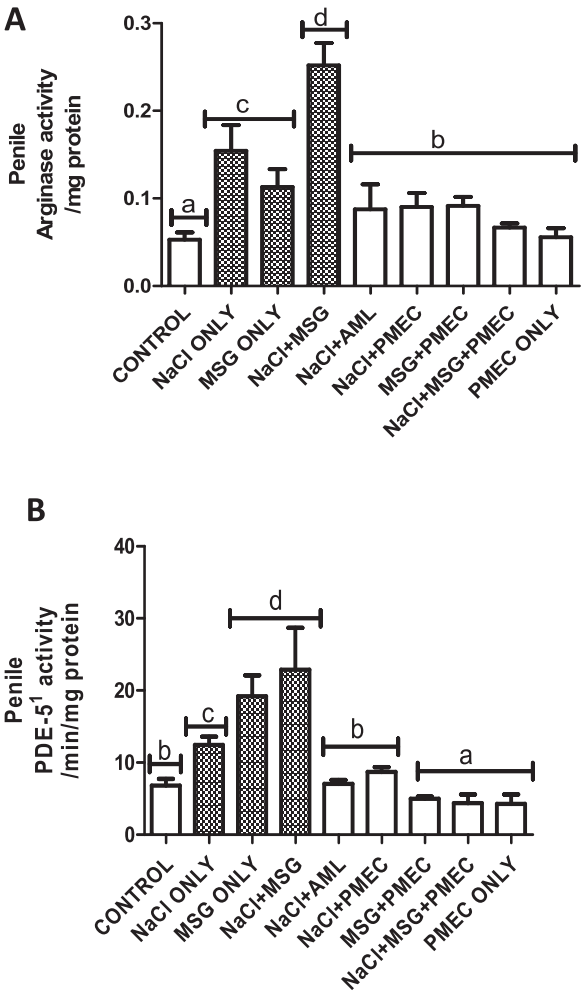

Fig. 2. Effect of PMEC on penile arginase activity (graph A) and penile PDE-5[1] activity (graph B) in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG).Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at [a] $p < 0.05$, [b] $p < 0.05$, [c] $p < 0.05$, [d] $p < 0.05$

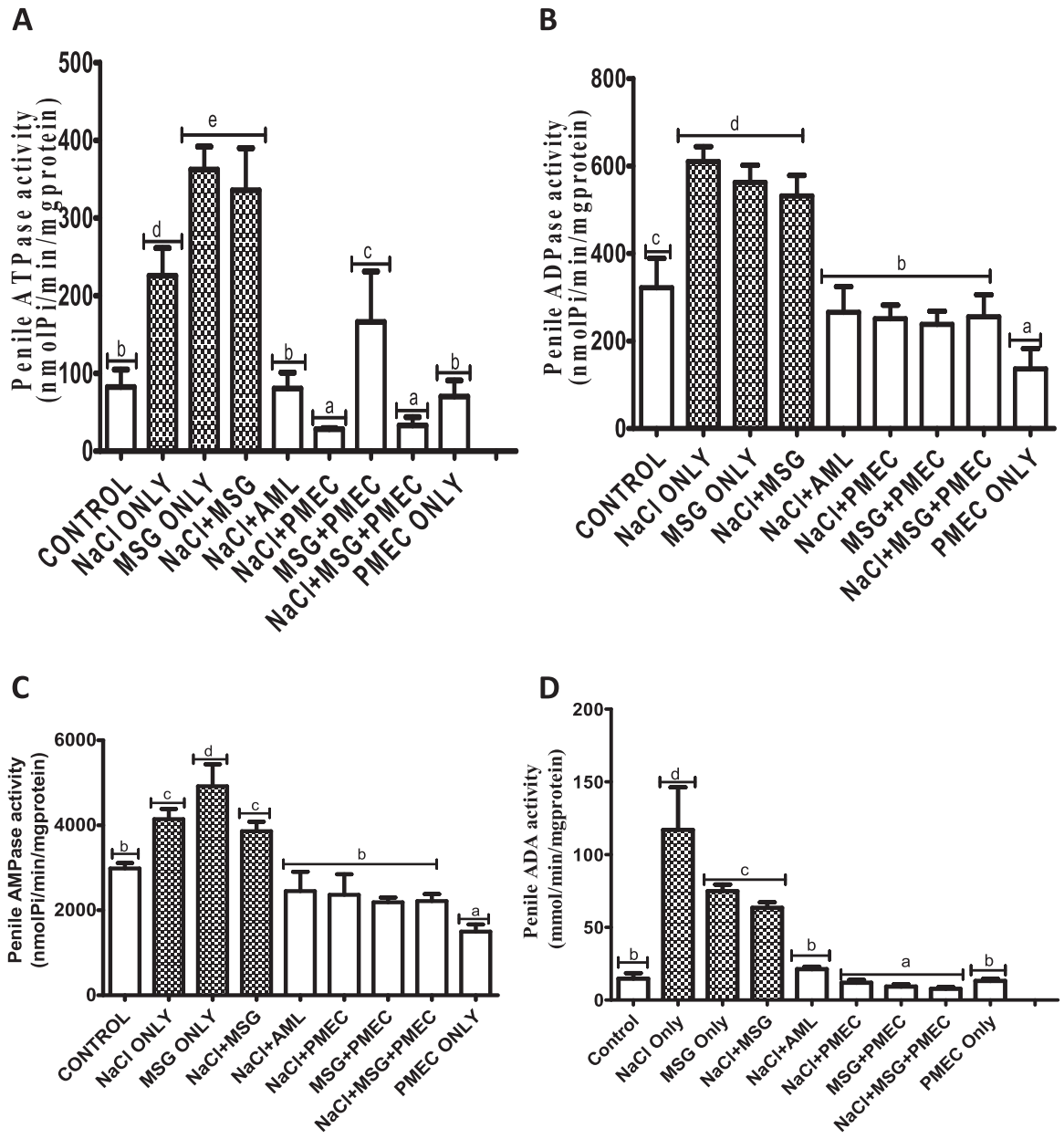

Fig. 3. Effect of PMEC on penile extracellular hydrolysis of ATP and adenosine deaminase activity in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). (ATP as substrate, graph A; ADP as substrate, graph B); 5^1-nucleotidase (AMP as substrate, graph C) and adenosine deaminase (adenosine as substrate, graph D). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at ^{a} $p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, ^{d} $p < 0.05$, ^{d} $p < 0.05$.

examined on exposure to mixture of NaCl and MSG (Fig. 1B). Post hoc analysis showed that NaCl only, MSG only and NaCl + MSG significantly ($p < 0.05$) increased the pMDA content when compared with the control group (Fig. 1A). The mixture of salt intake in rats (NaCl+MSG) did not show significant increase ($p > 0.05$) in relation to NaCl only and MSG only, on sub-acute ingestion for 14 days (Figure1A). The post-treatment of rats with PMEC, a dietary cake for 14 days significantly ($p < 0.05$) restored cell membrane integrity to the therapeutic drug (NaCl + AML) rats by inhibition of penile MDA level in NaCl only, MSG only and NaCl + MSG (Fig. 1A). Whereas, therapeutic drug (NaCl+AML) reduced the effect of the salt intake above the control rats. We also looked at the penile NO level, whether it follows NO-cGMP-PKG pathway. As observed in Fig. 1B, NaCl only, MSG only and mixture of NaCl+MSG pointedly ($p < 0.05$) depleted NO level when compared with the control group. The sub-acute mixture of NaCl and MSG (NaCl+MSG) to rats showed no significant penile NO decrease ($p > 0.05$) in relation to NaCl only and MSG only (Fig. 1B). However, the treatment with dietary cake significantly ($p < 0.05$) restored the penile function through up-regulation of NO content in NaCl only, MSG only and NaCl + MSG (Fig. 1B).

3.2. *Effect of 10% PMEC, a dietary cake on the activities of penile arginase and PDE-5[1] in rats exposed to mixture of NaCl and MSG*

To assess the effect of dietary cake (10% PMEC) on arginase and PDE-5^1 activities, both single and combined exposure to NaCl and MSG for 14 days were treated with 10% PMEC for 14 days (Fig. 2). Sub-acute exposure to NaCl only, MSG only and mixture of NaCl+MSG markedly ($p < 0.05$) increased the activity of penile arginase enzyme in relation to the control rats (Fig. 2A). Combined exposure (NaCl+MSG) for 14 days showed significant increase ($p < 0.05$) of arginase activity in relation to NaCl only and MSG only (Fig. 2A). Inversely, the noticeable hike in

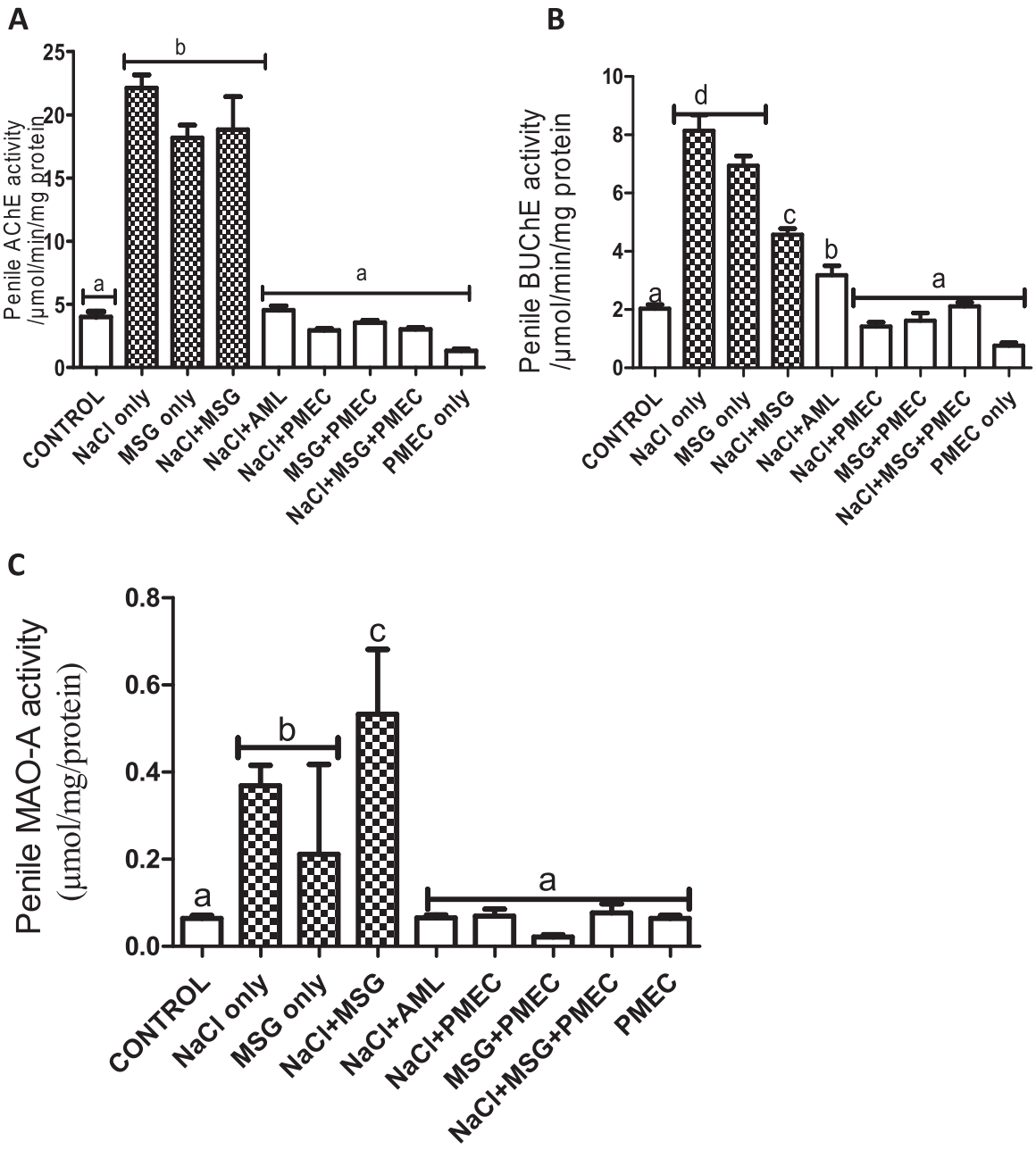

Fig. 4. Effect of PMEC on penile AChE activity (graph A), penile BuChE activity (graph B) and penile MAO-A activity (graph C) in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at ^{a} $p < 0.05$, $^{b}p < 0.05$, ^{c} $p < 0.05$, ^{d} $p < 0.05$.

penile arginase activity was significantly ($p < 0.05$) reverted to therapeutic drug rats (NaCl+AML) in all the groups treated with dietary 10% PMEC (Fig. 2A). Similarly, sub-acute exposure to NaCl only, MSG only and NaCl+MSG remarkably ($p < 0.05$) up-surged the activity of PDE-5[1] when compared with the control rats (Fig. 2B). Mixture of NaCl+MSG for 14 days showed significant increase ($p < 0.05$) of PDE-5[1] activity in relation to NaCl only and MSG only (Fig. 2B). However, the management of rats with 10% PMEC on sub-acute exposure to NaCl only (NaCl+PMEC) significantly ($p < 0.05$) down-regulated the penile PDE-5[1] activity to normal and therapeutic (NaCl+AML) drug rats (Fig. 2B). Whereas; MSG+PMEC, NaCl+MSG+PMEC and PMEC only significantly decreased ($p < 0.05$) the penile PDE-5[1] activity better than NaCl+AML and NaCl + PMEC rats (Fig. 2B).

3.3. Effect of 10% PMEC, a dietary cake on the extracellular hydrolysis of ATP and adenosine deaminase activity in rats exposed to mixture of NaCl and MSG

Fig. 3 shows the effect of PMEC on the action of ectonucleotidases, NTPDase (ATP as substrate, graph A; ADP as substrate, graph B) 5[1]-nucleotidase (AMP as substrate, graph C) and ADA (adenosine as substrate, graph D) in rats exposed to mixture of NaCl and MSG. Firstly, the set of animals exposed to NaCl only, MSG only and NaCl + MSG for 14 days significantly ($p < 0.05$) amplified the activity of (ATPase) ATP hydrolysis when compared with the control rats (Fig. 3A). The MSG only and the exposure of NaCl+MSG to rats showed significant elevation ($p > 0.05$) of ATP hydrolysis in relation to NaCl only (Fig. 3A). Management with therapeutic drug (NaCl+AML) and 10% PMEC only restored the ATP hydrolysis to normal (Fig. 3A). Ultimately, 10% PMEC

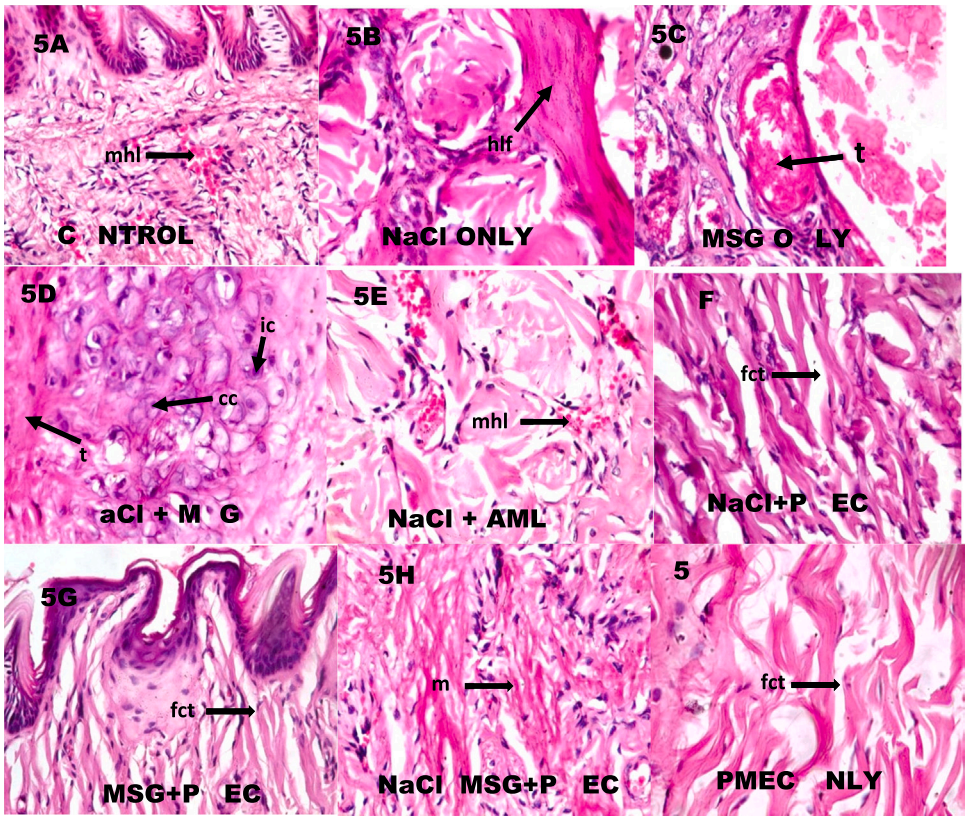

Fig. 5. Photomicrographs of penile histopathology (H & E X400).Control: The penile tissue shows minor hemorrhagic lesion (mhl). NaCl only: The penile tissue shows hemorrhagic lesion with fibrosis (hlf). MSG only: The penile tissue shows thrombus (t) in the corpus cavernosum. NaCl +MSG: The corpus cavernosum is calcified with numerous chondrocytes (cc), and the penile tissue shows trifling fibrosis (tf) with presence of inflammatory cells (ic). NaCl +AML: The penile tissue shows minor hemorrhagic lesion (mhl). NaCl + PMEC: The penile tissue shows free connective tissue (fct) in the corpus cavernosum. MSG +PMEC: The penile tissue shows free connective tissue (fct) in the corpus cavernosum. NaCl + MSG+PMEC: The penile tissue shows minor fibrosis (mf). PMEC only: The penile tissue shows free connective tissue (fct) in the corpus cavernosum.

treatment for 14 days on exposure to NaCl only and NaCl+MSG significantly ($p < 0.05$) normalized the ATP hydrolysis below therapeutic drug (NaCl+AML) and the control (Fig. 3A); and better in action than MSG+PMEC. Afterwards, as reported in Fig. 3B, exposure of rats to NaCl only, MSG only and NaCl+MSG for 14 days significantly ($p < 0.05$) increased the penile ADPase activity in relation to the control rats. Whereas, combined exposure to NaCl +MSG for 14 days did not show significant increase ($p > 0.05$) of penile ADPase activity in relation to NaCl only and MSG only (Fig. 3B). Treatment with 10% PMEC for 14 days significantly ($p < 0.05$) depressed the activity of penile ADPase to below normal in all the groups (Fig. 3B). Thirdly, as shown in Fig. 3C, the activity of 5^1-nucleotidase (AMPase) was aberrantly ($p < 0.05$) raised in NaCl only, MSG only and NaCl+MSG rats when compared with the control rats. The animal exposed to MSG only showed significant increase ($p > 0.05$) in relation to NaCl only and NaCl+MSG (Fig. 3C). Interestingly, management with dietary cake (10% PMEC) for 14 days significantly diminished ($p < 0.05$) the activity of penile AMPase to normal (Fig. 3C). Lastly, sub-acute exposure of rats to NaCl only, MSG only and NaCl+MSG for 14 days significantly ($p < 0.05$) hiked the penile ADA activity in relation to the control rats (Fig. 3D). The animals exposed to NaCl only remarkably increased ($p > 0.05$) the penile ADA activity in relation to MSG only and NaCl+MSG (Fig. 3D). But, treatment with dietary 10% PMEC significantly ($p < 0.05$) down-regulated the penile ADA activity to below therapeutic drug rats (NaCl+AML) and normal (Fig. 3D).

3.4. Effect of 10% PMEC, a dietary cake on the activity of penile neurotransmitter enzymes (AChE, BuChE and MAO-A) in rats exposed to mixture of NaCl and MSG

To explore the possible links between the neuro-transduction enzymes and penile function, we examined the effect of dietary cake (10% PMEC) on the activity of penile AChE, BuChE, MAO-A enzymes on exposure to mixture of NaCl and MSG in rats (Fig. 4). Firstly, the group of animals sub-acutely exposed to NaCl only, MSG only and NaCl + MSG for 14 days awkwardly ($p < 0.05$) mediated the penile AChE activity when compared with the control rats (Fig. 4A). The mixture of NaCl+MSG apparently did not show significant difference ($p > 0.05$) in penile AChE activity when compared with NaCl only and MSG only (Fig. 4A). The therapeutic drug (NaCl+AML) rats and the groups of animals treated with dietary cake (10% PMEC) significantly ($p < 0.05$) normalized the penile AChE activity (Fig. 4A). Again, as shown in Fig. 4B, post hoc study reported that the rats exposed to NaCl only, MSG only and NaCl+MSG for 14 days significantly ($p < 0.05$) triggered the penile BuChE activity when compared with the control rats. Whereas, set of rats sub-acutely exposed to NaCl only and MSG only significantly ($p < 0.05$) increased the penile BuChE activity in relation to NaCl+MSG rats (Fig. 4B). Post-administration of 10% PMEC for 14 days significantly ($p < 0.05$) lowered the activity of penile BuChE to normal in relation to therapeutic drug (NaCl+AML) rats (Fig. 4B). Finally, as shown in Fig. 4C, the activity of penile MAO-A was abnormally ($p < 0.05$) higher in NaCl only, MSG only and NaCl+MSG when compared with the control rats. Sub-acute exposure of rats to the mixture of NaCl and MSG significantly ($p < 0.05$) increased the penile MAO-A activity in relation to NaCl only and MSG only (Fig. 4C). However, post-treatment with 10% PMEC for 14 days significantly declined ($p < 0.05$) the activity of penile MAO-A to normal (Fig. 4C). The same trend of reduced penile MAO-A activity was discovered in animals treated with therapeutic drug (NaCl+AML).

3.5. Dietary cake (10% PMEC) reduces histopathological lesions in the corpus cavernosum

The penile tissue of the control rats shows minor hemorrhagic lesion (5 A). Whereas, sub-acute exposure of rats to NaCl and MSG for 14 days shows hemorrhagic lesion, fibrosis (5B) and thrombus in the corpus cavernosum (Fig. 5 C). The corpus cavernosum is calcified with numerous chondrocytes manifested by the presence of inflammatory

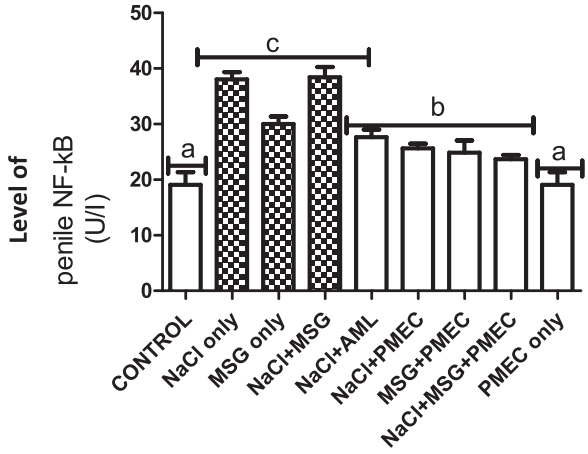

Fig. 6. Effect of PMEC on penile NF-kB level in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at [a] $p < 0.05$, [b] $p < 0.05$, [c] $p < 0.05$.

cells (5D). Thus, post-treatment with 10% PMEC for 14 days mitigated the morphological impairment by showing free connective tissue in the corpus cavernosum (Fig. 5 F, G and H) and minor fibrosis (5 H). Rats treated with therapeutic drug showed minor hemorrhagic lesion (Fig. 5E). Lastly, the animals treated with 10% PMEC only showed free connective tissue in the corpus cavernosum (Fig. 5I).

3.6. Effect of 10% PMEC, a dietary cake on the NF-kB dependent signaling pathway in rats exposed to mixture of NaCl and MSG

To have a better interpretation on the molecular signaling pathway of 10% PMEC in rats exposed to mixture of NaCl and MSG, we investigated the potential of 10% PMEC on the NF-kB dependent signaling pathway using ELISA method (Fig. 6). Result showed that sub-acute exposure of rats to NaCl only, MSG only and NaCl +MSG for 14 days significantly ($p < 0.05$) up-regulated penile NF-kB when compared with the control rats (Fig. 6). Joint exposure of NaCl+MSG showed no significant increase ($p > 0.05$) in relation to NaCl only and MSG only (Fig. 6). Post hoc analysis indicated that 10% PMEC treatment as well as therapeutic drug rats (NaCl+AML) for 14 days significantly ($p < 0.05$) down-regulated the penile NF-kB level (Fig. 6).

3.7. Effect of 10% PMEC, a dietary cake on the RT-PCR expression analysis of TNF-α, p53, IL-10 and HIF-1 in rats exposed to mixture of NaCl and MSG

To ascertain the possible molecular mechanism by which 10% PMEC inhibits inflammation, apoptosis and erectile vasoconstriction syndrome in rats, the expression of pro-inflammatory mediators (TNF-α and IL-10), apoptotic protein (p53) and erectile hypertensive marker (HIF-1) were analyzed by RT-PCR analysis. RT-PCR analysis revealed that sub-acute exposure to NaCl only, MSG only and mixture of NaCl+MSG significantly ($p < 0.05$) up-regulated the expression of penile TNF-α protein when compared with the control (Fig. 7A). Combined exposure of NaCl+MSG to rats for 14 days showed significant ($p < 0.05$) expression of penile TNF-α protein in relation to NaCl only and MSG only (Fig. 7A). Post-treatment of 10% PMEC for 14 days significantly ($p < 0.05$) down-regulated the expression of TNF-α protein to therapeutic drug rats (NaCl+AML). Again, as observed in Fig. 7B, exposure to NaCl only, MSG only and mixture of NaCl+MSG for 14 days remarkably ($p < 0.05$) up-regulated the expression of penile p53 protein when compared with the control. Joint exposure of NaCl+MSG to rats for 14 days showed no significant ($p > 0.05$) expression of penile p53 protein in relation to NaCl only and MSG only (Fig. 7B). However, post-treatment of 10% PMEC for 14 days significantly ($p < 0.05$) down-regulated the expression of penile p53 protein to therapeutic drug rats (NaCl+AML). Interestingly, PMEC only showed no expression of p53 protein (Fig. 7B). Similarly, sub-acute exposure to NaCl only, MSG only and NaCl+MSG for 14 days aberrantly ($p < 0.05$) up-regulated the expression of penile IL-10 protein when compared with the control (Fig. 8A). Whereas, MSG only and NaCl+MSG significantly ($p > 0.05$) up-regulated the expression of penile IL-10 protein in relation to NaCl only (Fig. 8A). Conversely, therapeutic drug rats (NaCl+AML) and NaCl+PMEC significantly ($p < 0.05$) attenuated the penile IL-10 protein to normal (Fig. 8A). While, MSG+PMEC, NaCl+MSG+PMEC and PMEC only significantly ($p < 0.05$) prohibited the penile IL-10 protein below normal and therapeutic drug rats (Fig. 8A). Lastly, sub-acute exposure to NaCl only, MSG only and NaCl +MSG for 14 days markedly ($p < 0.05$) heightened the expression of penile HIF-1 protein in relation to control rats (Fig. 8B). Whereas, NaCl only and MSG only significantly ($p < 0.05$) down-regulated the expression of penile HIF-1 protein when compared with NaCl+ MSG rats (Fig. 8B). Therapeutic drug rats significantly ($p < 0.05$) proscribed the increased HIF-1 to normal (Fig. 8B). Remarkably, dietary cake of 10% PMEC abolished the up-regulation of penile HIF-1 in all the treated groups (Fig. 8B).

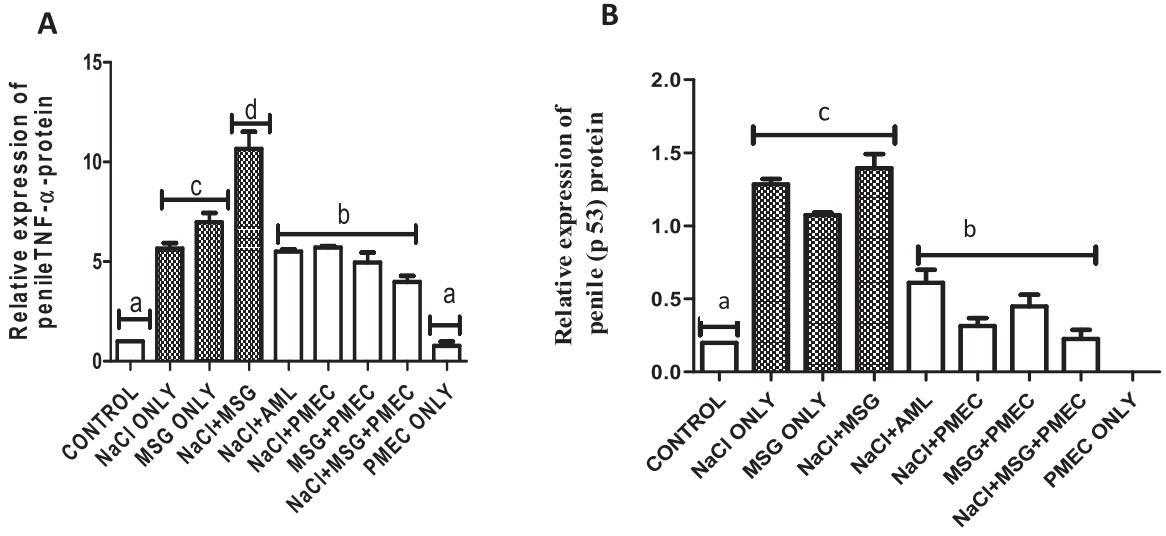

Fig. 7. Effect of PMEC on penile TNF-α protein (graph A) and penile p53 protein (graph B) in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at [a] $p < 0.05$, [b] $p < 0.05$, [c] $p < 0.05$, [d] $p < 0.05$.

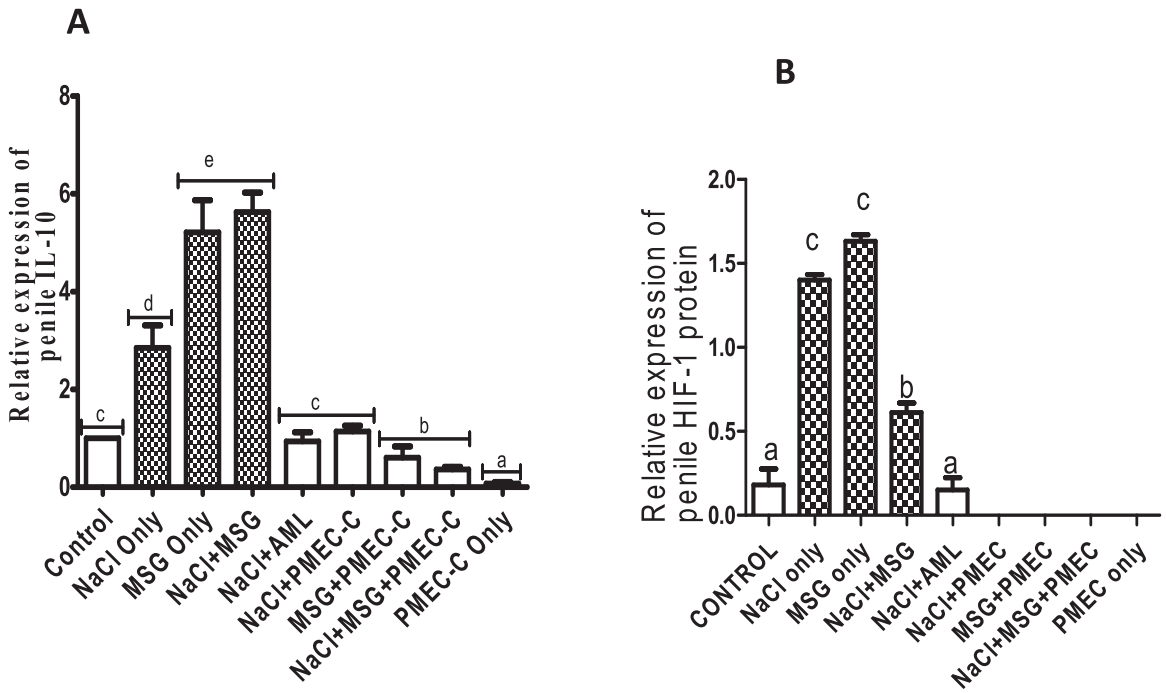

Fig. 8. Effect of PMEC on penile IL-10 protein (graph A) and penile HIF-1 protein (graph B) in rats administered with to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, $^{d}p < 0.05$, $^{e}p < 0.05$

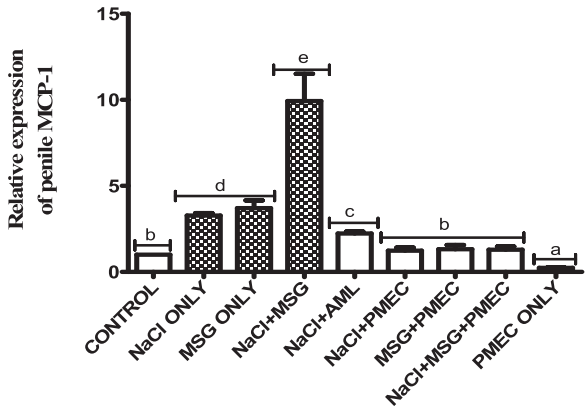

Fig. 9. Effect of PMEC on chemokine MCP-1 protein in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG). Results are expressed as mean ± SEM (n = 10). Bars with different letters are significantly different at $^{a}p < 0.05$, $^{b}p < 0.05$ $^{c}p < 0.05$, $^{d}p < 0.05$, $^{e}p < 0.05$.

3.8. Effect of 10% PMEC, a dietary cake on the RT-PCR expression analysis of chemokine MCP-1 in rats exposed to mixture of sodium chloride (NaCl) and monosodium glutamate (MSG)

The effect 10% PMEC (dietary cake) on RT-PCR expression analysis of chemokine MCP-1 in rats exposed to mixture of NaCl and MSG was presented in Fig. 9. As shown, sub-acute exposure to NaCl only, MSG only and mixture of NaCl+MSG for 14 days significantly ($p < 0.05$) up-regulated the penile MCP-1 expression than the control. Exposure of combined NaCl and MSG (NaCl+MSG) to rats for 14 days abnormally ($p < 0.05$) up-regulated the penile MCP-1 protein in relation to NaCl only and MSG only (Fig. 9). Post-treatment with therapeutic drug-amylopidin for 14 days showed no significant difference ($p > 0.05$) in comparison with NaCl only and MSG only (Fig. 9). Whereas, post- dietary intake of 10% PMEC significantly ($p < 0.05$) prohibited the up-regulation of penile MCP-1 expression to normal rats (Fig. 9).

4. Discussion

This study supports the hypothesis that 10% PMEC containing high proportion of protein by factor of 4 (25%), reduced inflammatory erectile dysfunction initiated by NaCl/MSG in rat model and its modulation was linked to NO-cGMP-PKG and NF-κB dependent signaling pathways. Fundamentally, reports have connected metabolic disorder and ED with inflammation [3,15] with limited understanding of its management. Also, the toxic effects of mixture of salt intake on ED associated with inflammation have not been highlighted in mammals. Evidently, our finding underscored that exposure to NaCl only, MSG only and their mixture compromised the penile membrane integrity within two weeks by augmenting the penile MDA, end product of lipid peroxidation. Compared with the normal rats, the up-production of penile MDA level on exposure to NaCl and MSG may be attributed to the metabolic disorder and reduction of penile endothelial dysfunction [3], causing increased inhibition of blood flow in the penile vessels. Individuals with increased penile MDA levels were linked to metabolic syndrome, reduced endothelial function score and ED [7]. Interestingly, the level of penile MDA, which can activate the inflammatory-endothelial response [3] was abrogated by dietary cake of 10% PMEC. Conversely, continuous exposure to NaCl only, MSG only and its mixture (NaCl+MSG) for 14 days caused penile low bio-availability of NO level. This signifies that the continual salt intake for 14 days prevented NO diffusion to the adjacent smooth muscle cells of the penis, which activates guanylate cyclase (GC). It can also be explained here that this enzyme (GC) that converts guanosine triphosphate to its active cyclic guanosine monophosphate (cGMP) had been impeded by continuous exposure to salt intake for two weeks. However, the supposed deactivation of protein kinase G, and cGMP by NaCl/MSG intoxication increased intracellular calcium levels that shrinks the vascular smooth muscle cells resulting in penile vasoconstriction known as erectile dysfunction. Nonetheless, the consumption of protein-rich-cake (%10 PMEC) by factor of 4 caused penile vasodilation through improved NO bio-availability. From this observation, it is essential to report here that the management of ED by protein-rich-cake

(10%PMEC) on exposure to NaCl/MSG intake may follow NO-cGMP-PKG signaling pathway.

Additionally, continuous exposure to both single and combined salt intake (NaCl and MSG) for 14 days impaired endothelial-derived NO signaling by increasing the expression of arginase activity. This contributes to the reduction of endothelium-dependent erectile responses [3] in rats via the scavenging of intracellular L-arginine. Study had shown that L-arginine is the common substrate for both NOS and arginase and arginase activity can inhibit endothelium-derived NO production via depletion of the available substrate responsible for its biosynthesis [8]. However, the protein-rich-cake (10%PMEC) by a factor of 4 (25%) pharmacologically inhibited the arginase activity to restore erectile function [39]. The decline in arginase activity in the present study may also be attributed to increased bio-availability of NO in the penile vascular cells [40]. It is very imperative to report here that protein-rich-cake (10%PMEC) that contain flavonoids, carotenoids and phenols may retard arginase activity. Inhibition of penile arginase activity has been depleted by a variety of natural antioxidants [41, 42, 43]. Correspondingly, continuous intake of NaCl only, MSG only and NaCl+MSG for 14 days remarkably up-surged the activity of PDE-5^1 in relation to normal rats. The increased PDE-5^1 on exposure to salt intake may bind to the catalytic site of cGMP or potentiate the phosphorylation of PKG to cause cGMP degradation [44]. As PDE-5^1 is specific to cGMP, study has reported that high expression of PDE-5^1 activity mediates muscle activation that results in penile dysfunction [39]. However, the consumption of protein-rich-cake (10%PMEC) by a factor of 4 (25%) noticeably down surged the activity of penile PDE-5^1, suggesting that the endothelial cells directly released nitric oxide in the penis, to stimulate guanylyl cyclase for production of cGMP levels and to lower the intracellular calcium levels [44]. Altogether, relaxing the penile smooth muscle, for its dilatation, and erection [8]. It is very imperative to report here that modulation of erectile dysfunction by protein-rich-cake (10% PME) on exposure to salt intake for two weeks was through NO-cGMP-PKG signaling pathways.

Moreover, adenosine is a potent vasorelaxant [8]. It can be both intracellularly and extracellularly synthesized through degradation of adenine nucleotides [45]. It has been reported that impaired adenosine signaling is associated with ED [46]. Excessive production of adenosine has been linked to priapism [46] and being used to diagnose and treat ED and priapism [47]. In our study, the set of animals repeatedly exposed to NaCl/or MSG intake for 14 days activated the activity of ATP hydrolysis (ATPase, ADPase and AMPase) and ADA. This validates that extracellular adenine nucleotides were dephosphorylated by the intake of NaCl/MSG for 14 days. This eventually hydrolyzes ATP to ADP and ADP to AMP [12], while AMP was catalyzed by 5^1 ectonucleotidase on exposure to mixture of salt intake. This metabolic reaction abnormally elongates penile erection called priapism [46]. Past studies had demonstrated that priapism (abnormal prolonged penile erection) in absence of sexual excitation could be linked to ischemia-mediated erectile tissue damage [46,47]. This suggests that chronic exposure to salt intake may initiate pathology (Priapism) in rats due to quick conversion of adenosine to inosine [48]. The reduced level of intracellular adenosine had been attributed to low NO bioavailabilty to initiate impair-mediated penile erection [10]. While, excessive accumulation of inosine can alter penile function [7]. The remarkable increase in penile ATPase, ADPase, AMPase and ADA activities corroborates our previous finding [15]. The post-treatment with protein-rich cake (%10PMEC) by a factor of 4 (25%) for 14 days decreased the activities of ATPase, ADPase, AMPase and ADA to normal, suggesting the stabilization of penile ATP. Similarly, elevation in the activities of penile AChE, BuChE and MAO-A on exposure to NaCl/MSG signifies impairment to penile cholinergic and dopaminergic cascades [49], whereas, their depletion by protein-rich-cake (10% PMEC) by a factor of 4, suggests normal functions of the synaptic cells in the penis.

Also, a pleiotropic transcription factor called NF-kB plays important roles in the immune system. Members of the NF-kB family regulate the transcriptional activity of several cell surface receptors, transcription factors, adhesion molecules, and promoters of pro-inflammatory cytokines that are implicated in inflammation [50, 51, 52]. Our finding shows that sub-acute exposure (14 days) of rats to NaCl only, MSG only and NaCl + MSG up-regulated the penile NF-kB molecule, reflecting endothelial dysfunction that contributes to erectile dysfunction [53]. The increased expression of NF-kB can implicate endothelial dysfunction. This causes the blood vessels to constrict and reduction in the blood flow [54]. Hence, the decreased blood flow triggered by NaCl and MSG intake in this study was responsible for erectile dysfunction through NF-kB dependent signaling pathway. It was also indicated that up-regulation of NF-kB transcript may be because the endothelial cells was malfunctioned and could not produce enough nitric oxide for the relaxation of penile blood vessels [55]. Thus, the blood flow needed to achieve penile erection have been depleted due to the generation of NF-kB. Study has shown that NF-kB is one of the inflammatory molecules in the body that gets elevated during the inflammation [53]. Thus, this study may report here that when NF-kB is elevated, endothelial cells may produce low nitric oxide. Also, the histology examination in this study, indicated penile hemorrhagic lesion, fibrosis while corpus cavernosum was calcified with numerous chondrocytes and the presence of inflammatory cells. This provides evidence for inflammatory erectile dysfunction [56,57]. A down regulation of penile NF-kB by protein-rich cake (% 10PMEC) for 14 days, in this study, suggests restoration of endothelial function to normal. This could further be linked to normalization of the penile function via NF-kB dependent signaling cascade.

High intake of salt (NaCl/MSG) promotes oxidative damage and inflammation through many mechanisms. These include alteration of cellular membrane function [58], apoptosis and up-regulation of cytokines [59]. In this study, we identified the potential role of TNF-α and p53 genes in relation to penile dysfunction using RT-PCR analysis. It was observed that exposure to salt particularly NaCl for 14 days initiated inflammatory erectile dysfunction by increasing the levels of penile TNF-α and p53 molecules. Also, increased levels of penile TNF-α and p53 mediated by MSG suggests its relationship between inflammation and ED [60,61]. Also, increased activity of penile arginase (regulatory enzyme for NO production) and PDE-5^1 further validated the mechanism of IED in rats. Remarkably, the post-treatment with protein-rich-cake by a factor of 4 (10% PMEC) down-regulated the levels of apoptotic cytokines (TNF-α and p53), signifying its protective potential to the penis through NO-cGMP-PKG signaling pathway. Equally, protein-rich-cake by a factor of 4 (%PMEC) reduced the structural damage in the penis provoked by the NaCl and MSG. The normal corpus cavernosum elcited by protein-rich-cake (%PMEC) was apparently connected to the low expression of penile apoptotic indicators, which was motivated by the active flavonoids in PMEC [22]. The 10% PMEC-treated rats also experienced a decrease in the level of penile TNF-α, whereas, this did not occur in NaCl-MSG induced rats, indicating TNF-α inhibition reduces penile trauma and inflammation [62]. A recent study has reported that TNF-α inhibition slowed the progression of inflammation to reduce cellular injury [63], suggesting the role of TNF-α in ED in rat model.

IL-10 is also known as human cytokine synthesis inhibitory factor (CSIF). It is a multi-functional cytokine with potent anti-inflammatory potential and encoded by the IL-10 gene. Contrary to the previous studies [64], intake of 80 mg/kg NaCl and 75 mg/kg MSG for 14 days increased the expression of penile IL-10 transcript. The increased IL-10 on exposure to salt was because the body produced more irreversible inflammatory responses to limit or terminate ED [65]. Consistent with our observation, a recent finding reported that high IL-10 level in systemic lupus erythematous (SLE) patients was pathogenic while its blockage ameliorates the disease [66]. In this perspective, we speculated that up-regulation of IL-10 on exposure to NaCl/MSG might have resulted into multi-organ systemic autoimmune disease [67] known as SLE i.e. a condition whereby immune system attacks its own tissues,

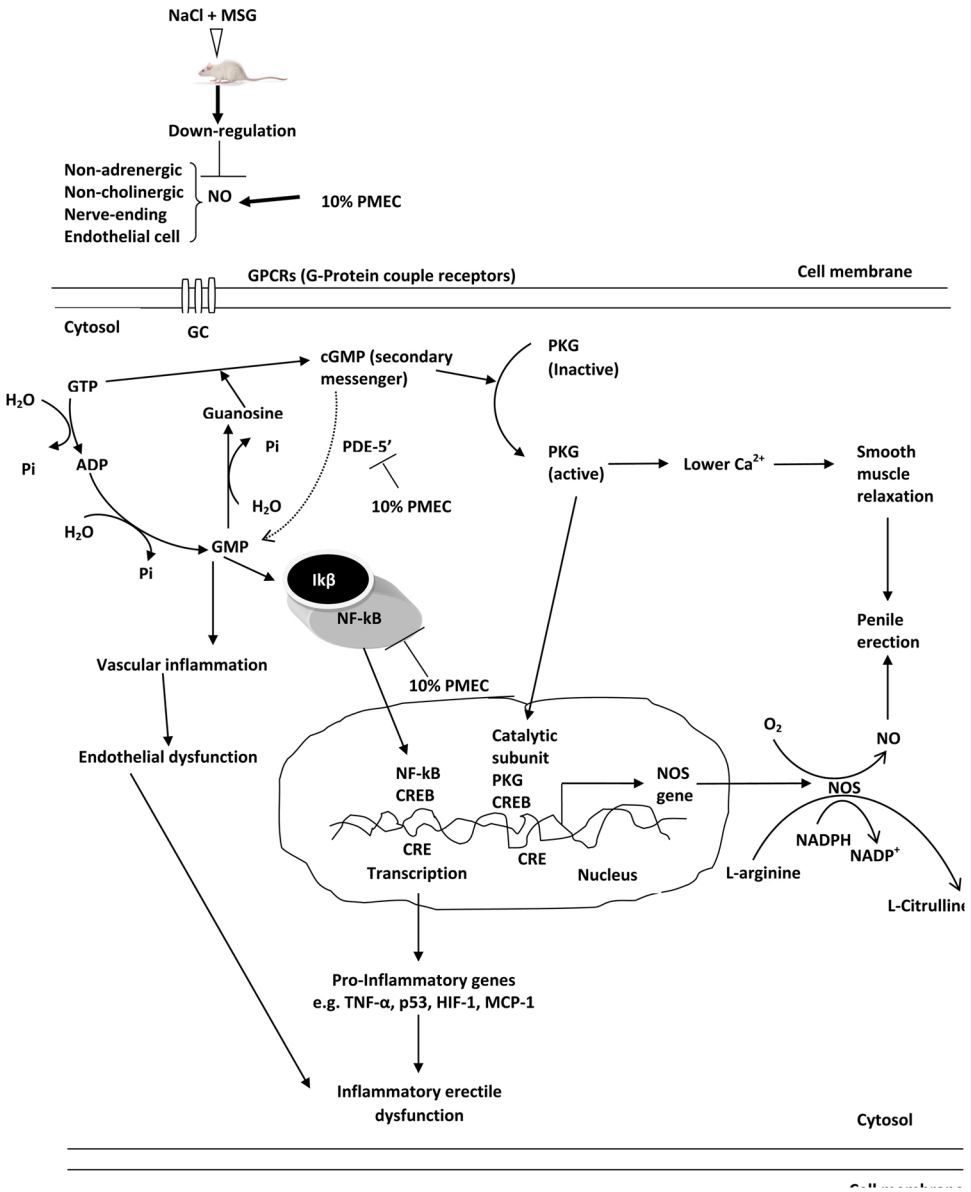

Fig. 10. Protein-rich cake (10% PMEC) by a factor of 4 downregulates cytokines/MCP-1 through NO-cGMP-PKG dependent βB signaling cascade and attenuates erectile dysfunction activated by mixture of salt intake in rat model. Intoxication with NaCl only, MSG only and its mixture (NaCl+MSG) increased the activities of arginase and phosphodiesterase-5[1] (PDE-5[1]) with corresponding decline of nitric oxide (NO). Post-treatment with protein-rich cake (10%PMEC) by a factor of 4 reverted the increased activities of arginase and PDE-5[1] with equivalent bio-availability of high NO. Up-regulated NO activates guanylyl cyclase to induce the synthesis of cyclic guanosine monophosphate (cGMP). NO-mediated cGMP induction is capable of inducing protein kinase G. The resultant decline in calcium/calmodulin-dependent myosin light chain phosphorylation advanced smooth muscle relaxation. Thus, the increased blood flow in the endothelial cavernosal smooth muscle cells triggered penile erection. Also, accumulation of GMP, cGMP and PKG induced phosphorylation and degradation of inhibitory kinase-β (Ikβ) for free translocation of NF-kB into the nucleus where it binds to the site with NF-kB consensus motif, thereby activating the transcription of pro-inflammatory target genes to initiate inflammatory erectile dysfunction. In contrast, 10% PMEC inhibits NF-kB, TNF-α, HIF-1, p53 and MCP-1 with corresponding translocation of PKG into the nucleus. PKG directly phosphorylates cGMP-response element binding protein (CREB) for CRE-dependent transcription of nitric oxide synthase (NOS) gene, resulting in penile erection.

causing inflammation and ED. This pathogenic mechanism of SLE may also be accompanied by immunological abnormalities including apoptotic cell clearance, hyperactivity of T and B cells to elicit auto-antibody production or immune complex formation or direct antibody-mediated cytotoxicity [66]. However, post-hock analysis revealed that administration of rats with protein-rich-cake (10% PMEC) by a factor of 4 blocked IL-10 production to decrease auto-antibody creation [67]. This finding further hypothesized that post-treatment with 10% PMEC by a factor of 4 may prevent Psoriasis by suppressing auto-antibody which may cause ED [68]. A study reported that men with Psoriasis are more susceptible to severe ED arising from sexual difficulties, stigmatization, depression or psychological impairment [69]. It is therefore pertinent to report here that 10% PMEC (protein-rich-cake by a factor of 4) may be an immunotherapeutic agent against SLE and psoriasis, although future studies are warranted on this.

Hypoxia-inducible factor 1 (HIF-1) is an heterodimeric transcription factor which mediates cellular responses in the state of low oxygen to activate the specific genes involved in tumourigenesis and angiogenesis [70]. We examined whether activated NF-kB and increased hypoxia inducible factor-1 (HIF-1) in the presence of low NO would trigger ED. It was apparently observed that high expression of penile HIF-1 upon exposure to NaCl and/MSG activated the NF-κB transcription with low production of NO level. This implies that penile cells showed high level of hypoxia upon excessive salt intake which in turns, produced pro-inflammatory mediators for ED [71]. Also, over expression of penile HIF-1 was linked to the penile intratumoral hypoxia [72] with low production of vasodilator (NO). This was to recruit more immune cells and several transcriptional factors including IL-6, COX-2 and NF-kB [73]. Altogether, HIF-1 signaling cascade mediates hypoxic inflammation [74] to inhibit penile cells or sex cells from differentiating causing male infertility and ED [75]. We therefore record here that up-regulation of penile HIF-1 transcript when exposed to NaCl/MSG for 14 days in rats promotes hypoxia condition and stimulates the release of penile NF-kB at the “on state” of the immune response by depleting the production of nitric oxide [76]. In this study, we postulated that the protein-rich-cake may restrict hypoxia inflammatory responses and whether it could prevent erectile dysfunction. Post-administration of 10% PMEC (protein-rich-cake by a factor of 4) for 14 days prohibited the over expression of HIF-1, suggesting preservation of penile stem cells and normal immune responses [77]. Thus, based on the present study, it was conjectured that NO bioavailability with declined HIF-1 could normalize NF-kB level. This could be a molecular mechanism for

restoring penile erection.

Monocyte Chemoattractant Protein-1(MCP-1) could be used to manage inflammatory-mediated erectile dysfunction (IMED). As discovered in this study, MCP-1 was highly expressed in penile homogenate of rats exposed to NaCl only, MSG only and NaCl+MSG. The up-regulation of MCP-1 suggested the production of adhesive penile molecules, to facilitate the macrophage infiltration into penile tissues [78] to elicit IMED. We are objective to report here that a chemokine-mediated inflammatory component is involved in the onset of ED on exposure to NaCl/MSG salt. However, this study also showed that the blockage of MCP-1 on treatment with protein-rich-cake (10% PMEC) by a factor of 4 suggested that penile inflammation was reduced on exposure to NaCl/MSG for 14 days. Interestingly, the low level of MCP-1 transcript was linked to the anti-inflammatory potential of the phenolic compounds found in protein-rich-cake (10%PMEC). This implies that protein-rich-cake reduced penile NF-kB and penile TNF-α activation. Studies had shown that consumption of protein- rich-diet can prevent hepatic inflammation [79] and that inhibition of NF-kB signifies little or no ED in inflammatory rats [80]. It was also reported that MCP-1 could be low in male folks without ED when NF-kB is inhibited [81]. Thus, one of the mechanisms for the inhibition of IMED in the present study was due to the anti-inflammatory effect of 10%PMEC via inhibition of MCP-1 and NF-kB depletion. This anti-inflammatory effect was likely because the agonist (10%PMEC) did not alter erection or it has boosted ATP for the erection of penis in rats.

Apparently, this study showed that TNF-α inhibition lowered NF-kB expression and MCP-1 was reduced, following the treatment with a protein-rich-cake (10%PMEC) by a factor of 4. Consistent with our finding, Park et al. [81] and Unsworth et al. [82] reported that MCP-1 activation induced NF-kB expression, while NF-kB inhibition was reported to prevent TNF-α and MCP-1 inflammatory responses in humans. Thus, it is possible that reduced MCP-1 activation by inhibiting NF-kB with down-regulation of TNF-α in rats treated with protein-rich-cake (10% PMEC) by a factor of 4 could account for the delayed progression of IMED. However, we can interpret here that reducing MCP-1 activation with protein-rich-cake (10% PMEC) may inhibit positive feedback stimulation of TNF-α, which in turns inhibits NF-kB activation to trigger the down-regulation of inflammatory responses by immune cells.

Finally, this study observed that the combined exposure of rats to NaCl and MSG remarkably hiked the expression of TNF-α, MCP-1 and arginase activity than NaCl only or MSG only. This showed that the retention of sodium ion was higher in the body than its excretion [5]. Furthermore, high accumulation of much sodium makes the body to retain more fluids which contributes to vasoconstriction of the penile vessels, to initiate heart attack, stroke, kidney failure and erectile dysfunction [83]. Whereas, p53, ATP hydrolytic enzymes, ADA, HIF-1, IL-10, PDE-5[1] and neurotransmitter enzymes showed no significant increase between combined exposure and single exposure. This implicates that increased salt ingestion triggered the body to maintain isotonic medium of the body fluids [84]. We therefore established here that the sodium volume in extracellular fluids have been increased due to a single or combined exposure to NaCl and MSG in rats. Hence, its accumulation in the body alters sodium homeostasis [85] during the process of IMED. It was concluded that inflammation is one of the major risk factors of ED, and dietary inhibition of chemokine/cytokines with protein-rich-cake may be an alternative anti-inflammatory therapy (as shown in Fig. 10), which could be of significance to clinical care in the treatment of ED and its related risk factors.

Source of funding

This research received no external funding.

CRediT authorship contribution statement

Akintunde J.K: Conceptualization, Methodology, Investigation, Supervision, Software, Writing – original draft, Writing – review & editing. **Olayinka M.C:** Investigation, Analysis. **Ugbaja V.C:** Investigation, Analysis. **Akinfenwa C.A:** Investigation and Analysis. **Akintola T.E:** Investigation, Supervision. **Akamo A.J:** Supervision **Bello A.J:** Research contribution.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgment

None.

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