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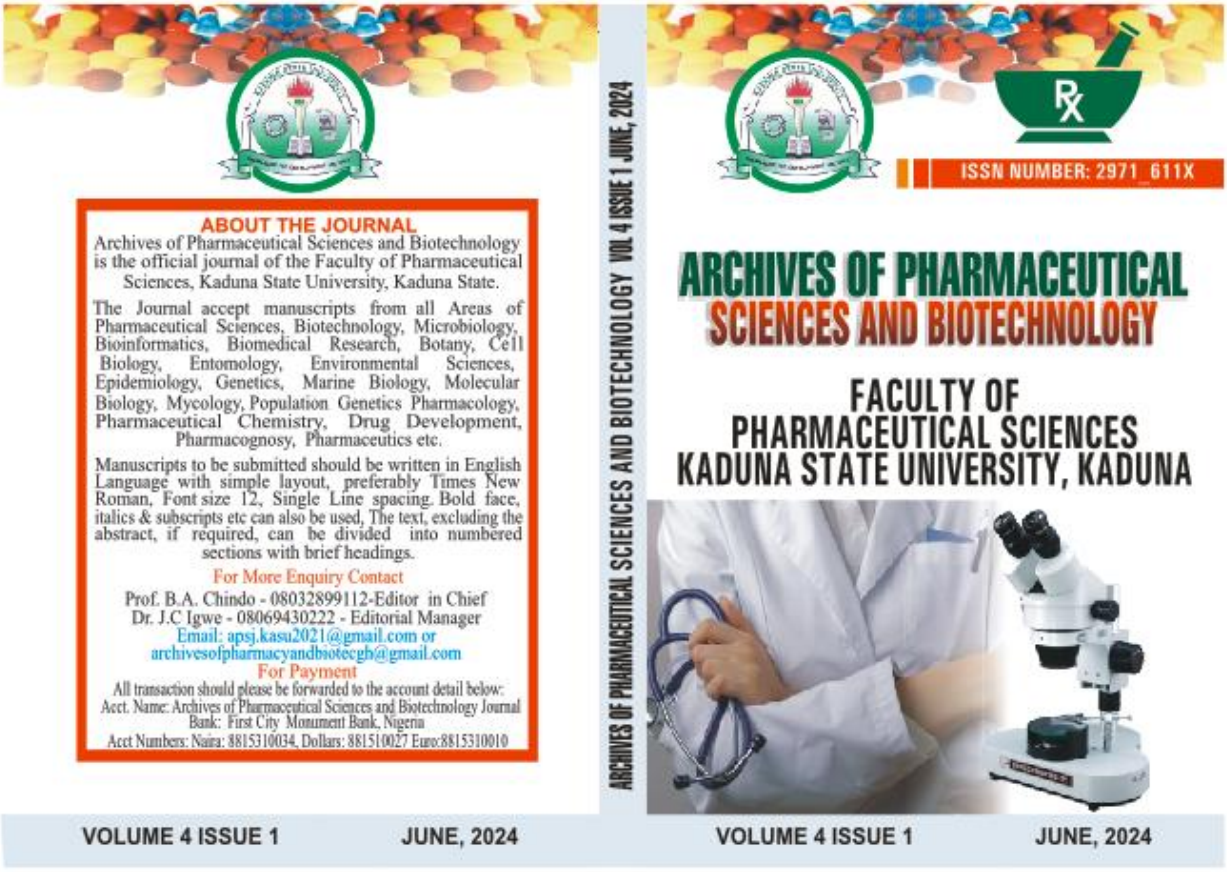

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THE ROLE OF LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS TO GASTROINTESTINAL MOTILITY

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ABSTRACT

Aim: This study is designed to investigate the role of large conductance calcium-activated (BK) channels in regulating gastrointestinal motility using mouse ileum strips in an organ bath preparation.

Place and duration of study: This study was conducted in the School of Pharmacy and Biomedical Science at the University of Portsmouth, UK for a period of four months, from September 2015 to January 2016

Methodology: Experimental procedures to test viability of mouse ileum and response to compound NS19504 and Tetraethyl ammonium (TEA) were conducted using organ bath preparation containing Krebs solution. Force of contraction and relaxation of each ileum section were measured and recorded using Labchart reader. Paired t-test conducted using GraphPad prism software for statistical analysis of results.

Results: Carbachol generated a concentration-dependent contraction in the tissue strip and NS19504 relaxed the tissue. TEA used as a large-conductance calcium-activated (BK) channel blocker raised the amplitude of contraction after Carbachol, and had no effect on relaxation generated by NS19504. Unpaired t-test produced a p-value <0.0001 (<0.05) meaning there is a significant difference between amplitude of contraction in the presence and absence of TEA. Mean ± SEM in the presence of TEA = 0.205±0.02, mean ± SEM in the absence of TEA= 0.05±0.01 (N=6)

Conclusion: Results from this study suggest that administration of NS19504 produced concentration dependent relaxation of the tissue and this relaxation is not blocked by TEA, which is a known BK channel blocker. Better understanding of the pathological roles played by ion channels may be useful in the development of more potent drugs targeting ion channels to treat gastrointestinal (GI) motility disorders.

Keywords: calcium-activated potassium channels, gastrointestinal motility, motility dysfunction

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INTRODUCTION

Multiple factors such as neurotransmitters, stress, peptides, hormones and genetics regulate the gastrointestinal system. These factors affect secretion, motor function and sensation of the tract (1). Neurotransmitter systems such as cholinergic, adrenergic and serotonergic systems are representative systems that have various effects on the gastrointestinal tract (2). Major functions of the GI tract are motility, digestion, absorption via villi in the small intestines and excretion of undigested substances as waste products through the anus.

Gastrointestinal motility refers to muscular contractions in the gut, which leads to mixing and propulsion of food and gastrointestinal secretions e.g. enzymes down the gut (3). Motility is determined by electrical activities, which occur in smooth muscle cells in the small intestine. Interstitial Cells of Cajal (ICCs) are the pacemaker cells in the GI muscle i.e. muscular regions of the tract e.g. stomach, intestines (4). Electrical signals from enteric neurons to SMCs are generated and transmitted via gap junctions (4). Basic electric rhythm i.e. slow waves are set by ICCs by establishing electrochemical equilibrium between spontaneous depolarising pacemaker currents and outward hyperpolarising potassium currents (4). T-type calcium channels are switched on by depolarisation of these membranes; this results in an influx of calcium ions resulting in generation of action potential (5). Action potential complex generated by slow waves, progress to smooth muscle cells through gap junctions of interstitial plexus triggering voltage-sensitive calcium channels especially L-type channels in the cells to start an excitation-contraction process resulting in smooth muscle contraction (6).

Relaxation of the GI smooth muscle occurs in two distinct ways i.e. relaxation triggered by contractile stimuli being removed or action of compounds or substances, which inhibit the contractile mechanism e.g., non-adrenergic non-cholinergic neurons in the Myenteric plexus, which utilise nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) and primary inhibitory neurotransmitters. Smooth muscle relaxation also requires a decrease in calcium influx into the cytosol and increase in MLC phosphate; this is achieved by reuptake of calcium by sarcoendoplasmic reticulum, which is dependent on ATP hydrolysis (5).

GI motility disorders refer to a group of diseases whose characteristic symptoms are related to motor, sensory or secretory functions in the system. About one in three persons present with GI motility disorder but diagnosis and treatment still remain challenging because symptoms are non-specific and include constipation or diarrhoea, nausea and vomiting, bloating and abdominal cramping. This results from a multisystem pathophysiology i.e. alteration of motor function of the gut wall, also enteric abnormalities. They may also result from complications from other systemic illnesses like diabetes as well as abnormalities of the enteric nervous system (7). Studies by Saito et al. (2009) showed sodium channel mutations in irritable bowel syndrome. Results from this study suggest that abnormal ion

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channel function/activity are related to human GI motility problems (8).

Large conductance calcium-activated potassium channels otherwise known as maxi-K or BK channels (KCa1.1) are activated by both calcium and voltage (membrane depolarization), they are expressed in both excitable and unexcitable cells (but predominant in excitable cells e.g. smooth muscle cells) in the central and peripheral nervous systems and are encoded by a single gene (*slo1*, *KCNMA1*) (5). Maxi-k channels are involved in a number of cellular functions including hormonal release, regulation of tone of gastrointestinal smooth muscle and release of neurotransmitters (9,10).

Opening of maxi-K channels increases potassium efflux, which in turn shifts the membrane potential, inducing hyperpolarization and deactivating the voltage-dependent calcium influx, thus, reducing contractility of muscle and causing relaxation of GI smooth muscle (11). Motility is also mediated by activation of autonomic nerves specifically parasympathetic nerves, which release neurotransmitter acetylcholine and ATP as a source of energy to increase action potentials and thereby contractility of the tissue (12).

Functional and motility disorders of the gastrointestinal system are very common and are responsible for morbidity and in some cases mortality of patients (13). Diagnosis and treatment of these conditions still remain challenging. This could be due to the multisystem pathophysiology of GI disorders, including motor wall dysfunction of the GI wall and abnormalities in the central and peripheral nervous systems (14). The pathogenesis of GI motility disorders is multifactorial; evidence from studies has shown the low-grade mucosal inflammation and immune activation is associated with impaired epithelial barrier function (15) ICCs play important roles in regulation of electrical activity of gut smooth muscle (4). Voltage-sensitive ion channels such as BK channels are central to triggering and regulating this electrical activity. Thus, BK channels are potential therapeutic targets for GI dysmotility like IBS. This study outlined how blocking BK channels could be an approach to targeting motility dysfunction and rational drug design can come from an integrated view of the mechanisms of activation of BK channels by voltage, ions or mechanical stress.

MATERIALS AND METHODS

Adult male and female mice (20g-30g) were obtained from the Animal House at School of Pharmacy and Biomedical Science, University of Portsmouth. The UK Animal Welfare and Ethical Review Body granted ethical approval for the experiment, with approval number UP677424. The Organ bath experiment was conducted using the Harvard Apparatus Tissue Bath and Perfusion Systems Selection Guide of 2014. The animals were euthanized by cervical dislocation and carbon dioxide asphyxiation for abdominal dissection. Sections of mouse distal ileum (2cm-3cm) were dissected and mounted on an aerator bubbled with carbogen (5% CO_2, 95% O_2), aerator is attached to a force transducer and inserted into the Harvard organ bath (10ml chamber) filled with Krebs solution. The system was set at 32^{o}C in order decrease autonomic contractions by the tissue. Preparation of Krebs solution (pH ~7.4) is shown in Table 1.

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Table1: Concentration of components of Krebs solution in mM

Conductance	Concentration
Sodium chloride (NaCl)	118
D^+ Glucose	25
Magnesium sulphate ($MgSO_47H_2O$)	11
Sodium bicarbonate ($NaHCO_3$)	4.7
Potassium chloride (KCl)	1.2
Potassium phosphate (KH_2PO_4)	1.2
Calcium chloride ($CaCl_2$)	2.5

25 litres of distilled water made up the solution and calcium chloride added last into the solution to avoid the "cloudy" reaction. This solution was used to mimic the internal environment of the mouse.

Experimental Procedures

Carbachol (100µl, 300µl) (10^{-2}M – 10^{-7}M) was added into each bath alternating between the two volumes for every concentration. This gave a final bath concentration of 10^{-4} M – 10^{-9} M. Tissue was left to contract in the bath for about 30 seconds to one minute after every concentration. This procedure was carried out on about 8 different tissue segments. NS19504 (0.0865g) made into solution using 0.1% ethanol as vehicle.100mM NS19504 (stock) diluted to: 60mM, 30mM, 10mM, 3mM, 1mM, 0.3mM and 0.1mM stored in the freezer. After pre-contraction with concentration of carbachol that produced about 50% maximum contraction for that particular tissue, 10µL of the least concentrated drug solution (0.1mM) is added into the bath for 3 minutes to measure relaxation response. After 3 minutes the tissue is washed and the process is repeated again in ascending order of drug concentration keeping carbachol concentration and volume constant.

A single piece of distal ileum was used from every animal and experimental data presented as mean ± SEM. Tissue responses were quantified by measuring size of contraction and relaxation.

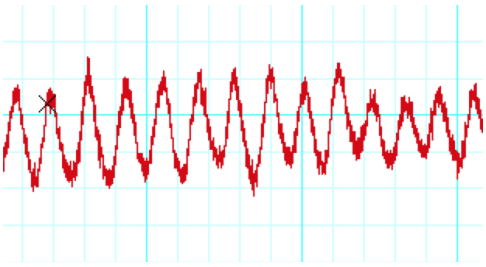

Figure 1: Representative trace of tissue contractions.

Statistical Analysis

Data was analysed using Microsoft excel as mean ± SEM. Statistical analysis carried out using software, Graph pad prism. Paired t-test used to compare tonic and peak contraction generated by carbachol, effects of ethanol on the tissue in the presence and absence of carbachol and response of tissue to NS19504. A p-value of less than 0.05 was taken to be significant.

RESULTS

In order to test viability of the tissue, muscarinic antagonist carbachol was used to test its response. Carbachol was added into the bath from the lowest to the highest concentration i.e. 1nM to 300μM. The experiment was conducted 12 times with the best 6 results used for statistical analysis. Maximal peak contraction observed at carbachol $3x10^{-5}$ M and tonic $1x10^{-4}$M as seen in the tables below. These maximal values correspond to EC_{50} of $1.03x10^{-6}$ ± 1.03 µM for peak response and $3x10^{-7}$ ± 1.59 µM for tonic response (95% confidence interval $-3.8x10^{-7}$ to $5.27x10^{-7}$). Force measurement was taken by difference between baseline and highest force observed. Concentration-dependent

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response produced by carbachol as peak contraction response (figure 2A) and tonic contraction response (figure 2B). Response data presented as normalised mean contraction (%) with SEM. N represents number of tissues used. From the curves it was observed that low concentrations of carbachol (1x10-7) do not produce any significant response. Paired t-test (figure 3) used to compare peak and tonic contractions indicated that EC50 of peak and tonic concentrations have no significant difference, as the p value is 0.4625. Values represent mean ± SEM (N= 6). An example of the traces produced by tissue contraction is shown in the figure below

Table 1 A&B: These tables show normalised mean and SEM of carbachol response values in increasing concentration for peak contractions

A

Concentration (M)	Mean	SEM	N
1 x 10^{-9}	-0.17	0.63	6
3 x 10^{-9}	1.20	0.78	6
1 x 10^{-8}	2.47	0.23	6
3 x 10^{-8}	2.41	1.05	6
1 x 10^{-7}	9.67	2.52	6
3 x 10^{-7}	19.17	1.67	6
1 x 10^{-6}	42.05	3.70	6
3 x 10^{-6}	71.48	4.76	6
1 x 10^{-5}	88.90	2.40	6
3 x 10^{-5}	98.66	1.33	6
1 x 10^{-4}	95.38	1.31	6
3 x 10^{-4}	91.70	2.47	6

B

Concentration (M)	Mean	SEM	N
1 x 10^{-9}	0.06	0.67	6
3 x 10^{-9}	1.07	1.14	6
1 x 10^{-8}	1.17	0.99	6
3 x 10^{-8}	1.79	1.37	6
1 x 10^{-7}	9.83	4.75	6
3 x 10^{-7}	17.4	4.97	6
1 x 10^{-6}	50.97	4.51	6
3 x 10^{-6}	80.67	4.37	6
1 x 10^{-5}	83.46	3.97	6
3 x 10^{-5}	94.80	3.11	6
1 x 10^{-4}	98.12	0.93	6
3 x 10^{-4}	94.07	2.55	6

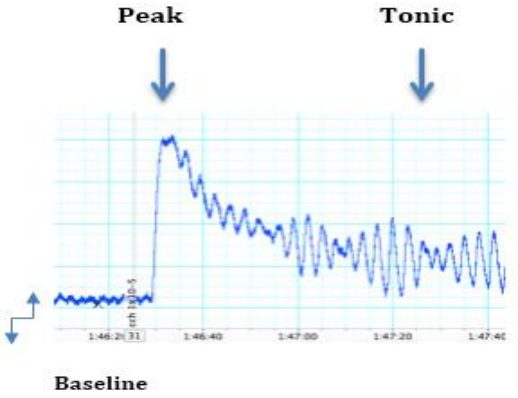
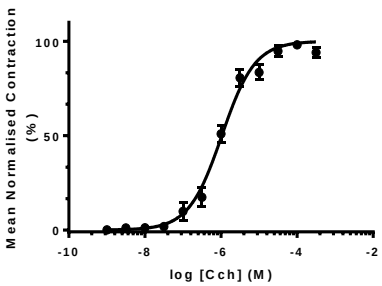


Figure 1: representative trace of Carbachol induced contraction response force, both peak and tonic contractions were measured in gram

A



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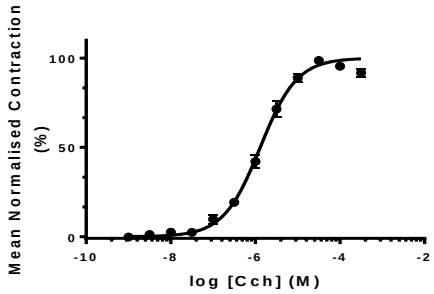

Figure 2: A) normalised peak concentration response of tissue to carbachol. B) Normalised tonic concentration response of tissue to carbachol

Both curves show carbachol response by ileum. Values used represent mean and error bars represent the SEM. N=6**.**

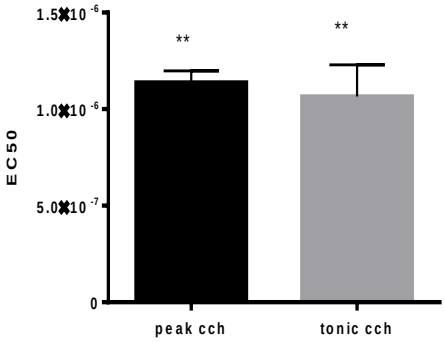

Figure 3: comparison of effect of carbachol on tissue contraction.
Result statistically insignificant if p value <0.05

Sub-threshold carbachol concentration from the first experiment above was used to pre-contract the tissue before testing relaxant property of BK channel activator, NS19504. NS19605 is soluble in ethanol therefore 0.1% ethanol was tested on the tissue as a control, percentage response of tissue to ethanol shown in table 2. Representative trace of response observed on the monitor shown in figure 5. Paired t-test conducted between two variables: Carbachol and 0.1% ethanol because the difference between the paired values is consistent. This test gave a p-value of 0.3041 indicating that there is no significant difference as p-value is greater than 0.05. This means that ethanol does not contraction generated by Carbachol in the tissue. 95% confidence interval -0.048 to 0.018. N= 6. (Figure 5).

Table 2: Tissue responses (%) with Ethanol 0.1% and without ethanol Tissues are numbered 1-6.

Tissues	0.1% Ethanol	Without Ethanol
1	0.63	0.58
2	0.35	0.37
3	0.54	0.51
4	0.50	0.47
5	0.73	0.70
6	0.72	0.75

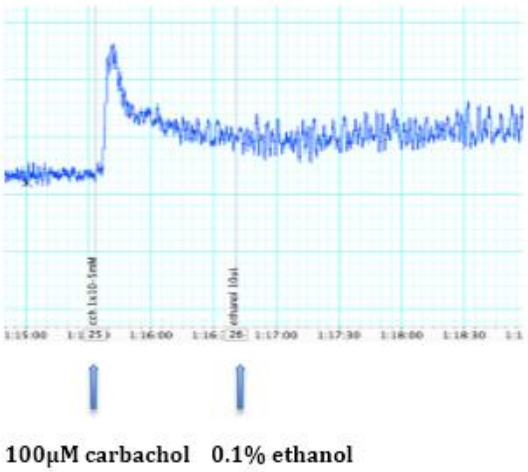

Figure 4: Effect of ethanol on tissue after pre-contraction with 100µM carbachol.

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It is observed that tonic contraction of the tissue is not altered by addition of 10µL ethanol into the bath.

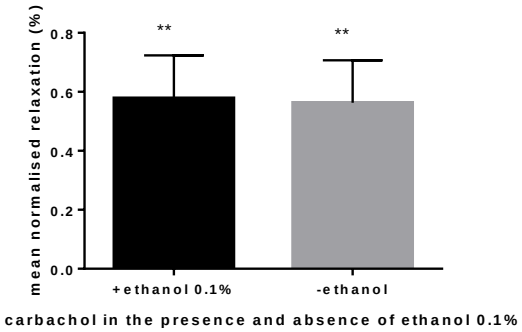

Figure 5: Ethanol used as control to test tissue response

NS19504 dissolved in 100% ethanol for experiment since ethanol had no effect on tissue contraction/relaxation. Addition of NS19504 to the bath produced relaxation response as seen in figure 6. Sample trace shown in figure 6 while the Response curve for relaxation of BK channel agonist is shown in figure 7. Concentrations of this drug were added into the bath in ascending order from 0.1μM to 100μM.

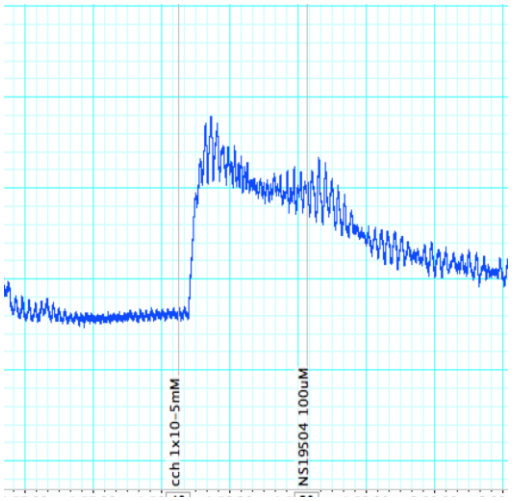

Figure 6: representative trace of relaxation generated by addition of 100µM BK channel agonist, NS19504 after pre-contracting the tissue with 100µM carbachol.

It is observed that this concentration of drug relaxed the tissue but not completely.

Table 3: normalised relaxation response of tissue to NS19504, data present as mean and SEM. Eight experiments conducted with the best 6 used for statistical analysis.

Concentration (μM)	Mean	SEM	N
0.1	100	0	6
0.3	87.51	3.31	6
1	81.07	3.68	6
3	75.55	5.15	6
10	64.51	6.39	6
30	49.77	5.27	6
60	29.11	5.43	6
100	15.51	2.95	6

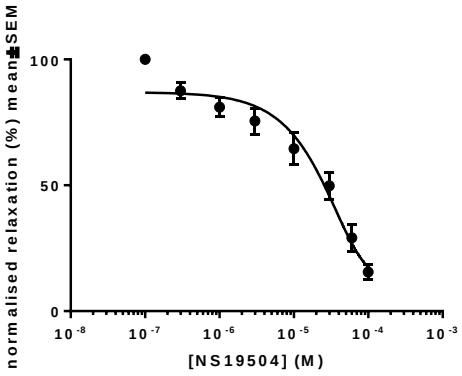

Figure 7: normalised relaxation response generated by potassium channel activator NS19504 after pre-contraction with Carbachol 100µM IC_{50} = 0.9999M

Two-way ANOVA test was used to compare concentrations of NS19504 used to produce relaxation (table 4), p value < 0.001 showed that concentrations used for experiments are significant statistically.

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Table 4: Relaxation response of mouse ileum to NS19504 after pre-contraction with carbachol

NS19504 (µM)	Tissue1	Tissue2	Tissue3	Tissue4	Tissue5	Tissue6
0.1	0.23	0.65	0.25	0.28	0.55	0.49
0.3	0.18	0.60	0.22	0.27	0.50	0.36
1	0.18	0.55	0.20	0.27	0.46	0.32
3	0.14	0.54	0.19	0.26	0.43	0.28
10	0.08	0.47	0.19	0.24	0.33	0.27
30	0.06	0.39	0.16	0.14	0.30	0.20
60	0.06	0.36	0.08	0.07	0.10	0.07
100	0.05	0.16	0.03	0.01	0.09	0.03

A BK channel blocker, tetraethyl ammonium used to test the selectivity of the BK channel agonist. 1mM TEA used to block BK channels and 60µM NS19504 used to induce relaxation. Values and statistical analysis generated from this experiment shown in table 5 and figure 8 respectively. Paired t-test produced a p-value of 0.8374. This means there is no significant difference between relaxation caused by NS19504 in the presence or absence of TEA. 95 % confidence interval -16.81 to 19.89. N=6.

Table 5: Experimental values produced when comparing relaxation generated by NS19504 in the presence and absence of TEA

60µM NS19504 + 1mM TEA	60µM NS19504 without TEA
-0.95	0.06
-1.00	0.36
-1.24	0.08
-0.95	0.07
-0.77	0.10
-0.91	0.08

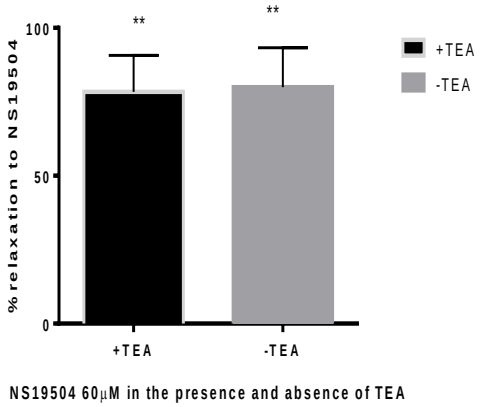

Figure 8: response of tissue to NS19504 in the presence and absence of BK channel blocker tetraethyl ammonium (TEA).

From the traces recorded, it was noticed that addition of TEA before carbachol changed the amplitude of contraction of the tissue, it was observed to be higher hence; statistical analysis was conducted to test if that is of any significant difference. Unpaired t-test produced a p-value <0.0001 (<0.05) meaning there is a significant difference between amplitude of contraction in the presence and absence of TEA. mean±SEM in the presence of TEA = 0.205±0.02, mean±SEM in the absence of TEA= 0.05±0.01. N=6 (figure 10).

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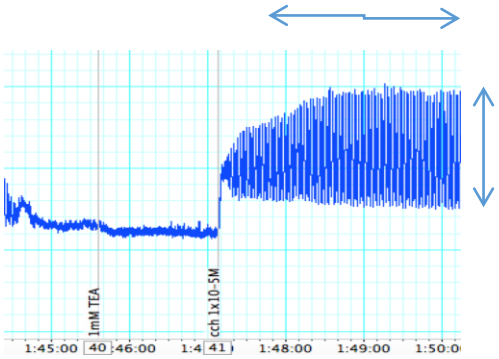

Figure 9: representative trace of the amplitude of contraction observed when 100µM carbachol was used to contract the tissue after using 1mM TEA as a BK channel antagonist

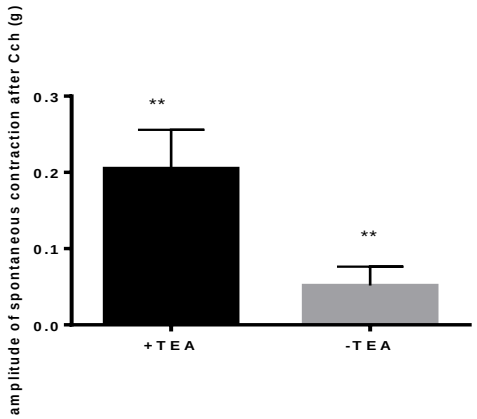

Figure 10: Amplitude of spontaneous contraction after addition of Carbachol in the bath

From the results generated overall, it can be seen that statistical tests provided evidence that high concentrations of NS19504 (30, 60 and 100µM) produced significant relaxation and 1mM TEA did not produce any significant blockade of relaxation.

DISCUSSION

The purpose of this study was to investigate the presence of BK channels in mouse ileum and the role they play in intestinal motility using BK channel agonist and antagonist. Results showed that carbachol generated a concentration-dependent contraction followed by relaxation after NS19504 was added into the bath and their activation is sufficient to cause this relaxation but the mechanism by which this drug relaxes the gut is not very clear.

Tetraethyl ammonium increased amplitude of contraction significantly and enhanced intestinal propulsion. This is because decrease in calcium concentration results in an increase in tissue sensitivity to carbachol and hence, the large amplitude of contraction observed in the trace as seen in figure 9. Sensitivity of TEA to variety of potassium channels differs, in canine colonic smooth muscle for example, TEA displays different sensitivity to components of delayed rectifier potassium currents (16). TEA is also a general potassium channel blocker; therefore it is not certain that it blocks only BK channels in the tissue. This is seen in figure 8 from the results where a statistically significant difference in force of contraction of the ileum was observed in the presence and absence of TEA after the addition of NS19504 to the bath. Furthermore, it has different effects on various regions of the GI tract since qualitative and quantitative expression of ion channels in each region differs. This could be reason for relaxation observed even after blocking BK channels with TEA in the bath. This suggests that other factors could be involved in the relaxation process. Some studies have shown that endogenous modulators such as metabolites of cytochrome P450, epoxygenase and lipoxygenase, have been found to be important regulators of vascular tone and activate BK channels (17) BK channels are also

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phosphorylated by a number of kinases including protein kinase C (PKC), PKG-1 and PKA (18,19). This phosphorylation increases voltage and calcium sensitivity of the channels, elevating its open probability. More specific channel inhibition is generated by peptides such as charybdotoxin and iberiotoxin (20) although they do not provide any therapeutic benefits. In addition to NS19504 other compounds such as benzimidazolone NS1619 has been shown to effectively activate BK channels by inhibiting calcium currents (21).

In a study using guinea pig bladder, NS19504 was seen to activate endogenous BK channels in native smooth muscle cells; this reduced spontaneous contractions in bladder sections used in an iberiotoxin-sensitive manner. The result suggested that NS19504 could be a useful tool to clarify functions of BK channels in other complex tissue.

Voltage-sensitive ion channels in general play essential roles in the mechanical and electrical activity of the GI tract, their pores have specific ion selectivity, and therefore they are reasonable targets for treating functional and motility disorders (22). Current approaches in electrophysiology and pharmacology are now being put together with structural information generated from X-ray crystallography to provide specific molecular binding sites and conformational states that are worthy to be targeted therapeutically (22) however, some difficulties are still experienced in these approaches. Furthermore, scientific attention is required for voltage-sensing mechanism of these channels since regulation of voltage sensor function enables modulation of channel function elaborately (22). Multiple conformational states involved in this mechanism can be utilised for therapeutic intervention.

Experimental tools developed for studying electrophysiology in gastrointestinal tissues have made elaborate understanding of electrical activities occurring at molecular levels possible. However, this progress is still yet to present the clinical tools, which can monitor and diagnose GI dysmotility. More effort to further understand mechanisms from cellular to tissue levels is needed, and also building rational models and more advanced ways to test these models. Voltage-sensitive ion channels should appear precisely in this plan, since their function and abnormalities is crucial to gastrointestinal functions. Computational models are emerging that once combined with experimental data on VSICs will be able to predict complex activities of these channels at the molecular and tissue levels (22). These efforts may result to a state where in vivo electrical activity will be able to be measured for GI dysmotility and compare with the normally predicted activity, hence, pointing out some molecular pathologies and thus potential therapeutic targets. Ion channels are generally good targets for drugs because they regulate a great number of physiological processes in different regions of the human body (4). Functional alterations of ion channels results in a wide range of pathophysiology i.e. channelopathies, hence they are important drug targets. Drug development to target these proteins has experienced challenges over the years because of their complex nature and selectivity.

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CONCLUSION AND RECOMMENDATION

Results from this study suggest that administration of experimental compound NS19504 produced concentration dependent relaxation of the tissue and this relaxation is not blocked by TEA, which is a known BK channel blocker. These results suggest that BK channels are functionally expressed in mouse ileum and targeting them could be potentially useful to treating GI dysmotility.

It is important to note that the phenotype of GI smooth muscle is similar to that of other organs. Therefore attempting to alter the molecular targets of the GI smooth muscle may lead to threatening consequences in other organs. There are currently few efficacious compounds for the treatment of motility problems. An improved knowledge of the cellular and molecular processes in human muscles and the discovery of more focused cell-specific pathways behind particular behaviours will provide hope for the future. Better techniques for specific manipulation of cell phenotype in addition to current knowledge may provide safe and efficacious therapeutics for gastrointestinal motility disorders.

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REFERENCES

1. Cheng L.K, O'Grady G, Du P, Egbuji J, Windsor J.A & Pullan A.J. (2010). Gastrointestinal System. *Interdisciplinary Reviews: Systems Biology and Medicine*. 2(1): 65-79.
2. Manabe N, Wong B.S, Camilleri M, Burton D, McKinzie S & Zinsmeister A.R. (2010). Lower functional gastrointestinal disorders: evidence of abnormal colonic transit in a 287 patient cohort. *Neurogastroenterology & Motility*. 22(3): 293-313
3. Sanders K.M, Koh S.D, Ro S & Ward S.M. (2012). Regulation of gastrointestinal motility – insights from smooth muscle biology. *Nature Reviews Gastroenterology & Hepatology*. 9(11): 633-645.
4. Garcia M.L, Priest B.T, McManus O.B, Kaczorowski G.J. (2008). Ion channels as drug targets: the next GPCRs. *The Journal of General Physiology*. 131(5): 399-405.
5. Cain S.M & Snutch T.P. (2013). T-type calcium channels in bursting and firing, networks synchrony and epilepsy. *Biochimica ET Biophysics Acta*. 1828(7): 1572-1578.
6. Andresen V, Camilleri M, Busciglio IA & Grudell A. (2007). Effect of 5 days linaclotide on transit and bowel function in females with constipation-predominant irritable bowel syndrome. *Gastroenterology*. 133: 761-768
7. Ghatta S, Nimmagadda D, Xu X & O'Rourke ST. (2006). Large-conductance, calcium activated potassium channels: structural and functional implications. *Gastroenterology*. 110(1): 103-116
8. Saito Y.A, Strege P.R, Tester D.J, Rae J.L et al. (2009). Sodium channel mutation in irritable bowel syndrome – evidence for ion channelopathy. *American Journal of Physiology*. 296(2): G211-G218.
9. Lu P, Quan X & Fan H. (2016). The role of substance P in the maintenance of colonic hypermotility induced by repeated stress in rats. Neuropeptides.

Available at http://www.sciencedirect.com/science/article/pii/S014341791600007X

10. Gribkoff VK, Startrett JE & Dworetzky SI. (1997) The pharmacology and molecular biology of large conductance calcium activated (BK) potassium channels. *Advances in Pharmacology*. 37: 319-348
11. Nausch B, Layne J.J, Olesen S.P & Nelson M.T. (2010). BK channel activation by NS11021 decreases excitability and contractility of urinary bladder smooth muscle. *American Journal of Physiology*. 298(2): 378-384.
12. Beyder A & Farrugia G. (2012). Targetting ion channels for the treatment of gastrointestinal motility disorders. *Therapeutic Advances in Gastroenterology*. 5(1): 5-21.
13. Van-Geldre L.A & Lefebvre R.A. (2004). Interaction of NO and VIP in gastrointestinal smooth muscle relaxation. *Current Pharmaceutical Design*. 10(20): 2483-2497.
14. Dong DL, Wang QH & Chen W. (2005). Contrasting effects of tetraethylammonium and 4-aminopyridine on the gastrointestinal function of mice. *European Journal of Pharmacology*. 509(2-3): 179-185.
15. Beaulieu G & Frank GB. (1967) Tetraethyl ammonium-induced contractions of frog's skeletal muscle: III. Mechanism of action by calcium release. *Canadian Journal of Physiology and Pharmacology*. 45(5): 845-855.
16. Kume H, Takai A, Tokuno H & Tomita A. (1989). Regulation of Ca2+-dependent K+ channel activity in tracheal myocytes by phophorylation. *Nature*. 341: 152-154.
17. Schubert R & Nelson M.T. (2001). Protein kinases: turners of the BKCa channel in smooth muscle. *Trends in Pharmacological Science*. 22: 505-512.
18. Galvez A, Gimenez-Galleg O & Reuben J.P. (1990). Purification and characterization of a unique peptidyl probe for high conductance calcium-activated potassium channel from venom of the scorpion Bathus tamulus. *Journal of Biological Chemistry*. 265: 11083-11090.
19. Holland M, Langton P.D, Standen N.B & Boyle J.P. (1996). Effects of BK channel activator NS1619 on rat cerebral artery smooth muscle. *British Journal of pharmacology*. 117: 119-129.
20. Heitzmann D & Warth R. (2008). Physiology and pathophysiology of potassium channels in gastrointestinal epithelia. *Physiological Reviews*. 88(3): 1119-1182.
21. Soder R.P & Petkov G.V. (2011). Large conductance Ca^{2+} - activated K^{+} Channel activation with NS1619 decreases myogenic and neurogenic contractions of rat detrusor smooth muscle. *European Journal of Pharmacology*. 670(1): 252-259.
22. Wulff H, Castle N.A & Pardo L.A. (2009). Voltage-gated potassium channels as therapeutic targets. *Natures Reviews Drug Discovery*. 8(12): 982-1001

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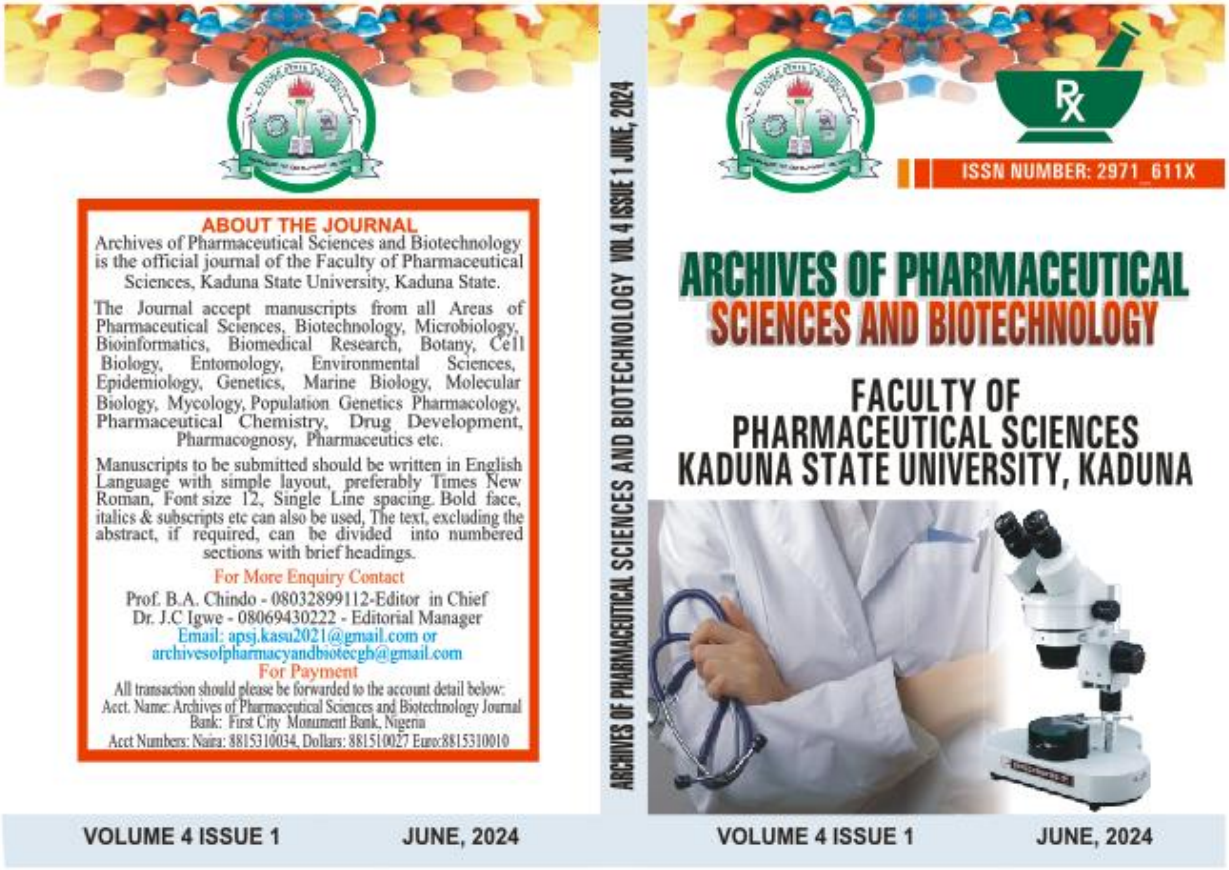

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KNOWLEDGE, ATTITUDE AND PERCEPTION OF ELECTRONIC MEDICAL RECORDS AMONG HEALTHCARE PROFESSIONALS IN NATIONAL EAR CARE CENTRE, KADUNA

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ABSTRACT

Background: Electronic Medical Records (EMRs) have the potential to revolutionize healthcare delivery by improving efficiency, patient safety, and data management. However, the successful implementation and utilization of EMRs depend on healthcare professionals' knowledge, attitude, and perception towards this technology. This study aimed to assess the knowledge, attitude, and perception of healthcare professionals regarding EMRs at the National Ear Care Center (NECC) in Kaduna, Nigeria.

Methods: A cross-sectional survey was conducted among healthcare professionals, including doctors, nurses, pharmacists, and other allied healthcare staff, working at NECC. A structured questionnaire was used to collect data on participants' demographic characteristics, knowledge of EMRs, attitude towards their implementation, and perceptions of their benefits and challenges. Data were analyzed using descriptive statistics.

Results: A total of 109 healthcare professionals participated in the study, with a response rate of 83.9%. The majority of respondents (92.6%) had a high level of knowledge about EMRs, while only 7.2% had a low level of knowledge. Regarding attitude, 65% of participants expressed a positive attitude towards the implementation of EMRs in NECC. However, concerns were raised that healthcare workers prefer paper based type than EMR (75.3%) due to the potential for privacy breaches. Perceived benefits of EMRs included increased work productivity (92.6%), enhanced patient care and safety (87.2%), and it enables tasks to be accomplished more quickly (85.3%).

Conclusion: The study found a positive attitude towards EMR implementation among healthcare professionals at NECC, but highlighted the need for education and training. Concerns were raised about EMRs negatively impacting doctor-patient relationships, suggesting strategies for maintaining strong communication and patient-centered approaches to improve healthcare delivery and efficiency.

Key words: Electronic Medical Record, Healthcare Professionals, Knowledge, Attitude, Perception

ABBREVIATIONS

EMR Electronic Medical Record
EHIMS Electronic Health Information Management Systems
WHO World Health Organization
NECC National Ear Care Center
HER Electronic Health record
HCW Healthcare Workers
HCP Healthcare Professionals
ICT Information and Communication Technology
IT Information Technology
HIT Health Information Technology
FMoH Federal Ministry of Health

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INTRODUCTION

Electronic Medical record (EMR) services are expanding rapidly and have the potential to improve the health of the community, enhance scientific understanding of health issues, and facilitate communication between health care providers and patients. The healthcare delivery system of a nation hinges, amongst other things, on how well its hospitals can deliver qualitative and affordable healthcare to its citizens. Thus, the role of hospitals in the healthcare delivery system of a nation cannot be overemphasized [1]. Health records are essential for good healthcare and good quality healthcare data play a vital role in the planning, development and maintenance of optimal healthcare [2]. The amount and quality of information available to healthcare professionals in patient care impact the outcome and continuity of patient care.

Furthermore, medical information needed for clinical decision making continues to increase, especially in developing countries. However, the organization and accessibility of medical information remain poor, usually resulting in inappropriate decisions and medical errors [3].

EMR systems in most developing countries such as Nigeria is in its early stage due to inadequate healthcare infrastructures, health professionals' attitude and awareness level, lack of proper management, resource shortage, skill related issues, users' resistance, policy related issues, poor commitments of staffs, and poor maintenance services among others [4,5]

EMRs are crucial for patient care, but sub-Saharan Africa still lags behind in Health Information Technology (HIT). A study is needed to assess the knowledge, awareness, and perception of EMRs among frontline healthcare workers in Nigeria, as the region accommodates 12% of the world's population and 27% of the global disease burden [6-8]

The findings of this study will provide a basis for the future adoption and utilization of standardized electronic health records in National Ear Care Centre. The study can lead to a good understanding of the knowledge, awareness and perceptions of EMR and thus offer opportunities and strategies that can enable a successful implementation of the system. In addition, it can serve as a source of information to the Kaduna state government as well as the federal government to develop strategic policies that can enable the delivery of quality healthcare to the people. The aim of the study is to assess the knowledge, awareness and perception of Electronic Medical record among healthcare professionals in National Ear Care Center Kaduna.

METHODS

Study Setting National ear care center (NECC) Kaduna is a public hospital within Kaduna state metropolis which was established in 1999 on the recognition of the World Health Organization (WHO), as a result of the high prevalence of hearing impairment in Africa. The center is the only specialists' hospital in Nigeria for diagnosis, treatment and research into the ear, nose and throat specialty, even though ENT services are available in many teaching hospitals in the country. The center renders many specialist services some of which are; Ear, nose and throat surgeries, audiology related services, school hearing screening services, speech therapy, laboratory, endoscopy, radiology and pharmaceutical services. The center has a 30 bed unit consisting of four (4) wards; Accident and emergency ward, amenity ward, female and children ward and male ward.

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Fig 1. A pictorial view of graphical location of National Ear Care Centre Kaduna

Study Population

The population of the study included all available healthcare which comprises of Medical Doctors, Pharmacists, Nurses, Medical Laboratory Scientists, Radiologists and non-healthcare professionals which comprises of Administration staff, Accountants, Medical records personnel etc. were included in the study to gain a broader perspective on the use of Electronic Medical Record (EMR).

Sample Size

The sample size for the study was determined using Cochrane formula for cross sectional study.

A total of 109 questionnaires that were completed was analyzed in the study.

The minimum sample size was determined using Cochrane formula as shown below:

$n = Z^2pq / d^2$

n = minimum sample size

Z= standard normal deviate = 1.96, corresponding to 95% confidence interval

d = desired precision or margin of error tolerable; at 5%

p = proportion of the study population with a positive attitude to electronic medical record in a previous study i.e. 82.9% [9] = 0.829

q = complementary probability = 1- p = 1 – 0.829

= 0.917

$n = (1.96)2 \times 0.829 \times 0.917 \div (0.05)^2$ = 116.8 +10%

n= 128.5

n=130

Study Design

A prospective cross sectional study survey was conducted, using a semi structured questionnaire to assess the knowledge, attitude and perception of health care professionals towards electronic medical records. Sampling was done using convenience sampling because it was easy to implement and the population of the institution is relatively small, however there could be high chances of sampling error.

Study Instrument

The study used a quantitative survey method in the form of a close-ended questionnaire to collect data from various healthcare workers in NECC. It contained four sections as follows; A- socio demographic information, B knowledge on

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electronic medical records, C- attitude towards use of EMR and D- perception on use of EMRs. The questionnaire was pre-tested in NECC among 35 healthcare workers to check for clarity of questions and to eliminate repetitive and ambiguous questions. Cronbach's alpha statistic was also employed to measure the reliability of the study instrument which was 0.694

Data Collection Procedure

Hard copies of the questionnaires were administered to the participants, a hard copy of the questionnaire was given to an identified representative person of each of the healthcare cadre (e.g. chief resident Doctor, chief medical officer, chief Consultant, In charge Pharmacist of various unit of pharmacy department, In charge medical laboratory scientist of various units in the Medical Laboratory department, In charge nurses of various units in the hospital etc.), to distributed to their staff and the completed questionnaires were collectively collected from the representative person.

Data Analysis

Data analysis was carried out using the SPSS software. Frequency distribution table were generated for all categorical variables. The mean and standard deviation (SD) were computed for quantitative study.

Ethical Consideration

Ethical clearance was sought and obtained from the ethics and research Committee of the National Ear Care Centre Kaduna with ethical approval number NECC/ADM/214/VI/145 (Appendix II). Written informed consent was obtained from the respondents after explaining the objectives and method of the study to them, also each participants was assured of confidentiality of their personal data.

RESULT

There were 130 questionnaires distributed in all, and 109 were successfully retrieved and evaluated generating an 83.9% response rate.

Demographic Characteristics of the Study Participants

The age range with the highest number of respondents was 36-46 years (48.6%). Majority of the respondents were Admin staff (29.4%), males (54.1%), (44.0%) had attained tertiary level of education, details in (Table 1) below:

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Table 1: Demographic characteristics of Participants

		Frequency (n = 109)	Percentage (%)
Gender	Male	59	54.1
	Female	50	45.9
Age	<35	49	45.0
	36-46	53	48.6
	>47	7	6.4
Profession	Physician	9	8.3
	Pharmacist	9	8.3
	Nurse	11	10.1
	Med lab Scientist	12	11.0
	Health Records	15	13.8
	Audit	8	7.3
	Account	13	11.9
	Admin	17	15.6
	Others	15	13.8
Level of Education	Diploma	46	42.2
	Bachelor Degree	48	44.0
	Master Degree	12	11.0
	Fellowship	3	2.8
Years of Experience	0-3years	40	36.7
	4-6 years	20	18.4
	7-10years	34	31.2
	10 years and above	15	13.8

Knowledge

The self-assessments of healthcare professionals using a dichotomous questions on knowledge revealed that 92.6% of the healthcare professionals have an excellent knowledge on what EMR is and its uses. The detail is in Table 2.

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Table 2: Health Care Professional Knowledge on use of EMR

Knowledge	YES (%)	NO (%)
I have heard about Electronic Medical Records.	97.2	2.8
An EMR is a digital record of patient's clinical data, management plan and treatment.	97.2	2.8
I know about the presence of Electronic Medical Records in our hospital.	85.3	14.7
Electronic medical record is synonymous with electronic health record.	89.9	10.1
Personal data, clinical data, laboratory data, radiology data, billing and payment receipts are components of EMRs.	93.6	3.7

Attitude

Respondent's attitude towards the use of EMR indicated that 45.9% agreed that the use of Electronic medical records will make patient management and follow up easier and 51.4% indicated that using e-health system will improves work performance. About 62% (61.5%) strongly disagree with the statement that EMR when introduced aids faster patient care, and about 1% (0.9%) of the respondents felt that EMR is detrimental to healthcare in the long run see Table 3.

Table 3: Healthcare professionals Attitudes towards the use of EMR

Attitudes	Disagree (%)	Neutral (%)	Agree (%)
Electronic medical records will make patient management and follow up easier	51.4	2.8	45.9
EMR when introduced aids faster patient care	61.5	3.7	34.9
Healthcare workers prefer EMR than the paper based type	56	19.3	24.8
Using e-health system improves my work performance	45	3.7	51.4
Training healthcare workers on EMR should be mandatory	45	9.2	45.9
Using e-health system improves the quality of the work I do	55	3.7	41.3
EMR is detrimental to healthcare in the long run	29.4	54.1	0.9

Perception

About 46% (45.9%) of the respondents strongly agree with the statement that Using e-health system enables me to accomplish tasks more quickly, and 53.2% Agree that using e-health system enhances my effectiveness on the job, while only 7.3% strongly agree that I feel electronic medical record affects doctor-patient relationship negatively see Table 4

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Table 4: Perception of Healthcare professionals on the use of EMR

Perception	SD (%)	D (%)	A (%)	SA (%)
Using e-health system increases my work productivity	3.7	2.8	48.6	44
I feel electronic charting would lead to better patient care and safety	1.8	10.1	51.4	35.8
Using e-health system enhances my effectiveness on the job	2.8	3.7	53.2	39.4
I believe electronic medical record increases cost of care for the patient	14.7	38.5	34.9	11
I feel electronic medical record affects doctor-patient relationship negatively	22	46.8	22.9	7.3
I feel electronic medical record does not protect patient privacy	20.2	53.2	13.8	11.9
When in use, I believe EMR will have a poor cultural acceptability	20.2	47.7	25.7	5.5
I believe that EMR should be used in the routine care of patient in our hospitals	5.5	5.5	46.8	41.3
I think EMR should only be used for specialized care of patients	14.7	58.7	17.4	8.3
Using e-health system enables me to accomplish tasks more quickly	6.4	7.3	39.4	45.9

Key: SD-strongly Disagree, D-Disagree, A-Agree, SA- Strongly Agree

DISCUSSION

The use of electronic medical record (EMR) in Nigeria has been a topic of interest, and several studies have been conducted to assess the knowledge, attitude and perception of healthcare professionals towards EMR. Electronic medical records system is one of the essential pathways to achieving and optimizing the delivery of quality of health care [10]. The introduction of EMRs in the study setting in 2021 necessitates a thorough evaluation of the knowledge of EMRs among its healthcare workers. The successful implementation and utilization of EMRs will heavily rely on the understanding and proficiency of the workforce in utilizing this technology. It is crucial to assess and address any gaps in knowledge to ensure the effective integration of EMRs into daily practice. This study focus mainly on the knowledge, attitude and perception of EMR among healthcare workers in NECC and also to access the relationship between profession and knowledge of EMR among the respondents.

The study found that 92.6% of respondents in the National Ear Care Center, Kaduna,

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have excellent knowledge about electronic medical records (EMRs). This indicated that healthcare professionals are well-informed and knowledgeable about the concept, purpose, and functionalities of EMRs. This knowledge is higher than the 71.5% found in a study conducted in Plateau State [9], suggesting that NECC had better access to information and also utilizes it in this era of unlimited medical information. The implication of the findings of this study to practice is that a relatively good knowledge base of EMRs exits, which will provide a good and smooth take off of such system, which has just being introduced.

Additionally, attitudes of the respondents towards electronic medical records (EMRs) provides insights into their perceptions and beliefs about the impact of EMRs on patient management, patient care, healthcare in the long run, and their own work performance, According to the survey data, approximately half of the respondents, specifically 45.9%, expressed a certain sentiment or perception that the use of EMRs would make patient management and follow-up easier. This suggests that an average proportion of the healthcare professionals in the NECC, Kaduna, recognized the potential benefits of EMRs in improving the efficiency and effectiveness of patient management processes. This finding is similar to the study obtained in 2016 by Farahnaz *et al* in Tehran. Which showed that 40.4% had good attitude and practice habits [11]. The belief that EMRs can streamline patient management aligns with the widely acknowledged advantages of EMRs, such as easy access to patient information, improved coordination of care, and enhanced communication among healthcare providers [12].

The majority of respondents (61.5%) disagree with the claim that EMRs improve patient care speed, suggesting that initial implementation may not be immediate due to factors like learning curves or system integration challenges, necessitating adequate training and support.

Nonetheless, only a small proportion of the respondents (0.9%) felt that EMRs are detrimental to healthcare system in the long run. This suggests that the majority of healthcare professionals in the NECC, Kaduna, do not perceive EMRs as negatively impacting healthcare delivery. Similarly, a study in Benghazi in 2005 reported that only 4% of the respondents had negative attitude [13]. This result aligns with the growing body of research supporting the positive impacts of EMRs, including improved accuracy, accessibility, and continuity of patient information.

An average 45.9% of respondents strongly agree that using an e-health system improves efficiency and time-saving, aligning with the advantages of Electronic Medical Records (EMRs), such as quick access to patient information and improved data management.

Only 7.3% of respondents disagree that electronic medical records negatively impact the doctor-patient relationship. However, concerns about increased screen time, distractions, and reduced face-to-face interaction can be addressed through appropriate training, communication, and workflow design to mitigate any negative effects [14].

The majority of respondents (53.2%) believe that e-health systems improve their job effectiveness due to improved patient data access, decision support tools, automated reminders, and efficient communication. However, a small proportion of respondents believe EMRs negatively affect doctor-patient relationships, requiring strategies to maintain strong communication and a patient-centered approach. . To preserve a patient-centered approach, strategies like establishing a personal connection, active

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listening, and allowing patients to set the visit agenda are recommended

CONCLUSION

The study highlighted the importance of ongoing education and training initiatives introduced at NECC, Kaduna to enhance EMR implementation, utilization and promoting a culture of understanding and effective usage of EMR in healthcare delivery.

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REFERENCES

1. Ojo, A. I. and S. O. Popoola (2015). Some Correlates of Electronic Health Information Management System Success in Nigerian Teaching Hospitals. Biomedical Informatics Insights **7**: 1-9 doi: 10.4137/BII.S20229
2. Adeleke, I.T. Sunday A.E, Ameenah M.N Tony C.A, Oluseye A.O and Danjuma A (2015). Health Information Technology in Nigeria: Stakeholders' Perspectives of Nationwide Implementations and Meaningful Use of the Emerging Technology in the Most Populous Black Nation. American Journal of Health Research **3**(1-1): 17-24. DOI:10.11648/j.ajhr.s.2015030101.13
3. Delpierre C, Cuzin L, Fillaux J, Alvarez M, Massip P, Lang T (2004). A systematic review of computer-based patient record systems and quality of care: more randomized clinical trials or a broader approach? International Journal of Quality Health Care. Oct;16 (5):407-16. doi: 10.1093/intqhc/mzh064. PMID: 15375102.
4. Yehualashet G, Andualem M, and Tilahun B (2015). The Attitude towards and Use of Electronic Medical Record System by Health Professionals at a Referral Hospital in Northern Ethiopia: Cross-Sectional Study. Journal of Health Informatics in Africa.;3(1):19-29. DOI: https://doi.org/10.12856/JHIA-2015-v3-i1-124
5. Al-Nassar BAY, Abdullah MS, Sheik Osman WR (2011). Health professionals use Electronic Medical Records System (EMR system) in Jordan hospitals 11(8):112-8. International Journal of Computer Science Network Security. Corpus ID: 45010005
6. Chaplin B, Meloni S, Eisen G, Jolayemi T, Banigbe B, Adeola J, et al. Scale-up of networked HIV treatment in Nigeria: Creation of an integrated electronic medical records system. Int J Med Inform. 2015; 84: 58-68.
7. Cline GB, Luiz JM. Information technology systems in public sector health facilities in developing countries: The case of South Africa.

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 1 June, 2024 ISSN: 2971_611X
Pages 114 - 124 Submitted 14/5/2024 Accepted 2/6/2024
APSBJournal

BMC Med Inform Decis Mak. 2013;13 :13.

8. Pantuvo JS, Naguib R, Wickramasinghe N. towards implementing a nationwide electronic health record system in Nigeria. Int J Health care Deliv Reform Initiatives. 2011;3: 39-55.
9. Afolaranmi T.O, Hassan Z.I, Dawar B.L, *et al*. (2020). International Journal of Research in Medical Sciences Nov;8 (11):3837-3843
10. Odekunle F.F, Odekunle R.O, Srinivasan S (2017). Why sub-Saharan Africa lags in electronic health record (EHR) adoption and possible strategies to increase EHR adoption in this region. International Journal of Health Sciences and Research. 7(1):282-290.
11. Farahnaz S. , Morteza H., Ali V. , Ali M. , Hesamedin A M. Assessment of Health Information Technology Knowledge, Attitude, and Practice among Healthcare Activists in Tehran Hospitals (2017). International Journal of Computer Science and Network Security, VOL.17 No.1
12. Anas Ali Alhur (2023). An Exploration of Nurses' Perceptions of the Usefulness and Easiness of Using EMRs ournal of Public Health Sciences 2(1) 20-31 DOI: 10.56741/jphs.v2i01.263
13. Medical Records in the General Hospitals in Benghazi, System, 2007, 3 (2):1-4.
14. Abdulmalek LJ. Doctors Knowledge, Attitude and Practice Towards Electronic

Archives of Pharmaceutical Sciences and Biotechnology Journal
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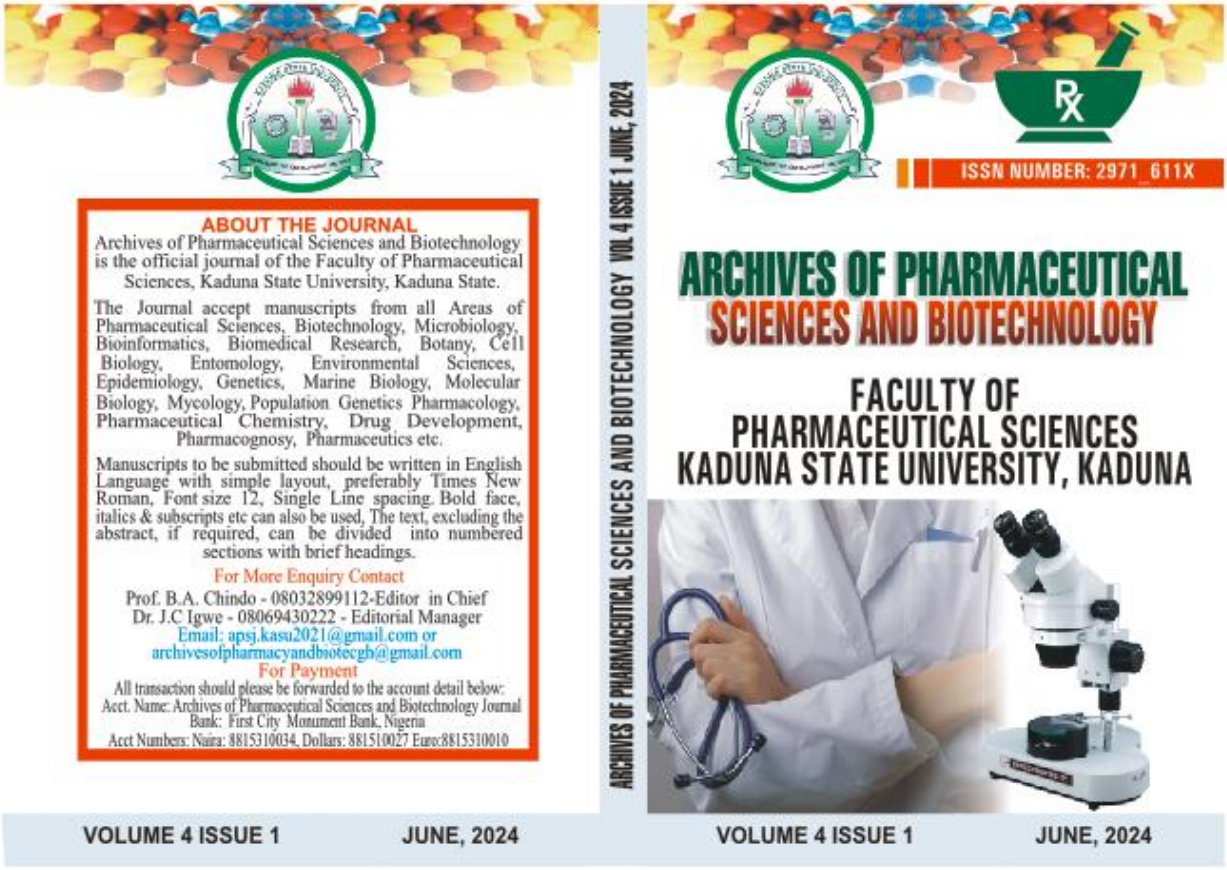

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ASSESSMENT AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS ISOLATED FROM HYDROLYZED GOAT MILK USED AS PEPTIDE INHIBITOR AGAINST HYPERTENSION

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ABSTRACT

Introduction: Hypertension has been a significant risk factor for cardiovascular illness. Synthesized angiotensin converting enzyme (ACE) inhibitors are active antihypertensive medicines but they commonly cause unwanted side effects.

Aim: This study describes the characterization of an ACE inhibitory peptide from goat milk hydrolysate.

Methods: Hydrolysates containing angiotensin converting enzyme (ACE) inhibitory peptide were prepared from goat milk using pepsin and trypsin.

Results: The purified peptide has an optimum temperature of 40 °C and optimum pH of 2. The activity of the purified peptide was not significantly ($P>0.05$) affected after *in vitro* incubation with gastrointestinal enzymes pepsin and trypsin. The purified peptide contained 18 amino acids with glutamic acid being the most abundant (13.63g/100).

Conclusion: ACE inhibitory peptide from purified goat milk hydrolysate digested with pepsin may be a helpful component to serve as drug against hypertension.

Keywords: Hypertension; Angiotensin Converting Enzyme Inhibitory Peptide; Goat Milk Hydrolysate; Purification; Ultrafiltration Membrane

1.0. INTRODUCTION

Worldwide, hypertension affects 15-20% of adults and is the primary cause of cardiovascular illness, accounting for 7.5 million deaths annually [1]. It is linked with cardiovascular disease, insulin resistance, obesity, carbohydrate tolerance, hyperuricidemia, and atherosclerosis [2]. Hypertension disturbs the structures and functions of arterioles, other blood vessels and can cause damage at variable rates to different target organs such as the brain, eye and kidney [2].

Angiotensin converting enzyme (ACE) inhibitors are used as the primary line of treatment for hypertension [3]. ACE is crucial for the physiological control of hypertension. ACE catalyzes the conversion of the inactive decapaptide, angiotensin I into an active vasoconstricting octapeptide, angiotensin II [3]. Lisinopril, captopril, enalapril, and other common ACE inhibitors are currently been used as synthetic inhibitors in the market, however they all have undesirable side effects such as cough, skin rashes, nausea, vomiting and dizziness. As a result, interest has grown in looking for all-

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natural remedies that could reduce hypertension [4].

Proteins source such as milk, soy, fish, and other proteins have been found to contain peptides with inhibitory activity against ACE. Many of these peptides that are bioactive are found to have anti-oxidative, anti-carcinogenic, antibacterial, immunomodulatory, antithrombotic, mineral binding, and ACE inhibitory properties [5]. Milk proteins serve as rich sources of bioactive peptides, as they are very well characterized, available in large amounts at a high degree of purity and low price [6]. As a result, the aim of this study was to characterizing an ACE inhibitory peptide purified from goat milk digested by pepsin and trypsin.

2.0. MATERIALS AND METHODS

2.1. Materials

Angiotensin converting enzyme from rabbit lungs, Hippuryl-histidyl-leucine (HHL), pepsin and trypsin (Sigma-Aldrich, St. Louis, MO 63103, USA). All other reagents used in the study were of analytical grade.

2.2. Equipment

Refrigerated centrifuge (MSB005.CR2.K, MSE Ltd, U.K), HPLC Machine (Adept CECIL CE4201) and spectrophotometer (JENWAY 6300) (Bibby scientific limited, UK), Spectrophotometer CE7400 (CECIL, Cambridge England), Oven (OV/100), Incubator (MINI/50/VIS) and pH meter (Genlab, Metrohm Ltd., Herisan, Switzerland), Electrical weighing balance and Freeze dryer (LabogeneApS, Denmark).

2.3. Effect of pH and Temperature on ACE Inhibitory Activity of the Partially Purified Peptide

The lyophilized ACE inhibitory peptide was dissolved in distilled water and incubated for 2 hours at various pH values ranging from 2 to 10. The inhibitor's ACE inhibitory activity was investigated. The influence of temperature on inhibitory action was also investigated by altering the incubation temperature from 20 °C to 80 °C [7].

2.4. Effect of Digestive Enzymes on ACE Inhibitory Activity

The peptide solution was incubated with pepsin at pH 2.0 and 37°C for 2 hours to imitate the enzymatic effects in the human intestines and stomach, as well as to analyze the stability of the ACE inhibitory peptide in vitro. After standing in boiling water for 10 minutes, the reactions were halted and centrifuged at 10,000 x g for 30 minutes. After that, the supernatant was collected to test for ACE inhibitory activity.

2.5. Determination of Amino Acid Composition

The amino acid profile in the sample was determined using the method described by [8]. Before loading into the amino acid analyzer, the sample was dried to a constant weight (10ml), hydrolyzed, and evaporated in a rotary evaporator.

2.6. Statistical Analysis

Data were expressed as mean of three replicates ± standard deviation (SD). The data were subjected to analysis of variance using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

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3.0. RESULTS

3.1 Effect of pH and Temperature on the Partially Purified Peptide

The inhibitory activity of the peptide on ACE was optimum at 40 °C (84 %), after which it decreased with increasing temperature having the lowest activity at 80 °C (71 %) (Figure 1).

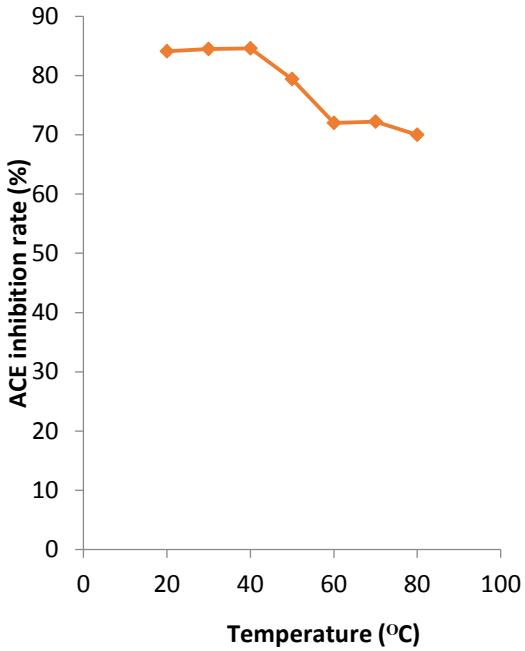

Figure 1: Effect of temperature on the ACE inhibitory activity of the partially purified peptide

The ACE inhibitory activity of the partially purified peptide was optimal at pH 2 (83 %), and remained stable between pH 3 and 4 due to slight decrease in inhibitory activity. The inhibitory activity of the peptide continues to decrease beyond pH 4 until it reached the lowest activity at pH 8 (58 %) (Figure 2).

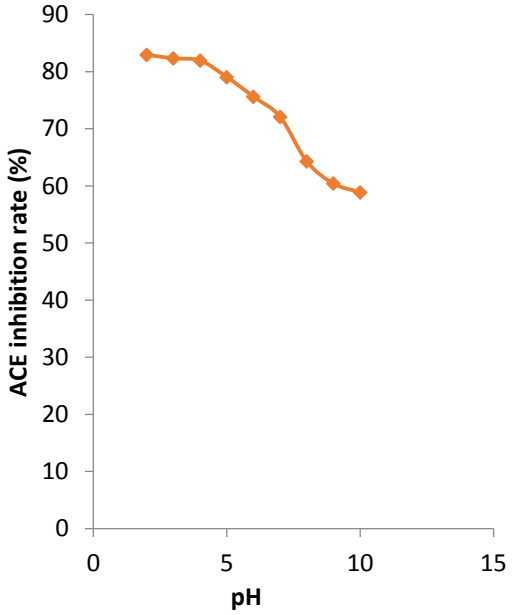

Figure 2: Effect of pH on the ACE inhibitory activity of the partially purified peptide

Figure 3 present the effect of pepsin and trypsin on the stability of the ACE inhibitory peptide. The results revealed that the ACE inhibitory activity of the partially purified peptide was not significantly ($P > 0.05$) affected after *in vitro* incubation with gastrointestinal enzymes. The value obtained for digestion with pepsin was 84.41 ± 0.06 %, while that of trypsin was 84.32 ± 0.04 % and control had 84.50 ± 0.26 %.

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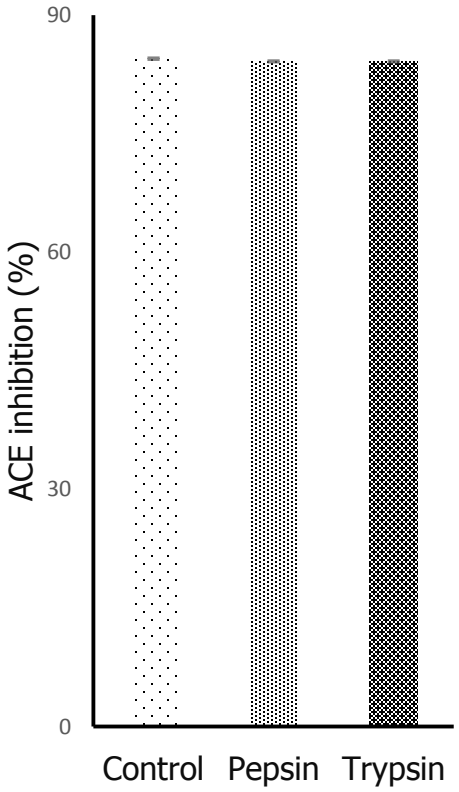

Figure 3: Stability of ACE inhibitory activity of the partially purified peptide after digestion with some gastrointestinal enzymes

Control = ACE inhibitory activity without gastrointestinal enzymes

Table 1 present the amino acid content of the partially purified peptide. The peptide contained eighteen (18) amino acids with glutamic acid having the highest percentage (13.63g/100 g), followed by leucine (10.25 g/100 g) and aspartic acid (8.20 g/100 g), while cysteine (1.33 g/100 g) was the lowest.

Table 1: Amino acids composition of ACE inhibitory peptide derived from enzymatic hydrolysis of goat milk

Amino Acids	Concentration (g/100g protein)
Leucine	10.25
Lysine	7.40
Isoleucine	7.34
Phenylalanine	5.23
Trytophan	2.36
Valine	5.44
Methionine	3.55
Proline	3.25
Arginine	6.54
Tyrosine	4.30
Histidine	3.13
Cystine	1.33
Alanine	4.02
Glutamic acid	13.63
Glycine	5.42
Threonine	4.80
Serine	4.86
Aspartic acid	8.20

4.0. DISCUSSIONS

The partially purified peptide from goat milk hydrolysate maintained their ACE-inhibitory activity after exposure to different pH values and different temperatures for 2 hours (Figures 1 and 2). The ACE inhibitory peptide was more stable at lower temperatures below 40 °C, but was more sensitive at higher temperatures, resulting in a drop in inhibitory activity. The decrease in activity of the partly purified peptide (Figure 1) indicates that they may be thermally denatured at high temperatures [9].

The effect of pH on the ACE inhibitory activity of the partly purified peptide suggests that acidic and slightly alkaline circumstances were more affirmative for

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maintaining inhibitory activity than high alkaline settings with an optimal pH of 2 (Figure 2). These findings showed that the partly purified peptide produced from goat milk hydrolyslate is thermally and pH stable. This is consistent with findings from other studies [9,10], who isolated stable ACE-inhibitory peptides from various sources, demonstrating that the peptides can be easily transported and stored at various temperatures and pH levels after being developed into a product.

For ACE inhibitory peptides to maintain their antihypertensive effect *in vivo* they must be ingested in their intact form from the intestine and further be resistant to plasma peptidase degradation to reach their target sites. This is because their activity may be affected if hydrolyzed *in vivo* [11]. The *in vitro* gastrointestinal enzyme stability studies of isolated ACE-inhibitors allow us to model their action *in vivo* following oral administration. The results showed that *in vitro* incubation with gastrointestinal enzymes had no effect on the partially-purified peptide's ACE-inhibitory action ($P>0.05$). This study is consistent with the work of [12] and [9], the results indicate that the partially purified peptide may be real inhibitor-type ACE-inhibitory peptides because it is capable of inhibiting ACE.

On assessment of the amino acids composition of ACE inhibitory peptide derived from enzymatic hydrolysis of goat milk. The findings from this study showed that partially purified peptide contain amino acid residues such as Leucine, Isoleucine, Tryptophan, Proline, and Glutamic acid were obtained; all of which have been shown to improve peptide ACE inhibitory activity [13,14], with Glutamic acid concentration being higher than other amino acids (Table 1). This is similar to an ACE inhibitory peptide isolated from *Moringa oleifera* leaves [15].

5.0 CONCLUSION AND RECOMMENDATIONS

This study showed the ACE inhibitory activity of the partially purified peptides at different pH and temperature, as well as the stability of the peptides in the presence of gastrointestinal enzyme. The amino acid composition of the peptides were also obtained and it contains 18 amino acid. The purified peptide isolated from goat milk hydrolysate possess potent ACE inhibitory activity and may be used in the development of reasonably cost, effective, and harmless antihypertensive peptides to serve as substitute to synthetic ACE inhibitory medication. Further studies are required to determine the *in vivo* activity of the purified ACE inhibitory peptide.

Authors Contribution:

Hashidu A.S., Babangida M. I. and Abdulazeez A.M. contributed to the study's conception, implementation, data analysis, and manuscript writing. Adekale I. A., Musa U. U., Efienokwu J. N. and Makeri M. S. contributed to data collection, processing and interpretation of the results. All of the writers examined the findings and approved the final manuscript version.

Conflict of Interest

The writers have all stated that they have no conflicts of interest.

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REFERENCE

1. Rosengren, A., Smyth, A., Rangarajan, S., Ramasundarahettige, C., Bangdiwala, S. I., Habib, K. F., and Gupta, R. (2019). Socioeconomic status and risk of cardiovascular disease in 20 low-income, middle-income, and high-income countries: The Prospective Urban Rural Epidemiologic (PURE) study. *The Lancet Global Health*, 7(6), e748-e760.
2. Bosetti, F., Galis, Z. S., Bynoe, M. S., Charette, M., Cipolla, M. J., Zoppo, G. J., and Lutty, G. A. (2016). Small Blood Vessels: Big Health Problems? Scientific Recommendations of the National Institutes of Health Workshop. *Journal of the American Heart Association*, 5(11), e004389.
3. Aluko R. (2015). Antihypertensive Peptides from Food Proteins. *Annual Review of Food Science and Technology*. 6:235-262.
4. Persson, I.A.L, Persson, K. and Andersson, G.G. (2009). Effect of *Vaccinum myrtillusa* nd its polyphenols on angiotensin-converting enzyme activity in human endothelial cells. *Journal of Agricultural and Food Chemistry*, 57: 4626 - 4629.
5. Nandhini, B., Angayarkanni, J. and Palanisamy, M. (2012). Angiotensin converting enzyme inhibitory activity and antioxidant properties of goat milk hydrolysates. *International Journal of Pharmacy and Pharmaceutical Science*. 4: 367-370.
6. Anusha, R., & Bindhu, O. S. (2016). Bioactive peptides from milk. *Milk Proteins*, 101.
7. Geng, X., Tian, G., Zhang, W., Zhao, Y., Zhao, L., Wang, H., and Ng, T. B. (2016). A *Tricholomamatsu* take peptide with angiotensin converting enzyme inhibitory and antioxidative activities and antihypertensive effects in spontaneously hypertensive rats. *Scientific Reports*, 6, 24130.
8. Benitez, L. V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies. *Fish Nutrition Research in Asia*, 23-35.
9. Qu W, Ma H, Pan Z, Luo L, Wang Z, He R, 2010. Preparation and antihypertensive activity of peptides from *Porphyrayezoensis*. *Food Chemistry*. 123(1):14-20.
10. Chen, J., Liu, Y., Wang, G., Sun, S., Liu, R., Hong, B. and Bai, K. (2018). Processing Optimization and Characterization of Angiotensin-I-Converting Enzyme Inhibitory Peptides from Lizard fish (Synodusmacrops) Scale Gelatin. *Marine drugs*, 16(7), 228.
11. Bougatef, A., Nedjar-Arroume, N., Ravallec-Plé, R., Leroy, Y., Guillochon, D., Barkia, A., and Nasri, M. (2008). Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (*Sardinella aurita*) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases. *Food Chemistry*, **111**, 350–356.
12. Pan, S., Wang, S., Jing, L., and Yao, D. (2016). Purification and characterisation of a novel angiotensin-I converting enzyme (ACE)-inhibitory peptide derived from the enzymatic hydrolysate of *Enteromorpha clathrata* protein. *Food chemistry*, *211*, 423-430.

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Volume 4 Issue 1 December, 2024 ISSN: 2971_611X
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APSBJournal

13. Lei, C., Huang, M., Zhao, D., Deng, Y., Qian, B., Zhang, L., and Wu, M. (2011). Amino Acid Composition, Antioxidant Property and Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity of Micropeptides in Skim Milk Powder. *The Philippine Agricultural Scientist*, 94(3).
14. Yu, D., Wang, C., Song, Y., Zhu, J., and Zhang, X (2019). Discovery of Novel Angiotensin-Converting Enzyme Inhibitory Peptides from *Todarodes pacificus* and Their Inhibitory Mechanism: *In Silico* and *In Vitro* Studies. *International journal of molecular sciences*, *20*(17), 4159.
15. Abdulazeez A. M., Yusuf R., Kurfi B., (2016). Isolation and Characterization of angiotensin converting enzyme inhibitory proteins from *Moringa oleifera* leaves. *Bayero Journal of Biomedical Science* 1(1): 8-17.