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Evaluation of the Apiacupuncture Against the Damages and Erythrocyte Osmotic Fragility Induced by Oxidative Stress in Egyptian Patients with Chronic Neck Pain

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Abstract

Many people suffer from neck pain due to a sedentary lifestyle. It has become imperative to study dealing with neck pain in Oriental medicine. Thirty female patients diagnosed with chronic neck pain were subjected to this study & thirty healthy control. The mean of the Visual analog scale (VAS) and of the Neck Disability Index Questioner (NDIQ) were significantly decreased ($P< 0.05$) after bee venom therapy. Also a significant decrease in Oxidative stress markers after bee venom administration, where malondialdehyde (MDA) & Glutathione S-transferases (GST) activities were decreased after treatment. On the other hand, catalase activities after bee venom were increased with a significant difference ($P<0.05$). Moreover, bee venom acupuncture diminished the elevated average osmotic fragility and decreased the elevated rate of haemolysis. Results suggested that bee venom could be a supportive antioxidant that plays an important role in damage control caused by oxidative stress. It can be concluded that Bee venom pharm-acupuncture has been reported to be effective in treating and dominating neck pain.

The aim of this study was to evaluate the role of bee venom acupuncture in chronic neck pain patients, concerning pain intensity and supported lifestyle quality with controlling oxidative damage.

Keywords: Chronic neck pain, Bee venom, Visual analog scale, Malondialdehyde , Neck Disability Index Questioner.

1. Introduction

Chronic neck pain (CNP) is a common medical disorder that impacts 30 to 50% of the overall population and is the maximum common in middle age [1, 2]. CNP causes a big socioeconomic problem due to health care costs and loss of productive capacity [3]. CNP can be associated with neck pain, upper limb pain, and headache, however, isn't always related to apparent radiculopathies or myelopathies, consisting of sensory and motor dysfunction [4]. Neck pain is categorized in Oriental medicine as the stiffness of the neck, headache, pain caused by stiffness, paresthesia, etc., which leads to the collateral meridian interruption and interruptions in the circulation of energy and blood [5].

Depending on the conventional therapeutic plans, different tools are available for neck pain, including medical drugs (non-steroidal anti-inflammatory drugs (NSAIDs), antiepileptics and serotonin reuptake inhibitors, physical therapy, spinal injection, and surgical interventions [6,7]. However, most of these previous therapies only have any effective cure in the short term, and most of these interferences are often accompanied by multiple side effects [8]. It has been reported that patients with (CNP) tend to use both complementary alternative medicine (CAM) and conventional treatment at the same time in the hope of having more positive effects and safety profiles [9].

Bee Venom (BV) contains bioactive molecules [10, 11] made of protein, peptides, amino acids, sugars, biogenic amines, phospholipids, enzymes, volatile compounds, and pheromones. Also, it is very aqueous as more than 80% of (BV) is water [12]. Melittin and phospholipase A2 (PLA2) are two more plentiful components of the (BV) [13]. Melittin accounts for around 50-60% of the dry weight. In addition, melittin is the most toxic compound of the (BV) molecule, but it has

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significant clinical and therapeutic effects [14]. It has anti-inflammatory activity by means of several mechanisms. Furthermore, it suppresses the nuclear factor Kappa-B (NF-kB) vital modulator [15]. All these mechanisms cause inhibition of the production of pro-inflammatory cytokines like prostaglandin E2 (PGE2), tumor necrosis factors (TNF), and nitric oxide (NO) [16]. Apamin has a cytotoxic and nociceptive action in nerves [17], so apamin has the capacity to be a part controlling of several (CNS) diseases [18]. Also, apamin has an anti-inflammatory effect through the suppression of (TNF-α), (IL-1), (IL-6), and (NO) [19]. With these pharmaceutical characteristics, (BVA) has been used to manage Parkinson's disease [20], knee pain [21], low back pain [15], and rheumatoid arthritis [22]. On the other hand, the bee sting can also be simulated directly with an injection of (BV) or indirectly by acupuncture needles [13]. Inflammation due to neck pain causes oxidative stress, which enhanced the production of reactive oxygen species which leads to damage lipids, proteins, and nucleic acids [23].

Experimental studies have shown that acupuncture can attenuate oxidative stress, which appears to be possible to explore the physiological antioxidative mechanism of acupuncture in various diseases [24].

The aim of this study was to evaluate the role of bee venom acupuncture in chronic neck pain patients, concerning pain intensity and supported lifestyle quality with controlling oxidative damage.

2. Materials and Methods:

Thirty patients with chronic neck pan and thirty healthy controls were recruited from the attendants at the Neurological Clinic & Complementary Medicine Clinic of the Medical Excellent Service Unit of the National Research Centre (NRC), Egypt.

Ethical Criteria

This study was approved by NRC ethics committee number 17091 as all patients were more than 18 years old; informed consent was obtained from each patient before being enrolled in the study. All patients were subjected to full history taking& clinical examination.

2.1. Inclusion Criteria

Thirty patients with chronic neck pain (CNP) & thirty healthy control were included in the study after advertisements in the local newspapers as well as on hospital websites. All participants were interviewed by telephone and scheduled for a screening visit by the clinical research coordinator. The present study started with 45 patients. Some cases (n=10) did not fulfill the inclusion criteria and were excluded from the study. Other cases (n=5) were irregular in attending apitherapy sessions. These cases were excluded as well, so as not to affect the statistical analysis. All patients were Egyptians their ages varied from 30-60 years. They have a painful restriction of cervical spine mobility >6 months and were not on any treatment for 2 weeks prior to the study, they were diagnosed and verified by cervical X-ray to diagnose and detect degenerative changes. Patients with grade II neck pain with no signs or symptoms of major structural pathology, but major interference with daily activities according to the neck pain task force [25].

2.2. Exclusion Criteria

The excluded criteria in this study were pregnant patients, patients having viral hepatitis (HBV, HCV), diabetes mellitus, and those with a history of malignancy, neurological deficits, pain below the elbow, ankylosing spondylitis, history of neck surgery or dislocation or fracture, epilepsy, organ failure, a severe psychiatric or psychological disorder, previous acupunctures, rheumatoid arthritis, organ failures, clotting disorders, anticoagulant therapy. Also, patients with hypersensitivity to bee venom were be excluded.

2.3. Radiological examination

Were be carried out of plain X-ray the cervical spine both AP, lateral views, and MRI to rule out surgical causes of neck pain.

2.4. Laboratory examination

Before and after treatment with(BVA), blood samples (5ml) were withdrawn from each patient then left to coagulate then centrifuged at 3000 rpm for 15 minutes, sera were separated into aliquots and stored at -70 till the determination of the activity of plasma oxidative stress and antioxidant parameters malondialdehyde (MDA) concentration [26], Glutathione S-transferases (GST) [27] and Catalase [28]. Liver and kidney functions were determined; Alanine transaminase (ALT) actively as the method [29,30] and Creatinine [31] by using the spectrophotometric assay method.

2.5. Erythrocyte osmotic fragility

Whole blood samples (3 ml) withdrawn in heparinized tubes were added to the hypotonic buffer saline in the proportion of 1:100 respectively. Hypotonic saline buffered to pH 7.4, with different concentrations (0, 2, 3, 3.5, 4, 5, 5.5, 6, 7.5, and 9 g/L) were used. The samples were incubated for 30 min at 37 ^{0}C, and centrifuged at 3000 rpm for 5 min

to precipitate the non-hemolyzed red cells. The osmotic lysis of red blood cells is detected by the release of hemoglobin into the extracellular fluid. The amount of hemoglobin appearing in the media was determined calorimetrically according to the method reported by [32]. The quantitative measurements [degree of hemolysis versus decreasing (NaCl)concentration] were plotted on a graph called the fragility curve [33, 34]. The experimental curves were normalized to 100% hemolysis to facilitate the comparison between different samples without the interference of the hematocrit changes. The average osmotic fragility (H50) (the (NaCl) concentration producing 50% hemolysis can be evaluated by the fragility curve. Other parameters can be obtained from the differentiation of the fragility curve, which represents a Gaussian curve [the rate of hemolysis (dH/ dC) versus (NaCl) concentration]. These parameters are the peak's position, the peak's width, the peak's area, and the peak's height. The peak's position on the x-axis is equivalent to the average osmotic fragility (H50). The width at half maximum reflects the dispersion of the hemolysis process. The area below the curve shows the rate of hemolysis of erythrocytes at certain intervals of (NaCl) concentration. The Gaussian peak indicates the maximum rate of hemolysis (dH/dC) max reached by the sample [35].

2.6.Assessment of patient

Pain intensity on a visual analog scale (VAS) was scored by the patients being asked to draw a mark on the line representing experienced pain, one end represents "no pain" while the other represents the worst pain. VAS was estimated at rest and scored the intensity of direction-related pain for the six directions [36].

The neck disability index questionnaire (NDIQ) is a condition-specific instrument for self-report of disability consisting of 10 items referring to various activities (personal care, lifting, driving, work, sleeping, concentration, reading, recreation and pain, pain intensity, headache) with 6 possible answers for each item. The score of each item varies between 0 (no pain and no functional limitation) and 5 (worst pain and maximal limitation [37].

The selected (CNP) patients were subjected to (BVA) treatment in the form of bees'stinging at standard acupoints (GB34, GB20, GB21, UB60, UB10, Liv3, SI3, LI4, LI11, TE5) according to Traditional Chinese Medicine [38]. All procedures, including the bee venom acupuncture increment protocol administered into predefined acupoints, were designed by a process of consensus with experts and previous researchers, according to the standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) [39], where each patient received 2 sessions weekly for 6 weeks.

2.7. Safety

BV is a toxic substance that causes some problems related to allergic reactions; however, a big dose is necessary to be lethal. The median lethal dose for an adult is 2.8 mg of venom per kg of body weight. Therefore, for a person of 70 kg, 196 mg of venom is needed to be a lethal dose. Nevertheless, this amount of venom is enormous because, in one single bee, there is only 0.15–0.30 mg of venom. Hence, as the number of stings necessary to be lethal is around 1300, (BV) has a reduced risk in terms of therapeutic uses [13]. Multiple bee stings always constitute a medical emergency, and patients who have suffered a mass attack by Africanized bees (>50 stings) should be monitored, as the venom may persist in the circulation for days and cause delayed reactions [40]. Inflammation results from an unbalanced immune response associated with the elevated production of pro-inflammatory cytokines, chemokines, and histamine. These are secreted through the degranulation of mast cells triggered by allergen stimuli, which are central in acute hypersensitivity reactions. This hypersensitivity inflammation has been associated with mitogen-activated protein kinase and nuclear factor-κB signaling cascade of mast cells and leukocytes with an inhibitory effect. This effect has been shown to be dependent on (BV) concentrations [41]. Moreover, (BV) action is dependent on the time, dose, and type of cell affected, producing a significant suppression of the immune response, with less leukocyte migration and concentrations of inflammatory mediators [42].

However, notwithstanding the latent therapeutic potential of (BV), there is a parallel reality of potential side effects or allergic reactions linked to (BV) composition. This aspect is one of the targets for the development of safe practices [43]. A systematic review provided a summary of the studies developed in apitherapy and their related adverse effects. The manuscript reviewed a total of 145 studies and found that 28.87% of (BV)-therapy-treated patients experienced adverse effects. Furthermore, given the allergenicity of the components of (BV), cases of allergenic reactions and anaphylactic responses to (BV) treatment have also occurred [44]. Other authors have emphasized that safety studies have not been developed for the

clinical application of (BV) and factors such as the incidence of adverse events, dose, frequency or form of administration have not been established or critically assessed in depth [45].

In our study prior to initiating (BVA)treatment and as a screening process for eligibility, a hypersensitivity test was performed for each patient by bee sting at (GV14) acupoints. Localized swelling greater than 10 mm in diameter or redness greater than 20 mm in diameter is considered a positive reaction; these patients were excluded from the study. A gradual increase in the dose of a bee sting was obtained. All adverse events and vital signs were noted and reported. The liver and renal functions were assessed for each participant before treatment and one week after the end of treatment.

2.8.Withdrawal and drop out

Participation in the study ended at any stage if the patient refused to continue, withdraws consent, violates inclusion or exclusion criteria or the trial protocol, or completes less than four treatment sessions as determined by the attending researchers. The trial was stopped if the principal investigator believed that there were unacceptable risks of serious adverse events.

3. Statistical analysis

Data were presented as the mean + Standard Deviation (SD) values. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Post Hoc and the least significant difference (LSD) tests using a statistical package for social science and also Pearson's correlation was used for statistical analyses by SPSS Statistics version 25 software. $P < 0.05$ was considered statistically significant.

4. Results and Discussion

Thirty female patients diagnosed with chronic neck pain were currently included in this study, their age mean was 45.8 ± 5.32, and their mean weight was 77.2 ± 8.3. The duration of the disease ranged from 1.54 ±0.28 years & thirty healthy control mean age was 48.5 ± 7.4 their mean weight was 69.2 ± 9.1 (in Table 1).

Table 1: Age and weight among the studied groups

Item	Chronic neck pain patient (n=30)	Healthy Control (n=30)
Age (years)	**45.8 ± 5.32**	**48.5 ± 7.4**
Weight (Kg)	**77.2 ± 8.3**	**69.2 ± 9.1**

Data are expressed as mean ± standard deviation (SD)

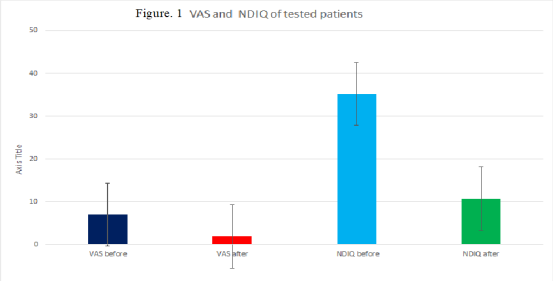
The mean of(VAS)for all patients studied before and after bee venom therapy was decreased from 7 ± 1.48 to 1.95 ± 1.46 with a significant P value ($P < 0.05$). All cases in the bee venom group achieved a decrease in the mean of (NDIQ)Questioner from 35.2 ± 6.23 to 10.75 ± 7.02 with significant P values ($P < 0.05$) (table 2) & (figure1).

Table 2: Mean of (VAS) and (NDIQ)of patients before &after(BV) therapy

Parameters	VAS	NDIQ	P values
Before bee venom therapy	**7 ± 1.48**	**35.2 ± 6.23**	**P < 0.05**
After bee venom therapy	**1.95 ± 1.46**	**10.75 ± 7.02**	**P < 0.05**

Data are expressed as mean ± SD $P < 0.05$ is significant

Figure 1:(VAS) and (NDIQ)of patients before and after Bee Venom therapy



In a Systematic review done by [46] the (VAS) was the most common evaluation tool, which was used in 30 studies of the 42 included studies. And three studies used the (NDIQ). A systematic review finding provided evidence of (BVA) for shoulder adhesive capsulitis, helping to obtain the best evidence in clinical practice [47]. Another study showed good results improvements of combined treatment of(BVA) and (NSAIDs) on people with non-specific chronic neck pain, showing excellent improvement of activity, but no long duration and there were no oxidative stress results [48].

In a study done by [49] the effects of Chuna manual therapy and Muscle relaxation pharmacopuncture were compared. They found that each group had significant effects in decreasing neck pain, but pharmacopuncture was more effective in improving (NDI). In a systematic study [50] from a review of the 17 clinical studies selected from Korean domestic databases. They found that pharmacopuncture therapy was effective in cervical pain induced by traffic accidents in all of these studies.

In this study, there were 30 neck pain patients treated with bee venom and 30 healthy controls. Oxidative stress markers were measured before and after bee venom administration, also (ALT) activity and creatinine concentration were measured. The results showed that, before treatment, (MDA) levels increased with a significant difference (P<0.05) compared with healthy controls, but after bee venom treatment there were decreases in(MDA) levels with a significant difference (P<0.05) compared with before treatment.

Also,(GST) activities increased in neck pain patients before bee venom treatment, but (GST) activities significantly decreased after treatment with bee venom (P<0.05).

On the other hand, catalase activities were lowered in neck pain patients before treatment with a significant difference of P<0.05 and after bee venom treatment the catalase activities increases with a significant difference P<0.05. In this study, we used (BVA) as a natural source of treatment to minimize hepatic, renal side effects and other hazards of chemical therapies, SO there was no significant difference between the values of (ALT) activities and creatinine concentrations before and after bee venom treatment, which is a good indication of the safety of the treatment (table 3).

Table (3): Effect of bee venom on oxidative stress caused by neck pain syndrome

Item	Healthy Control (n=30) Mean ± SD	Patients before bee venom therapy (n=30) Mean ± SD	Patients after bee venom therapy (n=30) Mean ± SD
MDA (nmol/l)	2.25 ±1.5	9.03 ± 2.2 (P<0.05)	4.7 ± 1.7 (P<0.05)
GST (U/L)	1.6 ± 1.06	6.97 ± 1.66 (P<0.05)	2.58 ± 1.14 (P<0.05)
Catalase (U/L)	88.25 ± 19	40.3 ± 11.31 (p<0.05)	73.25 ± 15.42 (p<0.05)
ALT (U/L)	22.7 ± 8.3	23.04 ± 9.1(P>0.05) (Non Sig.)	20.9 ± 7.66 (P>0.05) (Non Sig.)
Creatinine (mg/dl)	0.95 ± 0.21	0.88 ± 0.26 (P>0.05) (Non Sig.)	0.88 ± 0.05 (P>0.05) (Non Sig.)

Data are expressed as mean ± standard deviation (P < 0.05) is significant (P>0.05) is non-significant

Our data show that protection against oxidative damage has been obtained.

Oxidative stress is investigated in the pathogenesis of many diseases, including bone diseases and injuries [51]. Oxidative stress happens due to inequality between excessive generation and/or lowered degradation of oxygen species. It may cause destruction to lipids, proteins, and(DNA) [52]. There are different studies concordance with our result that inflammation has been recognized in the development and pathogenesis of a great number of different chronic disorders, including, chronic back pain, rheumatoid arthritis, nervous system diseases, metabolic diseases such as diabetes mellitus II, and cardiovascular diseases as atherosclerosis [53]. Higher values of oxidative stress in chronic back pain were due to an increase in lipid peroxidation and to the decreased level of anti-oxidants. The damage of joint spaces correlates with the overproduction of free radicals and lack of oxygen-processing enzymes and scavenging molecules [44].

The intracellular antioxidant mechanisms have non-enzymatic compounds such as reduced glutathione (GSH), Vitamins (C, E, Carotenoids) and minerals, in addition to enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [54]. Complementary and alternative medicine are the most frequently used to prevent cell damage caused by reactive oxygen species (ROS) [55]. MDA is an important indicator of oxidative stress and lipid peroxidation. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. It interacts with amino groups of proteins, phospholipids and nucleic acids and may have toxic effects [56, 57]. Also, high intracellular concentrations of GSTs are considered biomarkers for localizing and monitoring cell toxicity, infections, or injury [58]. There are different studies in agreement with our results, where (MDA) was studied as it has a role in pathogenesis also it was suggested that (MDA) can be used as a biological marker for different bone diseases including degenerative inflammatory rheumatic diseases [59], in fibromyalgia [60,61].

Oxidative stress is a biochemical and physiological process. The human body produces endogenous oxidative stress and free radicals. They are compatible with life and often the internal antioxidant mechanisms are overcome, but the internal antioxidant mechanisms are sometimes not sufficient to overcome the external free radicals that get inside the body with nutrient, drugs and other modern life conditions leading to disruption of normal metabolism [62]. It has been proven that some of these diseases are caused by oxidative stress, and fortunately, bee products have antioxidant properties [63]. The(BV) has an antioxidant action due to its mixture of melittin, apamin, and (PLA2) to increase super oxidase dismutase activity and stop the lipid peroxidation process [64].

Fig 2 revealed the erythrocyte osmotic fragility, the group of patients untreated with bee venom showed a shift to the right of the control curve, which indicates the elevation of the average osmotic fragility (H50). The widths at half maximum of these differential plots represent the relative elastic limit of RBCs membrane. The results indicated that both RBCs membrane elasticity and permeability decreased in untreated patients with bee venom. Acupuncture by Bee venom could modify the capability of erythrocytes to hemolysis when subjected to osmotic stress demonstrating a diminishing effect of free radicals on the structural integrity of RBCs. (Fig.2, Fig.3).

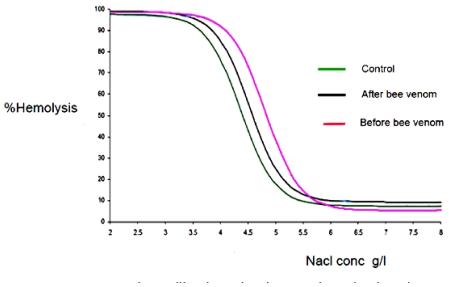


Figure 2: osmotic fragility alterations between the neck pain patients, bee venom treatment and healthy control group

Erythrocyte osmotic fragility is an indirect process for determining oxidative stress [65] as it detects the status of the red blood cell membrane & its metabolism. The hemolysis of the red blood cells reverberates the loss of integrity of the cells which can lead to the liberation of intracellular hemoglobin [66]. Membrane lipids are important for survival and cellular integrity maintenance [67].

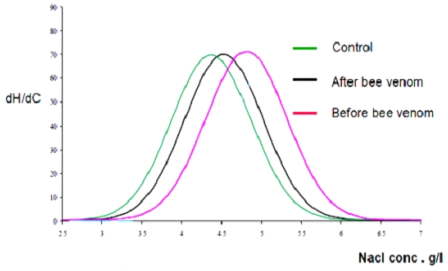


Figure 3: Gaussian curves of the neck pain patients, bee venom treatment and healthy control groups

The biochemical and physiological functioning of red blood cells can be disrupted by elevated(MDA) which is an indicator of lipid peroxidation. In the untreated group with bee venom, the fragility of red blood cells increased due to lipid peroxidative modification of the erythrocyte membrane. This leads to an increase in the maximal rate of hemolysis (height of the peak) and a reduction in hemolysis dispersion (lower dispersion than normally indicates sudden rupture of red blood cells). Also, it has been noticed that the center of the Gaussian peak (H50) shifted to greater (NaCl) concentrations..Changes in the red blood cell membrane's lipid composition are the sole factors that have a significant impact on mechanical behaviour; nevertheless, changes in the membrane's proteins structure are also important. [68]. Oxygen free radicals reduce red blood cell deformability, decrease cation permeability, and disrupt red blood cell membrane micro rheological characteristics (membrane rigidity increases) [69]. As a result, the osmotic fragility-induced alteration mirrored the cytoskeleton's degeneration.

5. Future Views and New Approaches

Although (BV) elements have been widely reported in the literature, little is known about their methods of action and metabolic pathways [70]. Also, further studies are required to establish specific protocols for ensuring management and safety [71, 47].

The possibility of acquiring what they call"essential bee venom" was investigated by some researchers. This material is made from Purified venom that has been clarified for histamine &(PLA2) to prevent its side adverse and allergic reactions while preserving its anti-inflammatory efficacy [72]. In our study, a hypersensitivity test was performed for each patient and gradually increasing the dose of a bee sting was obtained. All adverse events and vital signs were noted and reported. The liver and renal functions were assessed for each participant before treatment and one week after the end of treatment.

5. Conclusions

According to the results obtained it can be concluded that:

Bee venom pharmaco-puncture has proven effective in treating neck pain in addition to reducing elevated osmotic fragility, so it is effective in controlling oxidative damage.

6.Conflicts of interest

"The authors declare no conflict of interest." "The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results".

7.Formatting of funding sources

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9.Ethics approval and consent to participate

This study was approved by NRC ethics committee number 17091 &it was in accordance with the principles of Helsinki Declaration, as all patients were more than 18 years old; informed consent was obtained from each patient before being enrolled in the study. All patients were subjected to full history taking& clinical examination.

10.Author Contributions: Conceptualization, A.G.H.; methodology, F.I., A.E.G., S.W.A.and E.A.R.; validation, E.A.R., and S.W.A,; formal analysis, M.M.F.G,; investigation, M.M.F.G, E.A. R., S.W. A, A.M., , R.M. and A.E.G. resources, A.G. H, F. I.; data curation, A.G. H. and , F. I.; writing—original draft preparation, M.M.F.G, E.A. R., A.M., , R.M., A.E.G; writing—review and editing, A.G. H, F. I., E.A. R., and S.W. A,; supervision, A.G.H. and E.A. R.,.; project administration, A.G. H,; funding acquisition, A.G. H, and A.E.G. All authors have read and agreed to the published version of the manuscript.

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