RESEARCH PAPER

THE EFFECTS OF PAIN SENSITIVITY BEHAVIOUR ON SWISS WHITE MICE ADMINISTRATED WITH CHLOROQUINE PHOSPHATE

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ABSTRACT

This study evaluates the effects of *Chloroquine phosphate* on pain sensation in mice considering the fact that *Chloroquine* as s chemotherapic agent is known for its neurotoxicity effect. The mice were divided into three groups of 10 mice each. While group 1 as the control, 2 and 3 as the test groups and group 1 received 0.2ml physiological saline i.p. while test groups 2 and 3 received 10mg/k (human therapeutic dose) and 20ml/kg (pharmacological dose) of *Chloroquine* respectively. The tail flick and formalin tests were used to assess pain sensation. In the tail flick test, the latency of tail flick in group 2 and 3 were significantly lower compared to the control group in both phases, thus, showing an increase in pain sensation. In formalin tests, the frequency of right hind paw lick in group 2 was significantly higher compared to the control, representing an increase in pain sensitivity. The duration of hind paw lick was not significantly different among the groups. However, in phase 2, the duration of hind paw lick in group 2 was higher than control, showing an increase in chronic pain sensitivity. Our results suggest that, *Chloroquine phosphate* increases pain sensation in mice.

Keywords: Neurotoxicity, Chloroquine phosphate, pain sensation, tail flick tests, formalin tests.

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INTRODUCTION

In human history, malaria, especially that caused by plasmodium falciparum is the world's most devastating human parasitic infection. Malaria afflicts nearly 500 million people and causes some two million deaths each year (Breman,et at, 2001). It is widely distributed in the world, especially in the tropical Africa because of its ever increasing incidence of infection. Based on its wide spread and trenchant effects on human affairs, more efforts are being made to find a more convenient drug with minimal side effects for treatment of malaria. In view of this, *chloroquine* has been the drug of choice for malaria treatment.

Chloroquine is a synthetic 4 amino –quinoline that has been the mainstay of antimalarial therapy until the recent appearance of drug resistant strains of P.falciparum (Mycek et al, 2000).It is commonly used in the chemotherapy of malaria fever and as anti-inflammatory agent in patients with rheumatoid arthritis or systemic lupus erythematosus (Onigbogi,et al, 2000).

Chloroquine, however, has side effects among which are very common in black Africans (Ajayi,et al,1998). It should be used cautiously in patients with hepatic dysfunction and neurological or blood disorders. Also the drug

can cause electrocardiographic changes, since it has a quinidine like effect of inactivating sodium channels and preventing sodium influx, thus showing the rapid upstroke during phase 0 as well as decreases the slope of phase 4 spontaneous depolarization (Mycek et al., 2000).

Most of these side effects are infrequent or may be mild and tolerable at normal doses of the drug and they are reversible on withdrawal of the drug. For instance, corneal deposits of *chloroquine* may be asymptomatic or cause mild photophobia (Laurence et al, 1999). It has been reported that *chloroquine* causes blurred vision as one of its side effects, probably due to its effect on the lateral geniculation body of the thalamus (Ekanem and Caxton-Martins, 2000).

Amongst the known neurological side effects of *chloroquine* are psychosis, depression and dehlirum. It could depress invitroneuronal activity, perhaps, through inhibition of calcium channels as shown using the whole cell patch clamp method (O'shaughnessy et al., 2003). Also, Studies have showed that *Chloroquine* reduces locomotive activity and have a sedative effect (Musabayane et al., 1999; Etimita et al., 2005; Odo et al., 2007).

However, in view of the neurologic and neurotoxic effects of *chloroquine* as reported, no report has been established on its effects on pain behaviour. This study therefore, evaluates the effect of *Chloroquine* on pain in mice.

MATERIALS AND METHODS

Experimental animal/ groups: The animals were bred in the animal house, department Pharmacology, University Calabar. The animals were kept in the animal house of department of Human Physiology, University of Calabar. The animals have access to rodent laboratory chow from laboratory depot at Okigwe in Abia State of Nigeria and were fed for 3 weeks. The mice were used for the study after approval by the college ethical committee.

For the experiment, a total of 30 thirty swiss mice of both sexes were used. The animals were randomly assigned into three groups (1, 2 and 3). All animals were raised under standard laboratory conditions and given free access to normal feed and clan tap water. Each group was made of ten (10) mice weighing 18-28g.

Drug administration: Group 1 animals (control) were administered with 0.2ml normal saline intrapentoneally (i.p). Group 2 and 3 received 10mg/kg and 20mg/kg of *chloroquine phosphate* (Glaxo, Nigeria) (I.P) respectively, as safe dose having determined the LD50 as 367.18mg/kg. 10mg/kg of *chloroquine* served as the human therapeutic or low dose group while 20mg/kg served as the pharmacological or high dose group. The drug was obtained from University of Calabar Pharmacy and the administration lasted for 14 days.

Determination of pain sensitivity: The tail flick nociceptive assay was used to assessed the effort of pain (D'Amour and Smith, 1941). In the tail flick test, the pain thresholds of mice are measured by using warm water as the tail flick of each mice. The latency was determined by placing the distal part of the tail (about 2.5cm) in the warm water (45^{0}c – 49^{0}c) in order to minimize the damage of the tail skin while the thermometer was constantly immersed into the hot water to determine the temperature. A stopwatch was set and usually started immediately the tail of the mouse was immersed into the hot water at 49°c. The stopwatch was stopped exactly when the mouse flicked its tail from the hot water. The time was recorded as latency of tail flick. The experiment was repeated after one (1) hour for each mouse in the control and test groups.

On the other hand, the formalin test was performed on the mice. The animals were injected with 0.2ml of 25% formalin solution on their right hind paw with a syringe and needle according to Hunskwar and Hole (1987). After the formalin administration, the animals were isolated and observed for the first 5 minutes before it was taken back to the observation box. Each animal was then returned to its cage and allowed for thirty (30) minutes before it was taken back to the observation box to be re-observed for another 5 minutes.

Sample collection: The behaviours scored during the pain test include: Rearing frequency, grooming frequency, grooming duration, Frequency of right hind lick/scratch, frequency of right hind paw attention, duration of attention.

Statistical Analysist: Data were then analysed using one-way analysis of variance (ANOVA) followed by post hoc student´s t-test. Data were presented as means ± Standard error of the mean (SEM) and were regarded as statistically significant at a $p<0.05$.

RESULTS

The results from the tail flick test showed that in trial 1, both human therapeutic (Low) and pharmacological (High) doses were significantly ($p<0.001$) lower compared to the control. However, pharmacological dose was significantly ($p<0.01$) higher compared to therapeutic group

In trial 2, human therapeutic and pharmacological doses were significantly ($p<0.001$ and $p<0.01$) lower compared to control .While pharmacological dose was significantly ($p<0.01$) higher compared to the human therapeutic dose.

Latency of tail flick test in trial i & ii (Fig. 1):
Latency of tail flick is the time taken for the animal to flick its tail from the hot water.

The results from the tail flick test showed that in trial I, the mean latency of tail flick were 21.36 ± 2.20sec for control, 9.17 ± 0.89sec for therapeutic and 13.43 ± 0.75sec for pharmacological group(s). From the result, both human therapeutic and pharmacological groups were significantly ($P<0.001$) higher compared to the control. Also, pharmacological group was significantly ($P<0.01$) higher compared to the therapeutic group.

In trial II, the mean latency of tail flick were 25.63 ± 2.16sec for control, 13.52 ± 0.98sec for therapeutic group and 19.28 ± 1.33sec for pharmacological group. The result shows that therapeutic group was significantly ($P<0.01$) lower compared to the control. While the pharmacological group was significantly ($P<0.01$) higher compared to the control group and significantly higher than the therapeutic group ($P<0.01$).

Behaviours scored in the observation box of formalin test.

Frequency of right hind paw lick (Fig 2): The frequency of right hind paw in the control group with mean value of 8.40 ± 1.20/5min, and therapeutic group had 16.80 ± 2.67/5min, while the pharmacological group was 11.20 ± 1.39/5min,in the acute phase of the test. In chronic phase, the control was 1.20 ± 0.58/5min, 6.25 ± 1.03 for therapeutic group while 3.00 ± 0.70 for pharmacological group in the phase 2.

The result show that the therapeutic group was significantly ($p<0.001$) and ($p<0.001$) higher compared to the control in phase 1 and 2 respectively. While pharmacological group was not significant compared to the control group, but was significantly ($p<0.05$) and ($p<0.01$) lower compared to the therapeutic group in phase 1 and 2 respectively.

Duration of right hind paw lick (Fig 3): In acute phase, the duration of hind paw lick were 32.92 ± 6.53 sec for control, 52.72 ± 12.01sec for therapeutic and 34.00 ± 5.8sec for pharmacological groups. From the result, there was no significant difference among the groups. In chronic phase, the duration of right hind paw lick mean values were 3.04 ± 1.34sec,for control,14.70 ± 2.76sec,for therapeutic and 5.72 ± 2.23sec,for pharmacological groups. Result shows that therapeutic group was significantly ($p<0.001$) higher compared to the control group, While pharmacological group was not significant compared to the control group but was significantly($p<0.01$) lower compared to the therapeutic group.

Frequency of right hind paw attention (Fig 4): In acute phase, the mean values were:13.00 ± 1.51/5min, for control, 24.80 ± 3.6/5min for therapeutic and 16.80 ± 3.33/5min pharmacological group(s). From the result, therapeutic was significantly ($p<0.01$) higher compared to the control and the therapeutic groups. In chronic phase, the mean values were:1.80 ± 0.66/5min, for control, 2.60 ± 0.50/5min,for therapeutic and 1.50 ± 0.50/5min, for pharmacological group(s).The result shows that there was no significant difference among the groups.

Frequency of rearing (Fig. 5): In acute phase, the mean values were:7.00 ± 1.41/5min, for control, 1.40 ± 0.97/5min, for therapeutic and 2.40 ± 1.28/5min,for pharmacological group(s).The result shows that both therapeutic and pharmacological groups were significantly ($p<0.01$) lower compared to the control group. There was no significant difference between the test groups. In chronic phase, the mean values were 3.60 ± 1.07/5min, for control, 1.60 ± 0.37/5min for therapeutic, 2.40 ± 0.96/5min, for pharmacological group(s).From the result, there was no significant difference among the groups.

Frequency of grooming (Fig. 6): In acute phase, the mean values were 3.80 ± 0.86/5min,for control, 1.00 ± 0.44/5min,for therapeutic and 2.80 ± 0.66/5min for pharmacological group(s).The result shows that therapeutic group was significantly ($p<0.01$) lower compared to the control. The pharmacological group was not significant compared to the control group and therapeutic group. In chronic phase, the mean values were: 5.4 ± 1.20/5min

control,2.80±0.73/5min therapeutic group and 5.40±1.43/5min for pharmacological group.The result shows no significant difference among the groups.

Duration of grooming (Fig. 7): In acute phase, the mean values were 8.88±2.83 for control, 2.00±1.02sec therapeutic and 7.90±1.41 sec for pharmacological group(s). From the result, therapeutic group was significantly (P<0.01) lower compared to the control group while pharmacological group was not significant compared to the control but was significantly (p<0.05) higher compared to the therapeutic group.

In chronic phase, the mean values were 12.24±2.77sec for control, 6.58±2.14sec for therapeutic group and 12.06±3.08sec for pharmacological group. The result show no significant difference among the groups.
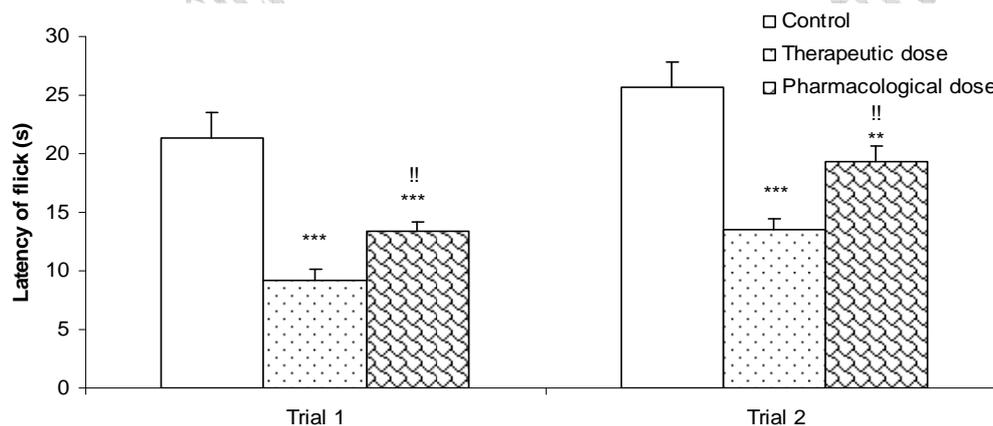

Fig. 1: Comparison of latency of tail flick in the tail flick test between mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
**=p<0.01 compared to control; ***=p< 0.001 compared to control; !!= p < 0.01 compared to therapeutic dose
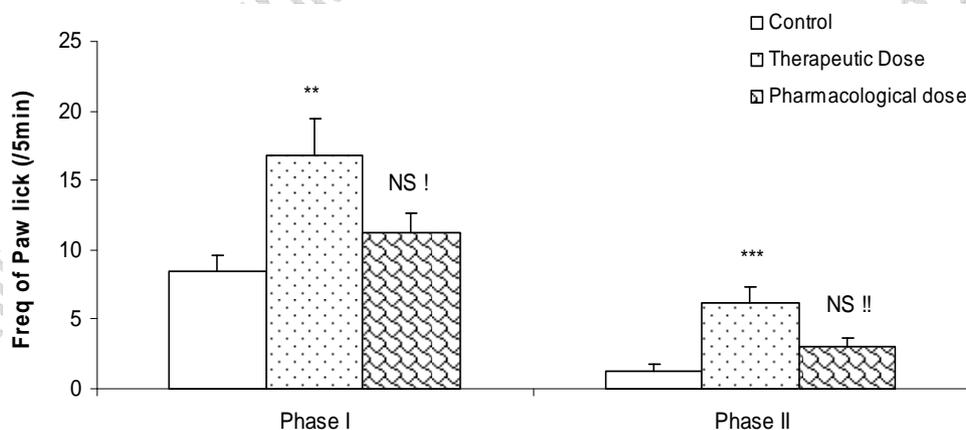

Fig. 2: Comparison of frequency of hind paw lick during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
**=p< 0.01 compared to control; ***=p< 0.001 compared to control; !=P<0.05; !!= p< 0.01 compared to therapeutic dose
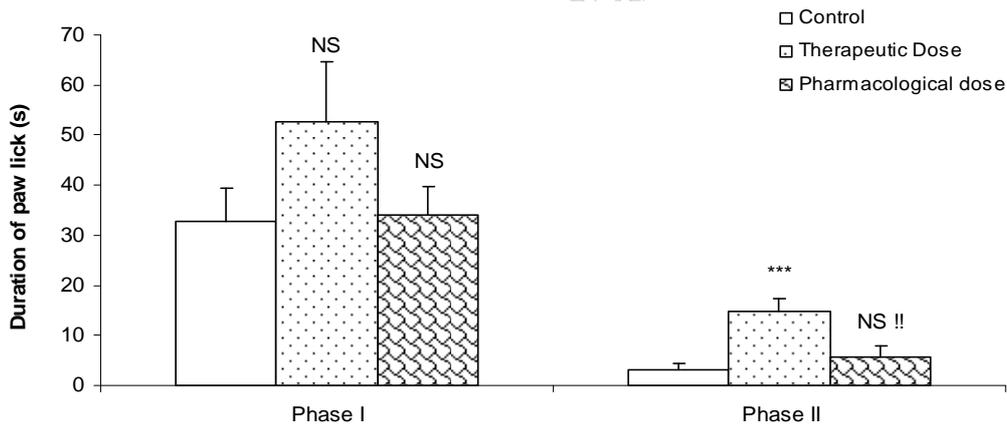
Fig. 3: Comparison of duration of hind paw lick during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
NS=Not significant compared to control; ***=p< 0.001 compared to control; !!=p< 0.01 compared to therapeutic dose.
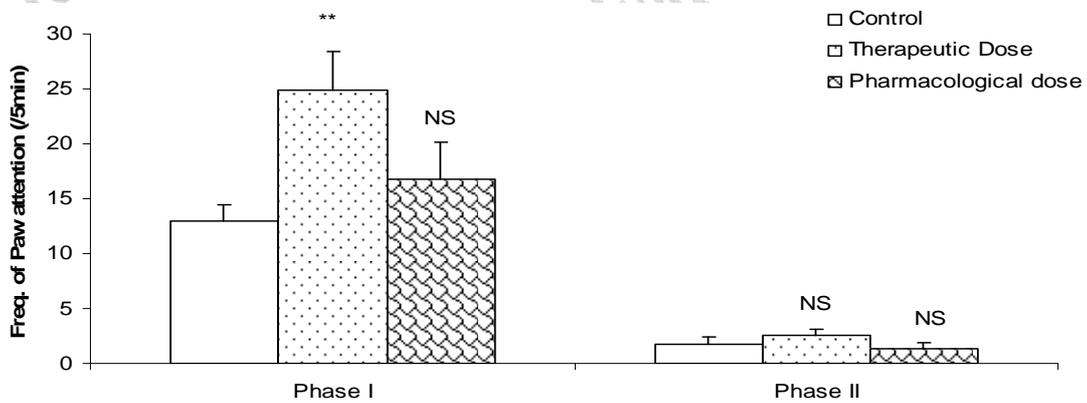

Fig. 4: Comparison of frequency of hind paw attention during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
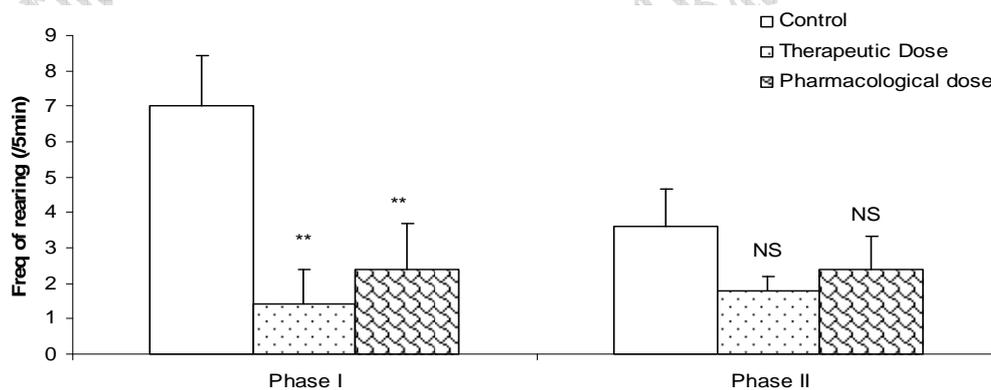NS=Not significant compared to control; **=p< 0.01 compared to control

Fig. 5: Comparison of frequency of rearing during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
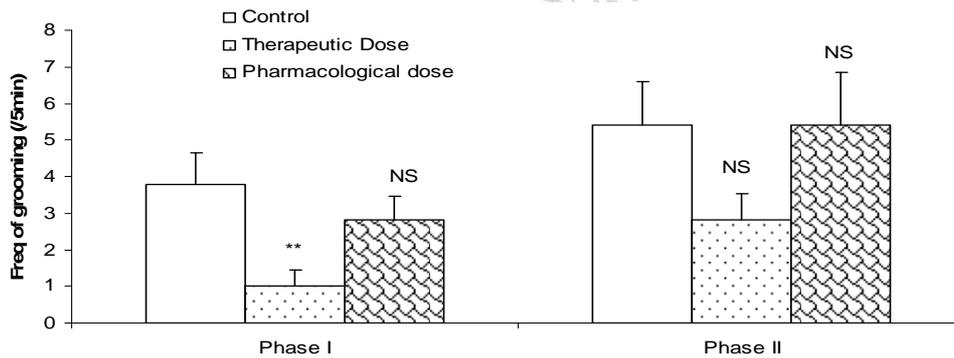NS=Not significant compared to control; **=p< 0.01 compared to control

Fig. 6: Comparison of frequency of grooming during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
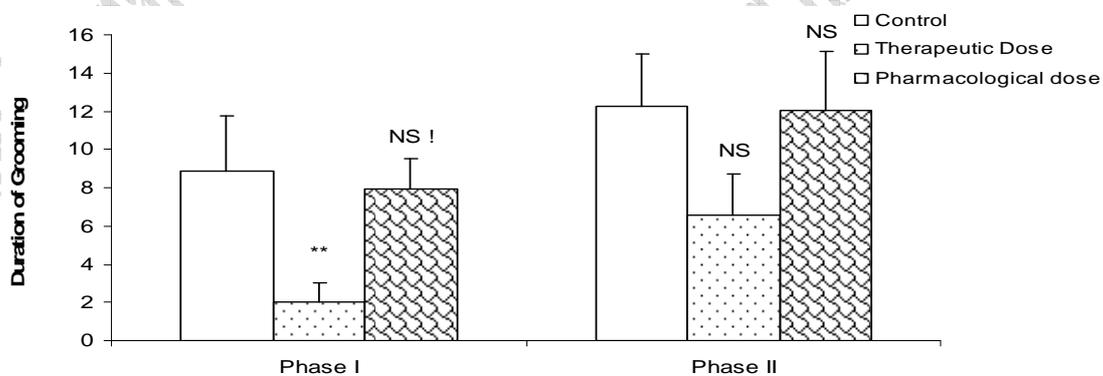NS=Not significant compared to control; **=p< 0.01 compared to control.

Fig. 7: Comparison of duration of hind paw grooming during the first and second phases of the formalin test in mice treated with therapeutic and pharmacological doses of *Chloroquine* with the control.
NS=Not significant compared to control; **=p< 0.01 compared to control; != p< 0.05 compared to therapeutic dose.

DISCUSSION

Tail flick is a process that involves radiant heat which is applied on the animals tail when the animal feels discomfort, there is a sudden tail movement (tail flick) as designed by D'Amour and Smith (1941). In the present experiment, the tail flick test showed significant differences (Fig 1) among the groups (1, 2 and 3) with the test groups (2 and 3) having low latency of tail flick indicating increase in pain sensitivity.

The formalin test was another model used in testing pain sensation in animals as designed by Hunskaar and Hole (1987).The formalin test is made of two phases, the acute phase and the chronic phase. In the case of the formalin test in the present experiment, the frequency and duration of hind paw lick, frequency and duration of hind paw attention were used to assessed the change in perception of pain sensation in the animals. Mice which feel more pain will exhibit more of these behaviours. The frequency and duration of hind paw lick and hind paw attention in the test groups were significantly higher compared to the control, thus, indicating increased in pain. Also, a decrease in frequency of rearing and frequency and duration of grooming shows an increased in pain sensation.

This study reveals that there was a significant increase in pain sensitivity both during the acute phase and chronic phase of pain in the experimental groups compared with the control. This increase may be due to the release of chemical pain excitants such as bradykinin, serotonin, histamine, acetycholine, prostaglandin and proteolytic enzymes which stimulate chemosensitive free nerve endings (pain receptors) and excite pain by making their membranes more permeable to ions and greatly decrease the threshold for stimulants of pain receptors (Guyton and Hall, 2006).

Also, experimental findings have indicated that substance P and bradykinins participate in early phase while histamine, serotonin and prostaglandins are involved in the late phase (Shibata et al., 1989). Moreso, it probably enhances the opening of the TRPV1(transient receptor potential vanilloid receptor 1) a ligand –gated cation receptor which is permeable to Na^+, Ca^+ and other cation causing depolarization and initiation of action potential (Wang and Woolf,2005). However, the mechanism by which *Chloroquine* increase pain sensitivity in mice is not certain.

In conclusion, the administration of *Chloroquine phosphate* may induce pain sensations in a dose dependent manner. However, our finding appears not to have any beneficial application in man.

ACKNOWLEDGEMENT

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AUTHOR(S) CONTRIBUTION

Lelei SA., Nneli R.O., Osim E. E. and Efeke B.G. were actively involved in the study, preparation of the first draft and correction of the reviewed version of this article.