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ABSTRACT

Although neonates with severe birth asphyxia are known to be at increased risk of early-onset hypocalcaemia, the magnitude of the problem is not well documented. The objective of this study was to determine the prevalence of early-onset hypocalcaemia in severely asphyxiated neonates.

In this case-control study, conducted at St Philomena Catholic Hospital (SPCH), Benin City, Nigeria, the total serum calcium concentrations of 31 full-term neonates with 1-minute Apgar score of 3 or less were measured using the O-cresulphthalein complexone method and the results were compared with those of their counterparts with 1-minute Apgar score of 7 and above. The total serum calcium concentration of severely asphyxiated neonates who received sodium bicarbonate therapy during resuscitation was compared with those of their counterparts who did not receive sodium bicarbonate therapy. All the study neonates (both the asphyxiated and the non-asphyxiated) were examined, at least, twice daily in the first 48 hours of life and their clinical findings were documented. Overall prevalence of early-onset neonatal hypocalcaemia (total serum calcium < 1.75 mmol/L) among asphyxiated neonates was 22.6%. Mean total serum calcium at the ages of 12, 24 and 48 hours were significantly lower among asphyxiated compared to non-asphyxiated neonates ($p<0.001$). The overall mean serum calcium concentrations were 1.68 ± 0.06 mmol/L (range 1.65 – 1.70 mmol/L) and 1.74 ± 0.07 mmol /L (range 1.74 – 1.77 mmol/L) in neonates treated with bicarbonate and those without bicarbonate therapy respectively ($p<0.01$). Asphyxiated neonates with normal total serum calcium concentrations at the age of 12 and 24 hours maintained this normocalcaemia at the age of 48 hours. The commonest clinical finding among asphyxiated neonates with early-onset hypocalcaemia was convulsion (57.1%). Carpopedal spasm was not a prominent clinical manifestation. Hypocalcaemia was common in the first 48 hours of life in asphyxiated neonates, particularly if they received bicarbonate therapy during resuscitation. Asphyxiated neonates whose serum calcium concentration was normal at the age of 12 hours tended to maintain this normal level at the age of 48 hours.

Key words: neonatal hypocalcaemia, birth asphyxia, bicarbonate therapy.

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INTRODUCTION

Early-onset neonatal hypocalcaemia (hypocalcaemia occurring within the first 48 - 72 hours of life) [1,2] accounts for 95% of all cases of neonatal hypocalcaemia [3]. It is related to the abrupt cessation of transplacental supply of calcium from the mother to the foetus. The healthy term infant experiences a physiological nadir in serum calcium between the age of 24 and 48 hours after which normal serum calcium levels are regained [4,5]. This normal pattern may be exacerbated with the nadir dropping to hypocalcaemic levels in neonates who have suffered severe birth asphyxia [3,4]. Tsang and Oh [6] reported a lower total serum calcium value at the age of 8 hours prior to the actual development of hypocalcaemia at the age of 29 hours.

The pathogenetic mechanism by which birth asphyxia causes hypocalcaemia is poorly understood. However, it has been speculated that delayed introduction of feeds, increased calcitonin production, increased endogenous phosphate load, transient functional hypoparathyroidism, target organ unresponsiveness and sodium bicarbonate therapy may play a role [2,5,7]. Of the total serum calcium, 40% is protein-bound, 10% is complexed with anions, such as citrate, sulphate, bicarbonate, phosphate and lactate and 50% is the free or ionized physiologically active form [2,4]. Changes in plasma protein concentration alter the total serum calcium concentration in the same direction as the protein concentration [4]. In neonates, it is unlikely that a change in the concentration of plasma protein that will significantly affect the total serum calcium concentration can occur in less than one week after birth [8].

Hypocalcaemia is defined as total serum calcium less than 1.75mmol/L in all infants including preterm infants; less than 2.0mmol/L in full-term infants and less than 2.2mmol/L in children and adolescents [9].

Information on the overall prevalence of neonatal hypocalcaemia is scarce in Nigeria, especially as it relates to birth asphyxia. Tsang et al, [10,11] in two separate studies reported a prevalence of 37.6% for preterm infants and 14.3% for infants with birth asphyxia. In a retrospective study in Benin City, Omene and Diejomaoh [12] reported a prevalence of 9.4% among their asphyxiated neonates. In that study, they did stated the method used in measuring total serum calcium concentration. Early-onset neonatal hypocalcaemia is often asymptomatic [7]. When symptoms and signs are present, they tend to be non-specific and mimic many other neonatal disorders such as hypoglycaemia, hypomagnesaemia, septicaemia, opiate withdrawal syndrome, and anoxic brain injury [13,14]. From the foregoing, it is obvious that there is a need for mandatory serum calcium determination in high-risk neonates to improve their perinatal health. Indeed, Speidel et al [4] recommended that

serum calcium concentration should be determined in all neonates with encephalopathy. However, routine sampling may not be feasible in resource-limited countries.

In Nigeria, birth asphyxia still remains a major cause of neonatal death and childhood disability. National estimates indicate that birth asphyxia is the commonest cause of neonatal mortality, accounting for 26% of all cases [17]. For each asphyxia-related neonatal death, many more neonates are left with permanent disabilities. A study from a secondary health-care facility in Nigeria reported a birth asphyxia prevalence of 84 per 1000 live-births and accounted for 20.9% of all admissions into their neonatal unit with a case fatality rate of 15.7% [18]. The present study sought to determine the prevalence of early-onset neonatal hypocalcaemia among asphyxiated neonates seen in a secondary health-care institution. This will highlight the magnitude of the problem and the need to be alert towards its occurrence.

MATERIALS AND METHODS

The study population consisted of full-term neonates with severe birth asphyxia (1-minute Apgar score of 3 or less) delivered at St. Philomena Catholic Hospital (SPCH), Benin City between 1st June 2007 and 31st May, 2008. The control group was made up of full-term neonates with 1-minute Apgar Score of 7 or more delivered in the same hospital during the same period. Birth weights of neonates in both groups were appropriate for gestational ages. The study protocol was approved by the hospital authority and consent was obtained from each of the mothers after explaining to each of them that those neonates found to have low serum calcium concentration will given appropriate therapy. Exclusion criteria included: (i) death within 48 hours of age; (ii) infants of diabetic mother; (iii) infants who required exchange blood transfusion; (iv) infants on frusemide (lasix) and

(v) admission after age of 48 hours. Using O-cresulphthalein Complexone Method of Baginski et al, [17] serial total serum calcium concentration were determined at 12, 24 and 48 hours of age. Thus, three samples per subject was analysed for total serum calcium. To avoid venous stasis and eliminate artefactual haemoconcentration, blood sample was collected (without applying a tourniquet) using the open-ended needle method recommended by Wilkinson and Calvert [18]. Each of the blood samples was processed within 24 hours of collection. Infants with two sequential total serum calcium values less than 1.75 mmol/L were considered to have significant hypocalcaemia and were treated with intravenous or oral calcium gluconate. No calcium supplement was given before the first serum calcium measurement. The corresponding serum albumin concentration was determined for each blood sample used for serum calcium measurement.

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Maternal age and parity as well as the infants' birthweight were recorded. Whether or not sodium bicarbonate was administered during resuscitation was noted. Both groups of neonates were examined, at least, twice daily in the first 48 hours of life and the findings were documented.

Neonates with 1-minute Apgar score of 6 and below were deemed to have had birth asphyxia. Student's t test was used in assessing the significance of the results which was set at p<0.05.

RESULTS

One hundred and twenty nine (9.5%) of 1,364 live-births had 1- minute Apgar score of 6 or less.

Of the 129 infants, 38 (29.5%) had 1-minute Apgar score of 3 or less (severe birth asphyxia) and they constituted the study population. More males (61.1%) than females (38.9%) suffered severe birth asphyxia with a ratio of 1.6:1.

Mean birth weight of severely asphyxiated and non-asphyxiated infants was 3.25 + 0.54 kg versus 3.30 + 0.33 kg p>0.05 respectively.
 Seven out of 31 (22.6%) of asphyxiated neonates and three out of 38 (7.9%) of control neonates had hypocalcaemia with an odd ratio of 2.7 (95% CI = 0.90 – 2.91). As shown in

Seven (18.4%) of the 38 severely asphyxiated babies died within the first 48 hours of life. Four out of the 7 deaths had bicarbonate therapy and low serum calcium concentration at 12 and/or 24 hours before their death.

The mean maternal age of severely asphyxiated and non-asphyxiated infants, was 24.2 + 0.9 years (95% confidence interval, CI = 23.9 - 24.5) versus 24.8 + 0.7 years (95% CI = 26.4 - 25.0) p<0.05 respectively. The mean maternal parity was 2.9 + 1.1 (95% CI = 2.5 - 3.3) for severely asphyxiated infants and 2.3 + 1.4 (95% CI = 1.8 - 2.8) for non- asphyxiated infants p >0.05.

Comparing total duration of labour in mothers of infants with severe birth asphyxia and those whose infants did not have birth asphyxia, it was 8.2 + 1.2 hours (95% CI = 7.8 - 8.6) versus 6.9 + 0.8 hours (95% CI = 6.6 - 7.2); p<0.01. Further details on maternal characteristics are shown in Table I.

Table 2, total serum calcium concentrations at the ages of 12, 24 and 48 hours were significantly lower in asphyxiated neonates compared to non-asphyxiated neonates, with lowest value at age of 24 hours (p<0.001).

Asphyxiated neonates with normal mean serum calcium at the age of 12 hours tended to

maintain normal serum calcium concentration at the age of 48 hours (Table 3).

The mean total serum calcium concentration of the 9 asphyxiated neonates who received sodium bicarbonate therapy was significantly lower than that of their counterpart who did not receive bicarbonate therapy (Table 4).

The mean serum albumin levels were 35.6 g/L (range 34.8 – 51.5 g/L) and 35.8 g/L (range 36.1 – 52.6 g/L) in asphyxiated and non-asphyxiated neonates respectively. As shown in Table 5, the leading clinical manifestation associated with hypocalcaemia in infants with birth asphyxia was convulsion. Carpopedal spasm was not a prominent sign.

Table 1: Maternal characteristics of study infants

Maternal characteristics	Asphyxiated infants No (%)	Non-asphyxiated infants No (%)
Maternal age (years)		
< 20	3 (9.6)	2 (5.2)
20 – 34	20 (64.5)	31 (81.6)
≥ 35	6 (19.4)	5 (13.2)
Unknown	2 (6.5)	0 (0)
Total	**31 (100.0)**	**38 (100.0)**
Maternal parity		
0	8 (25.8)	4 (10.5)
1 – 4	17 (54.8)	23 (73.7)
≥ 5	6 (19.4)	5 (15.8)
Total	**31 (100.0)**	**38 (100.0)**
Mode of delivery		
Caesarean section	9 (29.0)	7 (18.4)
Vaginal	22 (71.0)	31 (81.6)
Total	**31 (100.0)**	**38 (100.0)**

Table 2: Mean total serum calcium concentration in asphyxiated and non-asphyxiated infants.

Age of infant	Mean total serum calcium in mmol/L		p-value
	Asphyxiated infants	Non-asphyxiated infants	
At 12 hours	1.80+0.06	2.08+0.07	< 0.001
At 24 hours	1.72+0.05	2.04+0.06	< 0.001
At 48 hours	1.74+0.05	2.07+0.06	< 0.001

Table 3: **Mean serum calcium concentrations in 24 asphyxiated neonates with normal values.**

Age of neonate	Mean Serum Calcium conc.
At 12 hours	1.85 ± 0.05 mmol/L
At 24 hours	1.82 ±0.05 mmol/L
At 48 hours	1.83 ± 0.05 mmol/L

Table 4: Comparison of mean serum calcium concentration (mmol/L) in neonates treated with bicarbonate and those without bicarbonate therapy.

Age of Neonates	Neonates with bicarbonate therapy (n = 9)	Neonates without bicarbonate therapy (n = 22)	p-value
At 12 hours	1.65 ± 0.04mmol/L	1.74 ±0.06 mmol/L	0.01
At 24 hours	1.65 ± 0.07mmol/L	1.73 ± 0.06 mmol/L	0.01
At 48 hours	1.70 ± 0.06mmol/L	1.78 ± 0.07 mmol/L	0.01

Table 5: Clinical manifestations in 7 infants with severe birth asphyxia and hypocalcaemia

Clinical manifestations	Asphyxiated hypocalcaemic infants(n=7)	Non-asphyxiated hypocalcaemic infants (n=3)
Convulsion	4.(57.1)	1 (33.3)
High-pitched cry	3 (42.9)	0 (0)
Twitching of extremities	3 (42.9)	0 (0)
Hypertonia	3 (42.9)	1 (33.3)
Jitteriness	2 (28.6)	2 (66.7)
Carpopedal spasm	1 (14.3)	3 (100.0)

Figures in parentheses are percentages. Some infants had more than one of these clinical manifestations.

DISCUSSION

In this study, the overall prevalence (22.6%) of early-onset neonatal hypocalcaemia among neonates with birth asphyxia was three times higher than that reported by Omene and Diejomaoh [12] among their asphyxiated neonates. Their lower prevalence may be accounted for by the retrospective nature of their study and the fact that serum calcium was not measured in all their study population. In this regard, some case records may be missing and some neonates with hypocalcaemia may also be missed, leading to the lower prevalence reported in that study.

Data from the present study showed that neonates with 1-minute Apgar scores of 3 or less had significantly lower mean total serum calcium concentration than their counterparts with 1-minute Apgar scores of 7 or more. Tsang et al [11] have reported similar findings. This implies that birth asphyxia plays a separate role in early neonatal calcium homeostasis.

In this study, asphyxiated neonates whose serum calcium concentrations were normal at the age of 12 hours tended maintain normal serum calcium at 48 hours of age. The clinical implication is that asphyxiated neonates whose total serum calcium at the age of 12 hours are normal are less likely to develop hypocalcaemia at the age of 48 hours.

Asphyxiated neonates who had sodium bicarbonate therapy during resuscitation tended to have significantly lower serum calcium concentration compared to their

counterparts who did not have sodium bicarbonate therapy. Similar finding has been reported by Tsang et al [11]. The adverse effect of bicarbonate therapy on serum calcium concentration reported in present study is reinforced by the reports of previous studies which concluded that bicarbonate administration during resuscitation is not only useless, but also, detrimental to the asphyxiated neonate [19,20]. Administration of sodium bicarbonate to correct acidosis is believed to be associated with movement of calcium from blood to bone, resulting in hypocalcaemia [21].

In this study, the leading sign associated with hypocalcaemia in asphyxiated neonates was convulsion. It is worthy of note that carpopedal spasm was not a prominent physical sign among the hypocalcaemic neonates who suffered birth asphyxia.

Some limitations of the present study must be considered. Firstly, the use of Apgar score in defining birth asphyxia. The Apgar Scoring System, [22] though very useful in the measurement of birth asphyxia, has its shortcomings in that it does not fully define birth asphyxia [23,24]. It is known that factors (maternal medication) other than asphyxia may affect the Apgar score of an infant. However, in the review by Addy [27] he noted that Apgar score was the basis of many papers on the outcome of birth asphyxia, justifying its use in the present study. Secondly, our inability to

measure directly ionized serum calcium concentration and blood gases. This was due to lack of facility for their determination in our hospital. Future study will take this into consideration. Despite these limitations, the study gave an insight into the prevalence of the early-onset neonatal hypocalcaemia.

In conclusion, hypocalcaemia was common among asphyxiated neonates, particularly if they received bicarbonate therapy during resuscitation. Asphyxiated neonates whose serum calcium concentration was normal at the age of 12 hours tended to maintain this normocalcaemia at the age of 48 hours.

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CLUSTER ANALYSIS OF ANOPHELES STEPHENSI FOURTH INSTAR LARVAE BEHAVIOR TO

EXPLORE SEQUENTIAL ORGANISATION OF LARVAE MOVEMENT IN A MICROCOSM.

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CLUSTER ANALYSIS OF ANOPHELES STEPHENSI FOURTH INSTAR LARVAE BEHAVIOR TO EXPLORE SEQUENTIAL ORGANISATION OF LARVAE MOVEMENT IN A MICROCOSM.

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ABSTRACT:

Experiments were conducted on 20 fourth instar Anopheles stephensi larvae to explore behavior organisation. Twenty fourth instar larvae were placed in a glass aquarium and filmed using a handy cam video recorder and the recordings analysed in a laptop computer. Data of transitions from one behavior to another for all observations were collated into a matrix of preceding and succeeding behaviors to study sequential organisation and relationship among behaviors. Significant testing for first-order transition was done using G test at P< 0.005 and a kinematic graph constructed from significant transitions. A time budget and transition frequency data constructed for each behavior were subjected to cluster analysis to explore relationship between the behaviors.

Result of the analysis showed that fourth instar Anopheles stephensi larvae behaviors occur in clusters in specific locations in their aquatic environment. Furthermore, the sequential organisation of behaviors is influenced by behavior frequency and the amount time a larva spent doing that behavior. When food is kept constant, other factors such as gas exchange requirements, behavior variation due to day-night cycle, presence of a predator, interlarval competition for food and the size of the aquarium (depth and width) maybe determining behavior organization.

INTRODUCTION:

Global efforts to control malaria have been impeded by insecticide resistant vectors, drug resistant parasites and socioeconomic obstacles [1]. In an effort to search for novel control strategies larval behavior have been studied extensively [2, 3]. Certain mosquito species that were previously regarded as non-vectors have become a threat to humans as they find new habitats to establish themselves and the emergence of these potential vectors has been attributed to the changes in the environment and the influence of modern life [4, 5]. Anopheles stephensi has been observed to quickly establish itself in a new environment [6]. The ability of a mosquito species to establish itself in a new environment therefore is directly affected by the ability of the larva to exploit its aquatic habitat [6] and there is a need to re-examine larva behavior in its aquatic environment that may shed some light on factors determining a species' ability to establish itself in a new environment.

Differences in larval behavior, especially in relation to feeding, may result in different abilities to exploit their habitat [7]. Species-specific differences in larval behavior [3, 8] may also allow certain species to adapt more quickly to new environment and establish themselves rapidly [7]. Walker and Merritt [2] created a catalogue of larval behaviors using

Aedes triseriatus in an environmental with a constant food supply and noted that Aedes triseriatus used their entire habitat for feeding. Yee et al [3] compared the feeding behavior of Culex pipiens, Aedes albopictus and Ochlerotatus triseriatus (formerly Aedes triseriatus), all container-breeding mosquitoes, in two different food environments and found significant differences in larval behavior among the species.

Anopheles species generally feed at the air-water interface but they can also dive and feed at the bottom of their aquatic habitats [8, 9, 10, 11]. This diving ability in Anopheles species is most evident as an alarm response [9, 11]. Inter-specific competition between sibling species of Anopheles arabiensis and Anopheles gambiae has been reported by Schneider et al. [12].

Anopheles stephensi is a recognized malaria vector in Asia [6]. Using this species as a model, we conducted experiments to determine the behavior organisation of fourth instar Anopheles stephensi larvae in a microcosm to explore patterns of behavior that can provide insights into understanding this species' ability to establish itself in a new environment.

MATERIALS AND METHODS:

Twenty fourth instar Anopheles stephensi were used in the experiments. Mosquito larvae and adults were maintained using standard

protocols in an insectary with temperature maintained at 26°C and relative humidity 65 % with a 15 hours 8 hours day night cycle [11]. Light was provided by four 40-watt fluorescent light bulbs. Eggs were hatched in 250ml of de-chlorinated tap water in plastic cups (surface area = 95 cm²). At the late second to early third instar stage, larvae were transferred to 33- x 24- x 7-cm pans. Larvae were fed on ®Tetra Min baby fish food. Water was changed every other day.

The observation experiments were done in a 59- (length) x 28- (depth) x 35-(height) cm glass aquarium. The aquarium was filled with de-chlorinated tap water and incubated for one week to permit growth of microorganisms on the walls including the floor, in the water column and at the air-water interface [2]. A few pebbles were also put in the aquarium to allow microorganisms to grow on their surface. Twenty fourth instar larvae were individually pipetted into the aquarium and allowed to acclimate for one hour before larval behaviors were recorded. Observation data was collected from the 20 larvae.

Walker and Merritt [2] and Clements [10] have catalogued the list of behaviors observed in larval behavior studies. We used the same definitions with minor modifications to suit our experiment design. Some behaviors that were observed by Walker and Merritt [2] were not included in our study and others were combined. Briefly, the definitions of behaviors used in our study were as follows:

- Float/suspension feed – the larva is attached to the water's surface via its respiratory siphon with the body hanging obliquely into the water column. Anopheles larva lies horizontally in line with the air-water interface. The larva may be still or move slowly as a result of brush movements.

- Float/interfacial feed – the larva is attached to water's surface and its body bent into a U shape so that its mouthbrushes makes contact with the air-water interface. Anopheles larva attaches itself in parallel with the water's surface and rotates its head 180 degrees to make contact with the air-water interface.

- Autogroom – At either the surface or underwater, a larva bends its body into a U shape and works its mouthparts against its own body.

- Dive – A larva spontaneously descends from a position near the water surface using a wriggling, swimming motion.

- Brushwall – A larva that is underwater and its siphon detached from the air-water interface brushes the wall of the observation chamber with its mouthparts. The larva may be still or moving.

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- Wriggleswim – A larva moves through the water column by flexing and unflexing movements of its entire body forming a wriggling motion.

- Underwater/mouth swim – A larva moves forward in the water column as a result of its suspension feeding movements, not by flexing its body. The larva is not attached to the water's surface.

- Allogroom/feed – A larva directs its mouthparts against a nearby larva.

- Underwater/still – A larva remains motionless while underwater, usually at the bottom of the water column.

- Rise – A larva, when underwater, ascends through the water column to the surface.

- Float/brushwall – the larva is positioned at the surface while attached to the air-water interface via its siphon and brushes the wall of the observation chamber with its mouth parts. The larva may be still or moving.

- Bottom feed – A larva after diving and reaching the bottom of the water column brushes the floor, pebbles or chews a substrate. A larvae brushing the wall of the observation chamber approximately 1-2cm from the floor was also regarded as bottom feed. This behavior combines the float/substrate brush and chew substrate behaviors that were observed by Walker and Merritt [2].

A larva in the aquarium in any behavioral state was chosen at random and filmed for five minutes using a handy-cam (Sony Co. Japan) video recorder. To enhance image contrast, recordings were done with a white card placed behind the aquarium in daylight conditions [13]. Twenty fourth instar larvae were placed in the observation aquarium and allowed to acclimatize for one hour prior to filming. A larva was than chosen at random and filmed for five minutes. After videotaping 10 larvae, all 20 larvae in the aquarium were removed and replaced with a new group of 20 fourth instar larvae. This was done to ensure a larva was not filmed twice. Again one hour of acclimatization time was allowed and 10 larvae were videotaped at random, each larva being recorded for five minutes.

The focal-individual sampling method was possible because the tempo of larval behavior and the low density of larvae in the aquarium allowed the observer to track an individual larva [2] and videotape its behavior. Great care was taken not to videotape a larva more than once. The observer became familiar with larval behaviors through preliminary observation of more than 300 fourth instar larvae over a 10 month period.

Video tape recordings were converted to DVD and viewed on a Mac Os X version 9.3 laptop

computer. To study sequential organization and relationship among behaviors, data of transitions from one behavior to another for all observations were collated into a matrix of preceding and succeeding behaviors with the diagonal of the matrix held at logical zero, assuming that a behavior can not follow itself. The transition matrix was collapsed about the cell of interest to form a 2x2 contingency table [2] and first-order transitions for significantly greater occurrence than expected by chance was tested using G test at P <0.005 [14, 15]. Statistical significance testing was done using Excel set up on a website [15]. To visualize the organization of the behaviors in the microcosm, a kinematic graph of the behavioral sequences were constructed from the transition matrix by showing frequencies of significant nonrandom transitions between behavior states [2, 16].

Data from the time budget and transition matrix were subjected to hierarchical cluster analysis to explore relationships between the different larval behaviors. Cluster analysis was done using TANAGRA [17].

RESULTS AND DISCUSSION:

Behaviors were recorded from a total of 20 fourth instar Anopheles stephensi larvae. Each larva was recorded for five minutes totaling 110 minutes of observation time (Table 2). Anopheles stephensi larvae spent majority of

their time in the float/interfacial feed and bottom feed states (Table 2).

The 12x12-transition matrix (Table 1) shows the frequencies of transition among behavioral states. Zero entry indicates no transition was observed between these behaviors. Entries with an asterix indicates a significantly greater frequency of transition from the preceding to the succeeding behavior than expected by chance (G test, P<0.005).

The kinematic graph (Figure 1) shows statistically significant (G test P<0.005) patterns of association between behaviors based upon the frequency of occurrence of transitions between the paired behaviors. Patterns of association that were not statistically significant are not shown. The mean time spent in the behavioral state is shown in brackets. The numbers along the arrows represent percentage of transitions from preceding to succeeding behavior. Generally, the kinematic graphs show that there were a group of behaviors that occurred near the water's surface and another group of behaviors occurred underwater.

These two groups were connected by dive and rise behaviors. Wriggleswim was used to transit between one surface behavior with another. Larvae also used wriggleswim to move from one underwater behavioral state to another. These three behaviors dive rise and wriggle-

swim therefore can be called transition behaviors, as larvae used these behaviors to transit between one behavioral state and another.

The dominant activity at the surface was float/suspension feed and float/interfacial feed with frequent transitions occurring between these two behaviors. There were also frequent transitions between float/suspension feed and float/brushwall. This was because larvae that were in float/suspension feed state would frequently move along the water's surface and bump into the wall of the observation chamber and brush the wall. There were also frequent transitions between allogroom/feed and both float/suspension feed and float/interfacial feed. It was also interesting to note that Anopheles stephensi larvae immediately moved away from each other upon contact.

Transition from float/suspension to dive was not a significant behavior. Comparison of diving behavior between aedes, culex and anopheles species have shown that diving is not a frequent behavior in anopheles species [10, 11]. However, the few times that a larva did break from the water's surface; it would usually swim right to the bottom as reflected by significant transitions from dive to bottom feed (Figure 1). Once a larva dived to the bottom of the aquarium it spent a long time feeding before surfacing. The diving pattern of Anopheles stephensi fourth instar larvae was in

a zigzag manner with periods of sinking passively.

Cluster analysis of the frequencies of behaviors and the mean time allowed a different interpretation of the data. Cluster analysis of the time budget data showed the behaviors were to be clustered into five groups (Figure 2) while the frequency data clustered into seven groups (Figure 3). The dendrogram showing results of hierarchical cluster analysis on mean time (Figure 2) showed autogroom, allogroom/feed, brushwall and float/brushwall were closely related and formed one cluster that was closely linked with underwater/mouthswim. These five behaviors were loosely linked to the cluster formed by wriggle-swim and float/suspension.

Rise and underwater/still formed one cluster that was distantly linked to the cluster formed by float/interfacial feed and dive. These four behaviors were also loosely linked to floor feed. Hierarchical cluster analysis on data from the frequency transition matrix (Figure 3) showed dive and rise formed one cluster that was loosely linked to the cluster formed by floor feed and wriggleswim.

Autogroom/feed and underwater/mouthswim formed a cluster that was closely related to underwater/still. These three behaviors were loosely linked to the cluster formed by float/brushwall and brushwall. Allogroom/feed and float/interfacial feed formed a cluster that

was loosely linked to the rest of the behaviors. The larval behaviors observed in this study can be generally classified into three groups: (i) those that were performed near the water's surface, (ii) those that were performed at the bottom of the observation chamber and (iii) behaviors that can be termed as transition behaviors. Walker & Merritt [2] when cataloguing larval behaviors also classified the behaviors into a group that occurred near the surface and a group that occurred underwater and these two groups of behavior were connected by dive and rise behaviors. We have grouped dive, rise and wriggleswim into what we have termed as transition behaviors. Transition behaviors connected the near surface behaviors with the bottom behaviors, two surface behaviors or two bottom behaviors. Cluster analysis of the behavior frequency data revealed dive and rise formed a cluster supporting our hypothesis but wriggleswim formed a cluster with bottom feed. This is because wriggleswim was a frequent behavior performed while feeding at the bottom of the aquarium. Cluster analysis of the time budget data showed a different pattern. The transition behaviors were clustered with other behaviors, in particular float/suspension feed and float/interfacial feed.

The kinematic graph (Figure 1) obtained from significant preceding-succeeding transition showed patterns of behavior observed near the water's surface and at the bottom of the aquarium. Similar observations were obtained

when the same data were subjected to cluster analysis (Figure 3).

Allogroom/feed, float/interfacial feed, float/brushwall, brushwall and float/suspension feed were all behaviors observed near the water's surface and which were closely linked as shown by cluster anlaysis (Figure 3). The bottom behaviors - underwater/still and bottom feed formed also formed a cluster of their own (Figure 3).

Cluster analysis of the time budget data although revealed five clusters of behavior, was able to separate the bottom behaviors from the behaviors observed near the water's surface (Figure 2). Autogroom, allogroom/feed, brushwall and float/brushwall were behaviors observed near the water's surface and formed one cluster (Figure 2). Bottom feed and underwater/still also formed their own clusters in association with one of the transition behaviors (Figure 2).

We conducted our study without varying the food environment therefore we might have missed some larval behaviors such as patterns of intra-specific competition, use of air-bubbles to breath underwater, different modes of feeding and different modes of swimming that have been described in previous studies [2, 3, 12, 13].

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Our recording time of five minutes may have also limited us from videotaping other previously described larval behaviors. However, by grouping different specific behaviors into one general group with a common theme (e.g. bottom feed for different mode of feeding behaviors observed at bottom of aquarium) we tried to overcome this limitation.

The larval behaviors observed and described are for fourth instar larvae only. These behaviors may not be the same for other stages of larvae. We choose the fourth instar stage because they are larger and easier to observe and videotape their movements. Furthermore, the behaviors were recorded in day light conditions. This might have some influence on larval movement. We do not know if there will be any difference in larval movement in night conditions which has the potential to affect the overall behavior organization.

Microcosm scaling has been shown to affect experimental results when studying aquatic insects [18] and may have also influence the results of our study.

CONCLUSION:

This study showed that cluster analysis of Anopheles stephensi 4th instar larva behavior observation data when used in combination with a pictorial representation of significant sequential organisation of behavior can reveal patterns of behavior that has the possibility of being exploited to develop new larval control methods or improve existing ones.

However, further studies are needed to determine what the patterns of behaviors observed mean biologically.

Figure 1: Kinematic graph of Anopheles Stephensi 4ᵗʰ instar larvae.

{Kinematic graph showing statistically significant transitions (G test P<0.005) and mean time spent (seconds) performing each behavior. The percentage of total transitions from the preceding to the succeeding behavior is next to each arrow. The number in brackets next to the behavior represents the mean time}.
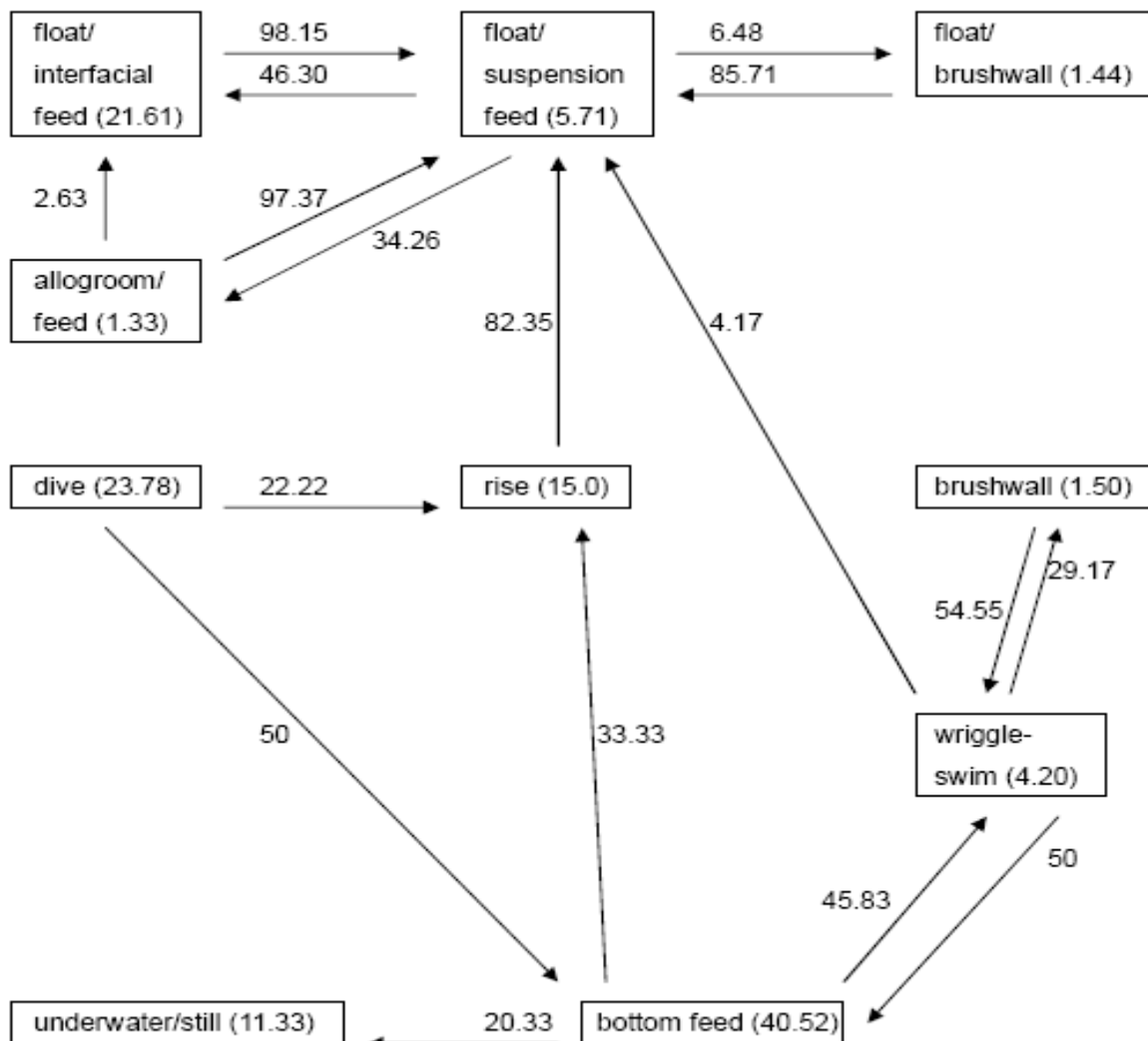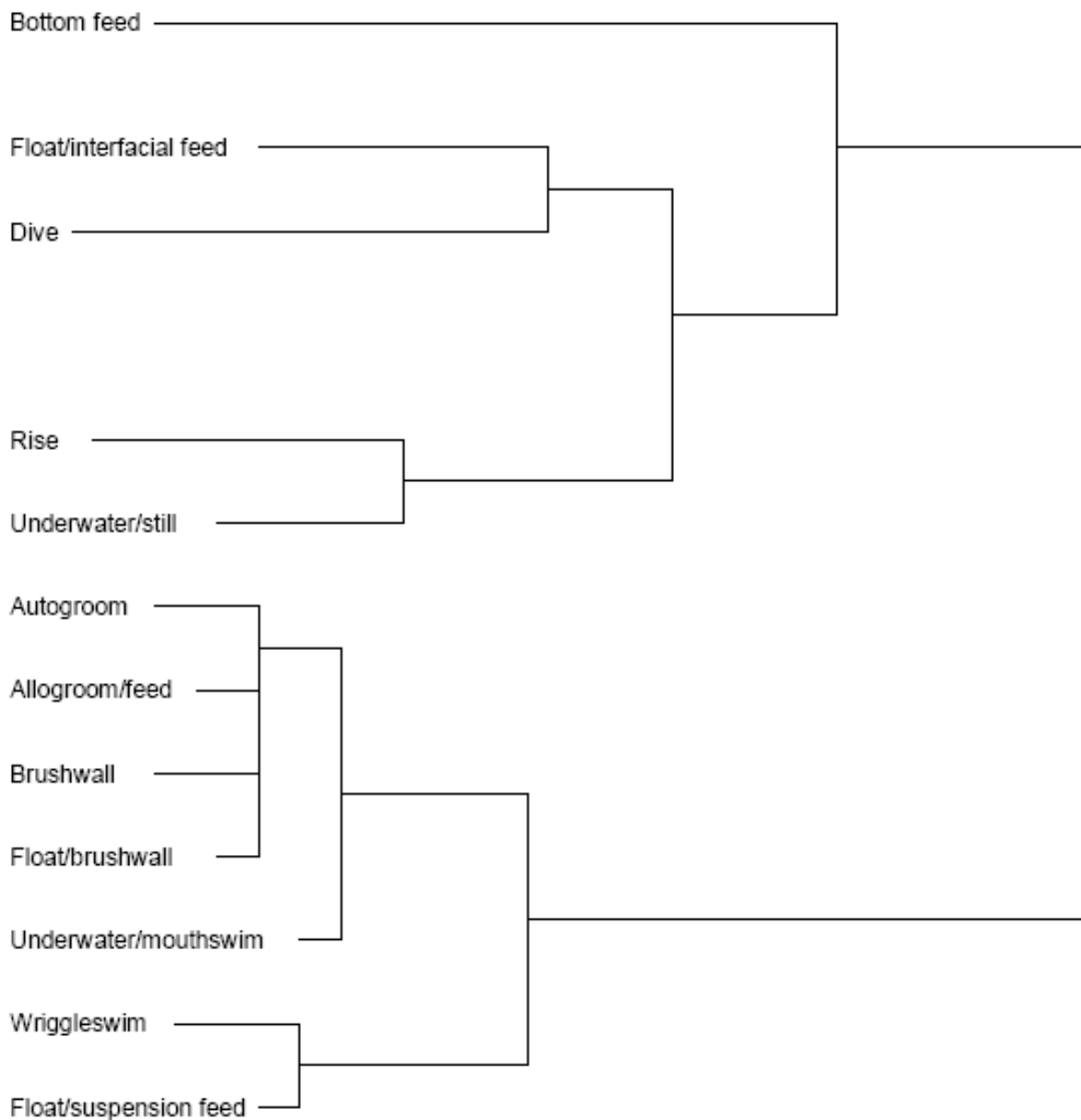
Figure 2: Dendrogram showing results of hierarchical cluster analysis of data from time budget for Anopheles stepensi 4th instar larvae.

{Dendrogram is not drawn to scale. Closely allied behaviors are indicated by relative length of the branches. Cluster analysis done using TANAGRA [17]}.
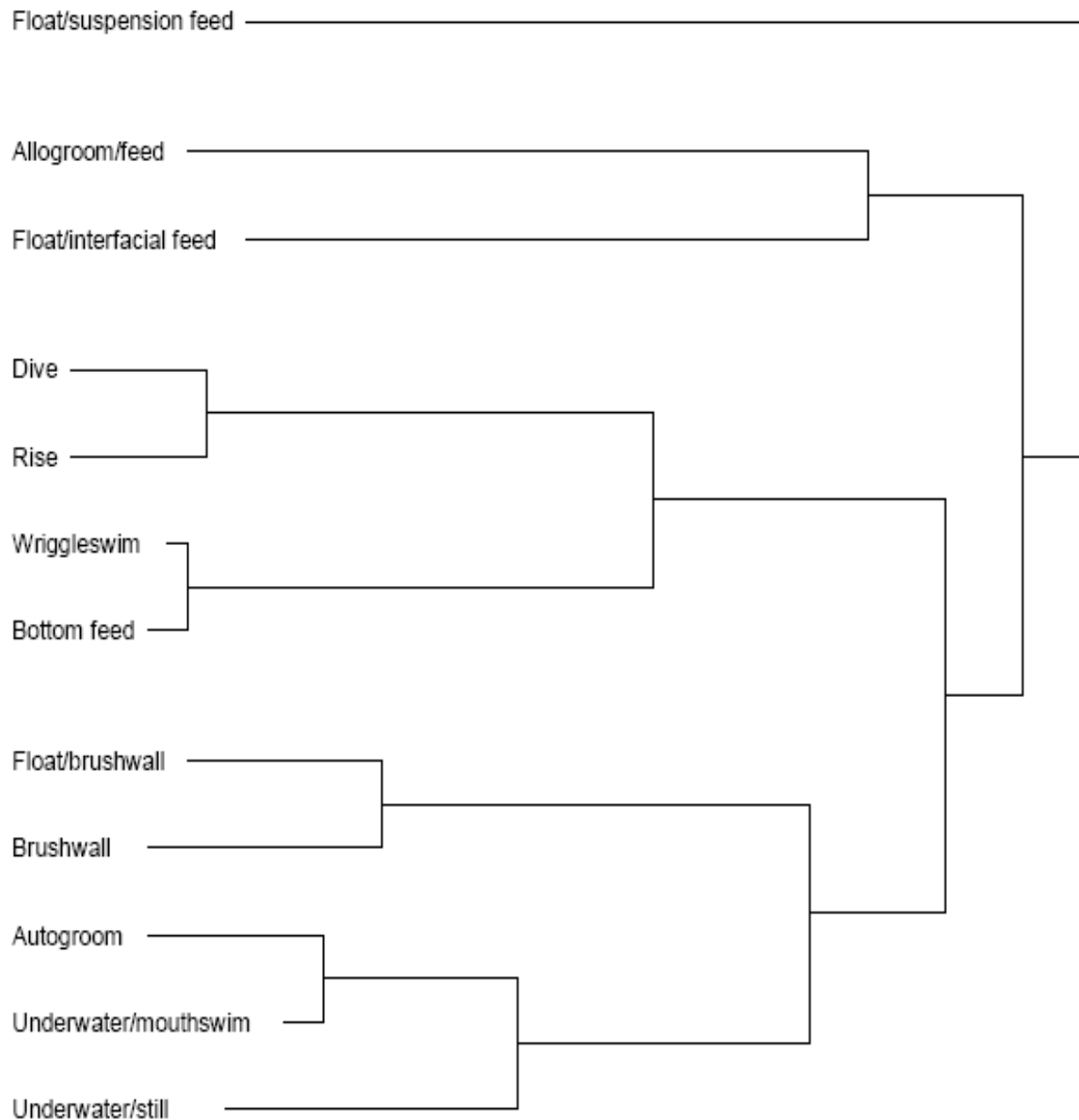
Figure 3: Dendrogram showing results of hierarchical cluster analysis of data from transition matrix for Anopheles stephensi 4th instar larvae.

{Dendrogram is not drawn to scale. Closely allied behaviors are indicated by relative length of the branches. Cluster analysis done using TANGARA [17]}

Table 1: Transition matrix for frequencies of transition for Anopheles stephensi 4th instar larvae; Entries with an asterix (*) indicate statistically significant transitions (G test, P<0.005).

Preceding behavior

Succeeding behavior

	Float-suspension feed	Dive	Wriggle-swim	Brush-wall	Bottom feed	Rise	Float/brush-wall	Float/inter-facial feed	Under-water/still	Allo-groom/feed	Under-water mouth-swim	Auto-groom
Float-suspension feed	-	11	0	0	0	0	7*	50*	0	37*	0	3
Dive	0	-	2	3	9*	4*	0	0	0	0	0	0
Wriggle-Swim	1*	1	-	7*	12*	1	0	0	2	0	0	0
Brush-wall	0	2	6*	-	0	0	0	0	0	0	0	0
Bottom feed	0	0	11*	0	-	8*	0	0	5*	0	0	0
Rise	14*	2	1	0	0	-	0	0	0	0	0	0
Float/brush-wall	6*	1	0	0	0	0	-	0	0	0	0	0
Float/interracial feed	53*	0	1	0	0	0	0	-	0	0	0	0
Under-water/still	0	0	0	0	6	0	0	0	-	0	0	0
Allogroom/feed	37*	0	0	0	0	0	0	1*	0	-	0	0
Under-water mouth-swim	0	0	0	0	0	0	0	0	0	0	-	0
Autogroom	2	0	0	0	0	0	0	0	0	0	0	-

Table 2: Time budget for Anopheles stephensi 4th instar larvae behavior (n=20).

Behaviour	Duration (s) Mean ± SD	Range (s)	% Total time (Total time = 18600 sec)	Frequency
Float/suspension feed	5.71±5.03	1-81	11.08	56
Dive	23.78±9.54	3-36	10.68	11
Wriggleswim	4.2±7.79	1-33	2.9	20
Brushwall	10.50±8.73	3-29	2.9	8
Bottom feed	40.52±35.36	2-149	29.43	21
Rise	15.0±3.67	9-19	7.78	15
Float/brushwall	1.44±1.5	1-5	0.45	9
Float/interfacial feed	2.68±27.79	2-128	32.88	44
Underwater still	11.33±9.61	1-20	1.18	3
Underwater mouthswim	-	-	-	-
Allogroom/feed	1.33±0.87	1-4	0.55	12
Autogroom	1.67±0.58	1-2	0.17	3

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HYPOGLYCEAMIC EFFECTS OF AQUEOUS EXTRACT OF *AFRAMOMUM MELEGUETA* LEAF ON ALLOXAN-INDUCED DIABETIC MALE ALBINO RATS

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Running Title: Hypoglyceamic effects of *Aframomum melegueta* leaf extract on alloxan-induced diabetic rats

ABSTRACT

Aframomum melegueta (Zingiberaceae) seeds are used in West Africa, as a remedy for variety of ailments such as stomach ache, snakebite, diarrhea and anti-inflammatory properties. The hypoglycaemic effects of crude leaf extract of *Aframomum melegueta* on the treatment of alloxan induced diabetes in male rats and non-diabetic rats (control) were examined in this study. Results obtained from the experiment showed that the elevated blood glucose level caused by oral administration of 250 mg / kg body weight of alloxan was reduced significantly ($p < 0.01$) by oral administration of *Aframomum melegueta* leaf extract doses of 50, 100 and 200 mg/kg with the exception of 20 mg/kg when compared to control groups. The non-diabetic groups that received the extract showed reduction in blood sugar level as the dose increases when compared to their control group. There was a final weight gain and organ restoration for both the diabetic and non-diabetic rats after treatment when compared with their controls. This study showed that the extract have hypoglycemic and prophylactic effects.

INTRODUCTION:

Diabetes mellitus (DM) is a group of syndromes characterized by derangements in carbohydrate, fat and protein metabolism. DM may be defined as a syndrome characterized by hyperglycemia due to an absolute or relative lack of insulin and/or insulin resistance (depressed target cell sensitivity) [1, 2]. The general characteristics of DM include glucosuria, osmotic diuresis, polyuria, polydipsia and polyphagia.

DM may either be primary and secondary [3]. Primary DM is associated with inadequate insulin production, circulation or resistance to insulin action. Secondary DM maybe associated with existing disease conditions such as infections, pancreatic damage, stress or environmental conditions. Virtually all forms of DM are due to insulin deficiency and a decrease in the response of peripheral tissue of insulin (insulin resistance).These abnormalities lead to alteration in the metabolism of carbohydrates, lipids and ketones [4].

Control of diabetes by spices and other natural products is becoming popular and is more appropriate and economical for use in developing countries like Nigeria. Spices come from dried aromatic plants or trees and may be the bark, root, seeds, fruit, buds or the berry of these plants/ trees [2].

Aframomum melegueta is a West African plant, with common (local) names as Alligator pepper or Guinea pepper and Grain of paradise. It is a member of the family Zingiberaceae. It is about 1.0 m tall with narrow lanceolate bamboo like leaves at base of leafy shoots on very short peduncles, with bracts, and pink or lilac labellum.The bracts enclose the developing flowers. The fruit is ovoid with reddish colour and numerous small brownish angular seeds with a cardamorn flavour. *Aframomum melegueta* are valued spice and this earned the plant the name 'Grains of paradise' [3]. They are also used for strengthening beer and other alcoholic drinks. In West Africa, the fruit pulp is chewed as a refreshing stimulant and the seeds and leaves are used for seasoning foods and in local medicine. It is also used as a remedy for variety of ailments such as snakebite, diarrhea, smallpox, chickenpox, wounds, cough, anaemia, rheumatism, measles, malaria, toothache, cardiovascular diseases, diabetics and fertility control [5, 6]. There have been claims by traditional herbalists that *Aframomum melegueta* can be used in treatment of diabetes.

The aim of this work was to investigate the hypoglyceamic effect of *Aframomum melegueta* (Alligator pepper) aqueous leaf extract on alloxan-induced diabetes mellitus in male albino rats.

MATERIALS AND METHODS:

Chemical

Alloxan was purchased from sigma chemical co U.S.A. All other chemicals used were of analytical grade.

Sourcing of Plant material

The leaves of *Aframomum melegueta* harvested in June were purchased from the local market in Lagos, Nigeria. It was authenticated by a taxonomist in the Botany Department of the University of Lagos. A voucher specimen was deposited (ascension number FHI108876) in the herbarium record.

Preparation of the aqueous extract of *Aframomum melegueta*

The leaves of *Aframomum melegueta* were sun dried and further dried in an oven at 25ºC for two days to remove moisture content. The dried leaves were ground to powdery form with blender. A 100.0 g of the ground leaves were dissolved in 250 ml of distilled water. The mixture was filtered with muslin cloth. The filtrate was emptied into two beakers and evaporated to dryness. The concentrate was weight, and then dissolved in 50 ml of distilled water.

Induction of Experimental DM

The animals were fasted overnight and diabetes was induced by a modification of method described by Sekar et al. [7]. Briefly, the animals were fasted overnight and DM was induced by a single oral administration of freshly prepared alloxan solution at a dose of 250 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia [8]. After a week time for the development of DM, the rats with moderate DM having glycosuria and hyperglycemia (blood glucose range above 250 mg/dl) were considered diabetic and used for drug treatment. The aqueous leaf extract was then administered orally with various concentrations given daily according to the weight of each animal. The rats were weighed every three days during the duration period of the study.

Experimental animal /Study design

The 30 albino rats used for this study were obtained from the Laboratory animal's centre of the University of Lagos. They were acclimatized for two weeks, and fed rats pellets and water *ad libitum.* The rats were divided into 5 groups with 5 rats per group. Groups 1-4 were the diabetic induced rats that received oral doses of 20, 50, 100 and 200 mg/kg body weight of the aqueous extract respectively. The group 5 rats served as the control, received only distilled water. The aqueous extracts were given the first day, after which the blood glucose levels of the rats in the experimental and control groups were taken and recorded.

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The administration of aqueous extracts continued after every 24 hours till the fifth day when the blood glucose measurements were repeated and the rats weighed. The rats were sacrificed on the sixth day, by cervical dislocation and blood samples obtained from ventricular punctures. The liver, pancreas and kidneys were harvested from each rat and weighed.

Estimation of blood glucose

Blood samples collected were used to estimate blood glucose levels using glucometer and strips. The Touch Basic made by Lifescan (Johnson & Johnson Company, California, USA) was used and the results were read off on the meter 45 seconds after application of blood samples to the strips [9]. The technical performance of the glucometer used was evaluated by comparison with standard laboratory method of blood glucose estimation (spectrophotometer) at the beginning, midway and at the end of the experiment as previously described by Ajala et al. [10].

Gravimetric analysis

The rats and the harvested organs were weighed at the end of experiment. Weighing was done using sensitive weighing machine [11] and values were expressed in grammes.

Statistical analysis

All results were analyzed using students t-test and ANOVA with the aid of SPSS (ver. 15) software package. The level of statistical significance was taken as $p < 0.05$. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [12] and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

RESULTS AND DISCUSSION:

The use of herbs is increasingly gaining acceptance among Africans and the world over as alternatives to orthodox medicine for the treatment of various types of diseases [13, 14, 15]. Regardless of the highly advanced orthodox medical therapies, substantial amount of medicinal plants are used for the treatment of ailments in some developed countries. In the United States of America, for example, medicinal plants constitute approximately 25% of all new refined prescriptions dispensed from community pharmacies [16]. The popularity of herbal product is being matched by a corresponding evidenced based research supporting their efficacy. *Aframomum melegueta* plant is one amongst such herbal products.

In the diabetic control group the increase in blood glucose level (Table 1) and reduction in weights of the pancreas, kidney and liver (Table 2) are indicative of the hyperglyceamic effects of alloxan resulting from its ability to destroy the pancreatic islets cells. Our findings support recent report by Mahesar et al. [17] that administration of alloxan (150 mg/kg) led to about 3-fold elevation of fasting blood glucose levels, which was maintained over a period of 4 weeks.

Our data indicated that when treated with graded oral doses of the aqueous leaf extract (20, 50, 100 and 200 mg/kg), a dose related decrease in blood glucose level were observed for both the diabetic and non-diabetic rats compared to their control counterparts ($p < 0.05$). The *Aframomum melegueta* aqueous extract resulted in significant decrease ($p < 0.05$) in the blood glucose levels in the diabetic groups especially at highest dose of 200 mg/kg (Table 1). Although similar effects were also recorded in the non-diabetic groups significant reductions were observed more on alloxan-treated diabetic rats than non-diabetic rats (Table 3). Our results are similar to that obtained in a recent study by Ilic et al. [18] in which the effect of ethanol extract of the seeds of *Aframomum melegueta* caused reduction in blood glucose level in male diabetic rats. The lowest dose (20 mg/kg) of our aqueous leaf extract produced non-statistically significant decrease in blood glucose level ($p > 0.05$) in the diabetic rats. This may be due to the low

concentration of the active phytochemicals in the extract.

After the initial shrinking in weights observed in the pancreas, kidney and liver in the diabetic groups, the weights of these organs increased with administration of the aqueous leaf extracts (Table 2). These increases in weights might be due to the regeneration of organ tissues that were damaged by alloxan [19]. The aqueous leaf extract did not have significant effect on the body weights of the diabetic and non diabetic rats, however at a higher dose of 200 mg/kg there was significant increase in the weight of the diabetic rats (Tables 1). This pattern is similar to that reported by of Prohpl et al. [15] in which the weights of the diabetic and non diabetic animals did not change significantly at lower dose of the extracts after treatment.

The actual mechanism of action of the aqueous leaf extract is not fully understood. However, a possible mechanism may include direct inhibition of alloxan by competing with the glucose receptors on the β-cell membrane on the pancreas or by increasing the β-cell resistance by activation of super oxide dismutase which scavenges super oxide radicals [20]. This may also be a determining factor of the toxic effect of alloxan [20]. Further, the anti-hyperglycemic activity of the aqueous leaf extract maybe associated with an increase in plasma insulin level suggesting an insulinogenic activity; stimulating insulin secretion from

the remnant β-cells or from regenerated β-cells [8]. Though data from this study indicate that the aqueous leaf extract has significant hypoglycemic and prophylactic effects (suggesting it could be developed as a drug for treatment of diabetes), long term treatment effect is yet to be ascertained. Further work should include estimation of the LD50, identification and isolation of the bioactive compound(s) and to elucidate their mechanism(s) of action.

Table 1: Effect of oral administration of *Aframomum melegueta* on blood glucose of diabetic and non diabetic rats[1] before and after treatment

Extract given in (mg/kg)	Non-diabetic group		Diabetic group	
	Initial mg/dl	Final mg/dl	Initial mg/dl	Final mg/dl
Control	39.00 ± 3.00	39.50 ± 3.50	90.50 ± 1.50	105.00 ± 5.00
20	53.66 ± 3.85	41.00 ± 4.89*	110.00 ± 5.71	110.30 ± 5.43
50	55.00 ± 10.42	39.00 ± 8.04*	104.00 ± 10.19	97.33 ± 9.97*
100	46.33 ± 5.79	39.00 ± 3.74	107.66 ± 8.37	76.66 ± 9.46*
200	56.00 ± 4.89	34.66 ± 5.24*	115.66 ± 3.68	48.00 ± 3.55**

[1]Values represent Mean ± SD for rat and triplicate determination; *$p < 0.05$

Table 2: Effect of oral administration of *Aframomum melegueta* on the weight of some internal organs of diabetic and non diabetic rats[1]

Concentration of extract (mg/kg)	Non-diabetic group (g)			Diabetic group (g)		
	Pancreas	Kidney	Liver	Pancreas	Kidney	Liver
Control	0.69±0.10	1.15±0.15	5.75± 0.04	0.24±0.04	0.55± 0.05	3.40± 1.05
20.00	0.71±0.10	1.17±0.12	5.76±1.02	0.44± 0.04	1.00± 0.21	6.03±1.72*
50.00	0.73±0.04	1.2± 2.10	6.00±0.80	0.51±0.09*	1.03±0.20*	5.26±0.57*
100.00	0.74±0.10	1.2± 0.10	7.36± 0.60	0.64±0.04*	1.20±0.08*	6.16±0.41*
200.00	0.8±0.10	1.36±0.12	7.8± 0.30	0.65±0.10*	1.33±0.09*	6.70±0.73*

[1]Values represent Mean ± SD for rat and triplicate determination; *$p < 0.05$

Table 3: Effect of oral administration of *Aframomum melegueta* on body weight of diabetic and non diabetic rats[1]

Doses of extracts (mg/kg)	Non-diabetic group		Diabetic group	
	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
Control	89.75 ± 2.15	89.35 ± 1.85	124.6 ± 5.60	112.25 ± 7. 05
20.00	95.73 ± 14.21	88.00 ± 9.14	150.66 ± 11.89	148.76 ± 10.77
50.00	95.00 ± 6.09	87.03 ± 5.52	146.53 ± 24.27	143.93 ± 26.54
100.00	125.00 ± 26.99	133.40 ± 30.04	132.66 ± 35.33	137.76 ± 33.01
200.00	111.90 ±15.94	114.60 ± 16.08	106.63 ± 37.93	123.4 ± 37.80*

[1]Values represent Mean ± SD for rat and triplicate determination; *$p < 0.05$ considered significant when compared to initial weight

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EFFECT OF *MOMORDICA CHARANTIA* ON ESTROUS CYCLE OF SPRAGUE-DAWLEY RATS

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Running title: Effect of *Momordica charantia* on estrous cycle, morphology of ovary and uterus in rats

ABSTRACT

Bitter Melon (*Momordica charantia*) is a plant known widely particularly in the Indo-Malayan region for its uses in various ailments as reported in literature. However, little is known for its effect on the female reproductive system. The aim of this project was to evaluate the effect of oral administration of methanolic seed extract of *Momordica charantia* on the estrous cycle and the histology of the ovary and uterus in Sprague-Dawley (S-D) rats. A total of 20 adult cyclic female S-D rats (4-day cycles), weighing between 110– 140 g were used. These were divided into 4 groups (A, B, C and D) of 5 rats/ group. The dose of the extract administered was 25 mg/100g body weight and the route of administration was oral by gastric gavages with a metal canula. Groups A and B were both treated with a daily dose of the extract for 28 days (7cycles) and vaginal smear monitored within this period between 9.00– 10.00 am daily. Animals in Groups C and D (control) were fed distilled water and vaginal smear monitored daily throughout the duration of the experiment. The effect of withdrawal of the extract was studied in Group B which was treated with distilled water for another 28 days. The rats were sacrificed by cerebral dislocation. Groups A and C at the end of the first 28 days while Groups B and D at the end of the second 28 days. The ovaries and uterii were harvested for histological studies. Irregular changes in the phases of the estrous cycle in all the treated rats were observed. The diestrous phase was increased while the proestrous and estrous phases were decreased significantly. These effects were reversible on withdrawal of the extract. Histological sections did not show any difference between the ovarian and uterine tissues of the treated and control respectively. The extract resulted in a reversible variation in the estrous cycle pattern. Histological sections reviewed were essentially normal.

INTRODUCTION:

Momordica charantia (Bitter melon) is a monoecious climber. It grows in tropical areas including parts of the Amazon, East Africa, Asia and the Caribbean and is cultivated throughout South America as food and medicine [1, 2]. In Nigeria, especially in folkloric settings, its culinary usage is largely hinged on its relief to various ailments. Studies have demonstrated its antimicrobial (against viruses, bacteria and fungi) [3 – 6], anticancerous (cytotoxic) [7, 8], antidiabetic (hypoglycemic) [9 – 11], anti-oxidant [12, 13] and anti-ulcer actions [14, 15]. Phytochemical profile shows it contains an array of biologically active substances that include triterpenes, proteins and steroids. Its actions in the female reproductive system have also been documented [16]. The fruit and leaf have demonstrated an *in-vivo* anti-fertility effect in female animals and in male animals to affect the production of sperm negatively [17]. The seeds however have exhibited ability to induce abortions in rats; the roots shown to have uterine stimulant effect [18]. In another study, ethanolic extract of the roots of *Momordica cymbalaria fenzl* at doses 250 and 500 mg/kg body weight was found to have abortifacient and anti-ovulatory effects in rats [19]. Scanty literature exists on its effect on the estrus cycle and histology of the ovary and uterus of which this work is designed to address. The results therefore will help in assessing its suitability as an anti-fertility agent.

MATERIALS AND METHODS:

Plant Materials (Collection and identification)

The procurement of fresh fruits of *Momordica charantia* seed (MC) was done in a local market at Mushin, Lagos State Nigeria. The same was identified and authenticated by Professor J. Olowokudejo, a taxonomist in the Botany Department of the University of Lagos, where the voucher specimen was deposited (ascension number FHI 108422).

Processing/Preparation of seed extract

The processes leading to the constitution of the appropriate formulation of 230 g of MC in 1000 ml of methanol was done in the Pharmacognosy Department of College of Medicine, University of Lagos (CMUL). These included drying the fruits to get seeds which were then weighed and Soxhlet extraction done using alcohol (absolute methanol) as solvent. The percentage yield of the seed extract obtained was 23.0% w/w.

Animals

This study was done with 20 healthy adult female albino rats of Sprague-Dawley strain. These rats, 8-10 weeks old, weighing 125±15 g were obtained from the Animal Breeding Laboratory Centre of CMUL. They were housed in well-ventilated plastic cages in the rat room, Anatomy Department of CMUL under standard animal house conditions (temperature: 28°C to

31^0C; light: approximately 12 hours natural light alternating with 12 hours darkness per day; humidity; 50 to 55 %). The rats were allowed free access to pelleted food (Pfizer Nigeria Limited) and water *ad libitum*. Lighting in the room was by sunrays (natural light) reflecting through the glass windows. Illumination periodicity plays a dominant role in the incidence and duration of the stages of the ovarian cycle [21]. Animals were also allowed to acclimatize to the laboratory environmental condition for two weeks.

They were weighed at procurement and weekly subsequently. Each animal had a 4-day estrous cycle, confirmed through vaginal smears taken and examined daily between 9.00-10.00 am for 16 days (4 cycles).

Experimental Protocol

A total of 20 adult female Sprague-Dawley rats, weighing between 110-140 g were used. These were divided into 4 experimental groups of 5 rats per group. The groups were designated A, B, C and D. The rats were weighed weekly from the onset of the experiment. The dose of the extract administered was 25 mg/100 g/body weight of rat [19, 20]. When required, they were fed orally with the extract/distilled water. From the percentage yield of the seed extract obtained, the treatment dose (25 mg/100 g) suspended in distilled water was prepared20. Appropriate volumes based on the animal's individual weight were calculated by simple proportion and administered orally by gastric gavages with a metal canula [20].

Groups A and B were the treatment groups; C and D were the control groups. These rats showed 3 consecutive regular 4-day estrous cycles. The first day of the estrous cycle called the diestrous phase, showed predominance of leukocytes and a few large nucleated cells. This is designated "L2". The second day showed large nucleated cells with the leukocytes, called the proestrous phase and designated "N". The third day, called the estrous phase, showed large flake of squamous cells with small pyknotic nuclei. This was designated "C". The metestrous, which was on the fourth day showed leukocytes amidst remnants of large squamous cells with pyknotic nuclei and was, designated "L".

Groups A and B (treatment groups) were both treated with a daily dose of the extract while Groups C and D (control groups) were fed equal volumes of distilled water (5 ml), all for 28 days (7cycles). Vaginal smears from each animal were examined daily between the hours of 9.00-10.00 am during this period; while the assessment of the weight done weekly. The vaginal smears were collected using a small rubber suction manual pipette and normal saline. The normal saline was first drawn into the tip of the pipette. This was then introduced into the vaginal canal and the normal saline released. The vaginal fluid, by negative pressure is suctioned into the tip of the pipette. The fluid was then smeared on a glass slide

and examined under the light microscope immediately before drying up. It is important to note that the treatment for 7 cycles was to cover three regular estrous cycles and to exclude the possibility of irregular cycling being caused by pseudo-pregnancy [22]. At the end of this period, Groups A and C rats were sacrificed by cervical dislocation, a laparotomy done and the ovaries and uteri harvested *per* abdomen, for histological studies. The effect of withdrawal of the extract was studied in Group B rats. These rats were discontinued from the extract and together with the group D rats fed with distilled water for another 28 days (7 cycles) and vaginal smears monitored within the same period (reversal effect). These rats again were sacrificed by cervical dislocation at the end of this period and the ovaries and uteri harvested as described above for histological studies (reversal studies). It is important to mention that Groups A and C rats were sacrificed at the end of the first 28 days while Groups B and D at the end of the second 28 days.

Tissue processing for histological studies

The harvested organs were carefully dissected out, trimmed of fat and connective tissue. The tissues were processed by the method described below with slight modification [23]. The steps involved in tissue processing included fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning, and staining. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol (50%, 70%, 90%, absolute alcohol), then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and made into blocks of wax. Microtome whose sectioning size knob was adjusted to six Microns was then used to section the block, fixed on clean slides and later stained with haematoxylin and eosin.

RESULTS:

Estrous Cycle

Analysis of the Estrous cycle revealed that oral administration of 25 mg/100g body weight of Methanolic seed extract of *Momordica charantia* produced an irregular pattern of cycling in all the treated rats (100 % of the treated rats). The length of the estrous cycle was significantly increased (Tables 1) and this was marked in the diestrous phase.

Cycles up to 8 days were observed when compared with the control at $p < 0.05$. A significant decrease in the duration of the proestrous and estrous phases were also observed throughout the treatment period.

However these effects were reversible on withdrawal of the extract and it took the rats an average of 8 to 10 (2– 3 cycles) days to regain their normal 4-day cycle. No death was recorded as a result of the test extract.

Control rats showed a regular 4-day cycle.

Histology of the uterus and ovary

Histological analysis of the uteri of the treated groups showed a well differentiated serosa, muscularis and endometrial layers. The glands were numerous and well developed. There were no overt histoarchitectural (uterotrophic) changes compared to those of the control groups (Figures 1-3).

The ovaries of the treated groups were characterized by the presence of growing follicles, matured follicles, numerous ruptured follicles and some atretic follicles (Figures 4-6).

Body Weight

There was a general downward trend in the weights of all the rats treated with Methanolic seed extract of *Momordica charantia* at the specified dose (25mg/100g body weight). This is shown in table 2 which depicts the mean body weights of all the animals while on treatment with the extract for 28 days and on withdrawal. There was a significant mean loss of weights ($p < 0.05$) in the animals while on treatment with the extract for 28 days compared to control. In the mean weights of the animals for reversal studies; animals were initially treated with the extract for 28 days. On withdrawal of the extract, a slight weight gain was observed although this was not statistically significant ($p \square 0.05$). The difference in weight after experiment was not statistically significant in the treatment group but was significant ($p < 0.05$) when compared to the control group.

Table 1: The estrous cycle analysis in experimental and control Sprague-Dawley rats

Estrous cycle phase (%)	Treatment with *Momordica charantia* extract/distill water			
	Before administration	During administration	Withdrawal of extract	Control
Normal	100.0	0.0	0.0	100.0
Irregular	0.0	100.0	100.0	0.0
Metestrous	24.4	16.5	16.5	24.3
Diestrous	25.8	35.5	35.5	25.9
Proestrous	24.6	20.5	20.5	24.7
Estrous	25.2	27.5	27.5	25.1

Table 2: Effect on the mean body weights during extract administration and following extract withdrawal (Reversal effect)

Mean body weight	Experimental	Control	Withdrawal	Control
Before experiment	127.5±12.5	128.6±11.0	124.2 ±11.5	128.6 ±11.0
After experiment	113.6 ±11.5*	137.4±11.0	121.6 ±11.5	146.6 ± 8.5
Difference	-13.90*	+ 08.8	- 2.6	18.0

Values are expressed in mean ± SD; Weight in grammes; *Level of significance is at $p < 0.05$; Significant difference between values of experimental and control groups

DISCUSSION

The anti-fertility potentials of many plants have been investigated [19, 24 – 26]. In this study, oral administration of *Momordica charantia* methanolic seed extract caused irregular changes in the phases of the estrous cycle in all the treated rats.

The duration of the diestrous phase was significantly increased while the proestrous and estrous phases were decreased. These effects were reversible on withdrawal of the extract. This disruption of the estrous cycle may be due to the effect of this extract on the ovary which controls ovarian functions and estrous cycle via ovarian and extra ovarian hormones [27, 28].

The diestrous phase is maintained by the activities of the corpus luteum which produces progesterone in the absence of pregnancy and

terminates with the regression of the corpus luteum. Earlier studies with extracts of *Abrus precatorius* seed, castor bean and cotton seed have all shown to cause a significant but reversible alteration in the estrous cycle of Sprague-Dawley rats [25, 26, 29].

Histologically, there is no difference between the ovarian and uterine tissues of the treated and control respectively. This could be due to the anti-oxidant and anti-inflammatory potentials of MC.

The results in this study also indicated that oral administration of methanolic extract of MC seeds to adult cyclic female Sprague-Dawley rats have effects on the mean body weight which was seen to be decreasing with administration of the extract.

On withdrawal of the extract, there was a slight trend towards weight gain although this was not

statistically significant.

In conclusion *Momordica charantia* (25 mg/100g body weight) caused a reversible alteration in the estrous cycle pattern. Histological sections reviewed were essentially normal.

These properties, among others will be helpful in assessing the suitability of MC as a good anti-fertility agent. However, further studies are recommended to look at the histology at the level of electron microscopy.

The potential to cause weight loss will be beneficial in combating metabolic disorders such as obesity which is becoming an epidemic and posing serious health problem.
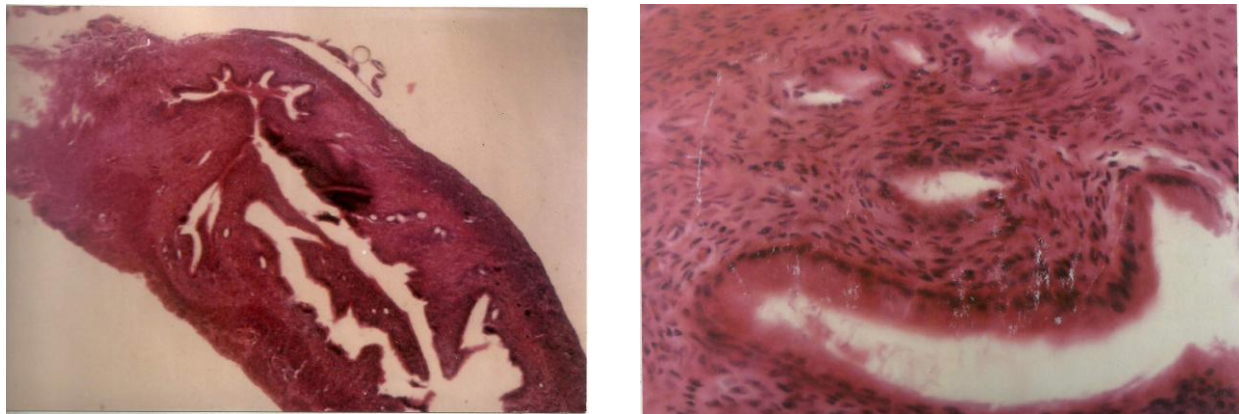
Figure 1: Photomicrograph of the uterus of a control rat stained with Haematoxylin and Eosin. Magnifications; ×40, x 100
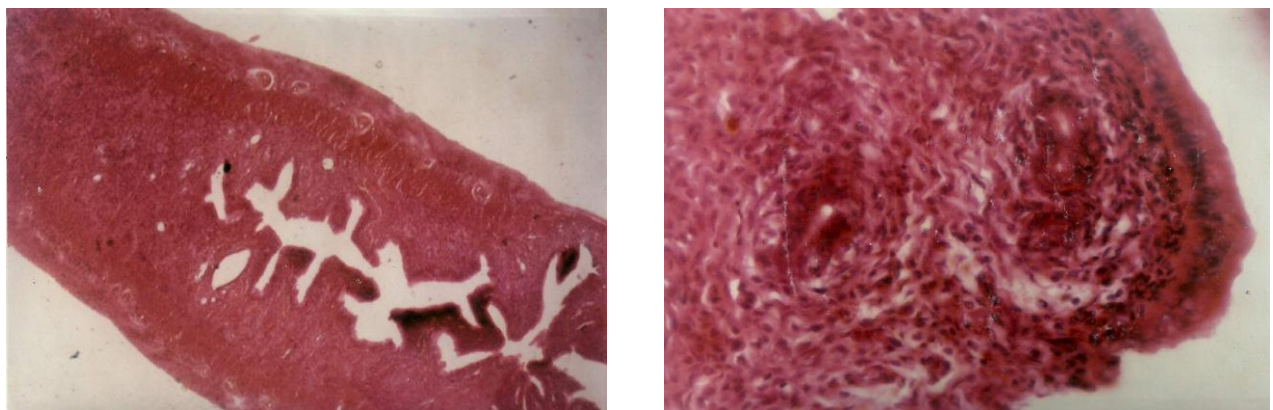

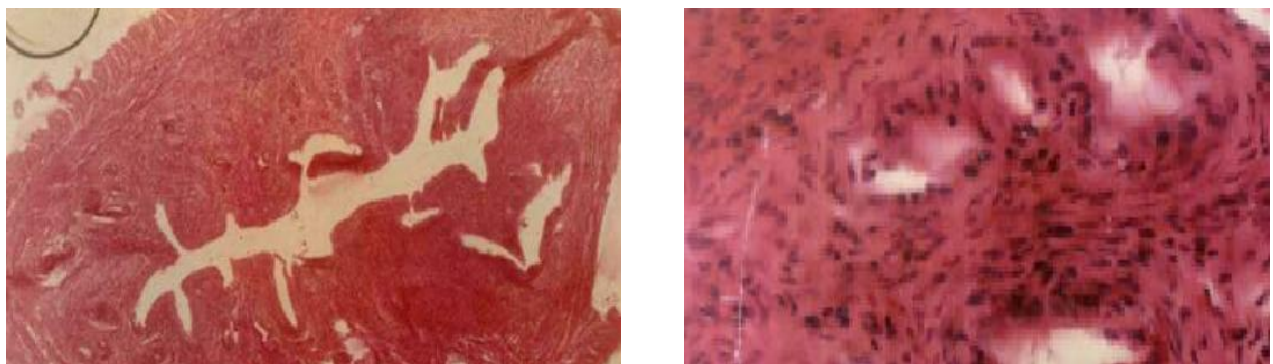Figure 2: Photomicrograph of the uterus of a treated rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100

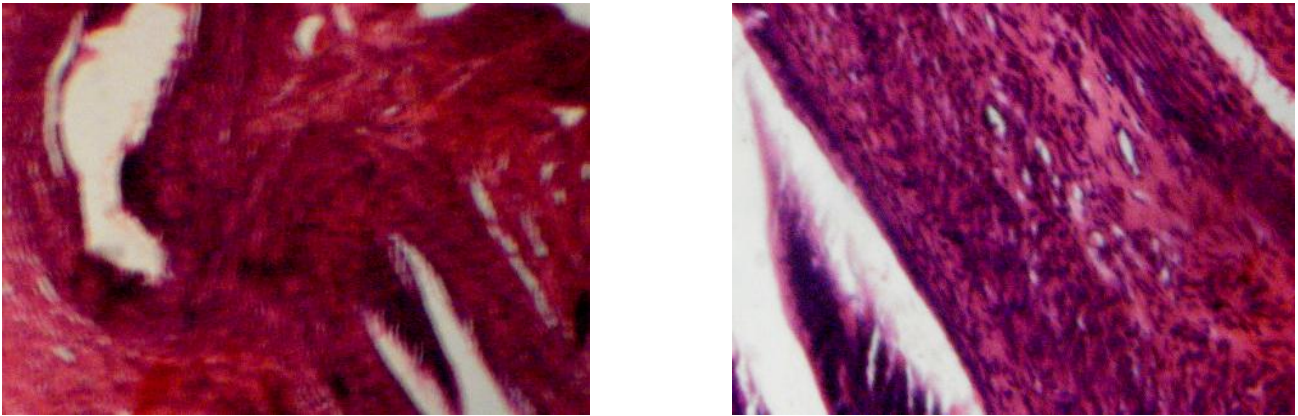Figure 3: Photomicrograph of the uterus for withdrawal rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100

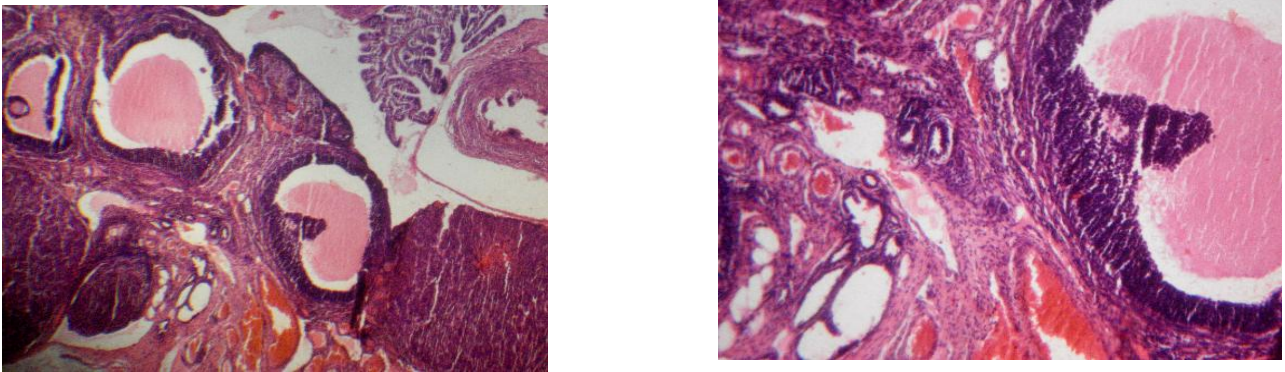Figure 4: Photomicrograph of the ovary of a control rat stained with Haematoxylin and Eosin. Magnifications; ×40; ×100

Figure 5: Photomicrograph of the ovary of a treated rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100
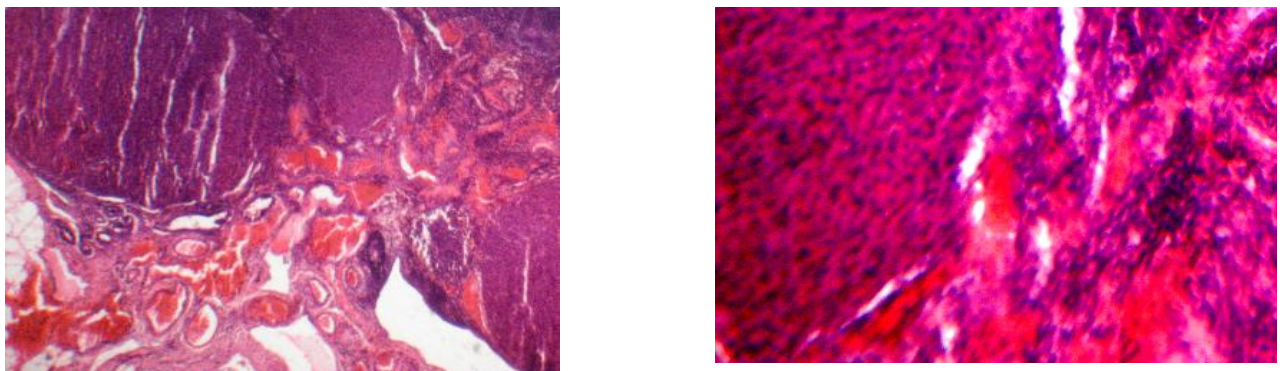

Figure 6: Photomicrograph of the ovary of a rat for withdrawal studies stained with Haematoxylin and Eosin. Magnifications; ×40, x100

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FREQUENCY OF ALLELES D16S539, D7S820, D13S317 IN A POPULATION SAMPLE IN NATIONAL CAPITAL DISTRICT, PAPUA NEW GUINEA

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ABSTRACT:

The allele and genotype frequency values of the three tetranucleotide short tandem repeat (STR) loci, D7S820, D13S317 and D16S359, were analysed in blood samples of 25 unrelated randomly selected individuals in the National Capital District, Papua New Guinea.

Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) was used for the PCR amplification in GeneAmp®PCR System 9700 thermal cycler (Applied Biosystems). Data analysis was carried out using the PowerStatsV12.xl workbook template obtained from Promega Corporation. The three STR loci were in Hardy-Weinberg equilibrium.

Five alleles (9 – 13) were identified for D16S539, five alleles (8 – 12) for D7S820 and six alleles (8, 9, 11 – 14) for D13S317. No new or microvariant alleles were observed. The most frequent genotypes for D16S539 were 11-11 and 11-12; for D7S820 were 10-11 and 12-12; for D13S317 was 8-12. Observed Heterozygosity was highest in D13S317 (0.880). The combined power of discrimination was 0.99733 and the combined power of exclusion was 0.9363.

The data suggests that the three loci are useful for identity testing, forensics and for solving paternity cases among the population in National Capital District, Papua New Guinea.

INTRODUCTION:

The non-protein-coding segments in the DNA consist of tandemly repeated sequences of about 2 – 7 base pairs in length [1-3]. The short tandem repeats (STR) loci are well distributed throughout the human genome, are polymorphic in nature and represents important genetic markers for population studies [1 – 3].

Polymerase chain reaction (PCR) can be used to amplify the STR loci in low amount or degraded DNA in biological samples [2 – 6]. STR data can be highly informative and extremely effective for individualizing a wide range of biological samples, especially when multiplex PCR is used for simultaneous amplification of the STR loci [2 – 6].

Polymorphic loci whose alleles are the result of STR are the most informative PCR-based genetic markers for human identity testing, personal identification, and paternity testing because of their high statistical capability of discrimination and individualization [2, 4, 5].

The many uses of STR loci in forensics and criminal investigations have been highlighted by many researchers [1 – 6]. Selected STR loci are currently used for DNA typing and for setting up of DNA database in various countries [2,3]. Determination of the allele frequencies and distribution of genotype are prerequisites for DNA typing of any population.

In resource limited countries like Papua New Guinea DNA typing can be carried out with the multiplex STR system using silver stain for manual detection [7,8]. The Gene Print Silver STR III multiplex system containing three tetra-nucleotide repeat STR loci, D16S539, D7S820, and D13S317 can be used for DNA typing [7,8]. The characteristics of these STR loci are presented in Table 1.

There are no published data on the allele frequency of STR loci in the DNA of the population in the National Capital District (NCD) in Papua New Guinea (PNG). This project was an attempt to initiate the setting up of a DNA database for forensic testing in NCD.

The aim of this project was to determine the allele frequency of the three tetra-nucleotide STR loci, D7S820, D13S317 and D16S359, in a population sample in NCD, PNG.

MATERIALS AND METHODS:

The study site was the NCD, which is the incorporated area around Port Moresby the Capital of PNG. The study population consisted of 25 randomly selected and consented unrelated individuals resident in the NCD.

Whole blood was collected by finger stick and spotted onto special filter paper, which was then left to dry overnight [9,10]. Each dried blood spot obtained was then stored in a refrigerator until required for analysis [9,10].

DNA extraction was carried out using 5% Chelex®100 (Bio-Rad Laboratories, Hercules, CA) extraction procedure [10]. Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) was used for the PCR amplification, of the loci D16S539, D7S820 and D13S317, in GeneAmp®PCR System 9700 thermal cycler (Applied Biosystems), following the standard procedures [9]. DNA amplification was done in 25µl per reaction sample.

The master-mix contained 140µl of primer pair mix (Multiplex 10x) and 8.4µl of Taq DNA polymerase mixed into 140µl of STR buffer (10x) and 971.6µl of sterile water, which gives a total volume of 1260µl.

A master-mix of 22.5µl was placed into each reaction well and the reaction plate was placed on ice, after which 2.5µl of template DNA was added to each reaction well. A total of 31 cycles of PCR were performed [9].

The amplified PCR products were separated in 6% Polyacrylamide denaturing gel on vertical Sequi Gen GT gel apparatus (Bio-Rad Laboratories, CA) using standard protocol [9, 11]. After electrophoresis the gel was stained according to the Promega Multiplex STR Silver Staining protocol [9]. The allele bands were identified by comparison with the allelic ladders in the Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) Allelic Ladder Mix.

Ethical clearance and permission for this study was obtained from the ethics and research grant committee of the School of Medicine and Health Sciences, University of Papua New Guinea. Signed informed consent was obtained from all selected participants.

Data analysis was carried out using the PowerStatsV12.xl workbook template obtained from Promega Corporation [12].

The alleles were assigned their numerical values according to the reference information in the Promega technical manual [9].

These figures were used in the PowerStatsV12.xl software to calculate the allele frequencies and forensic efficiency parameters, such as Power of Discrimination (PD), Probability of Match (PM), Polymorphic Information Content (PIC), Power of Exclusion (PE), Heterozygosity (H), and Typical Paternity Index (PI).

Table 1: Characteristics of the STR loci D16S539, D7S820 and D13S317 [9]

STR Locus	Chromosome Location	Repeat Sequence	Allele Size Range (Bases)	Known Alleles
D16S539	16q24-qter	AGAT*	264 – 304	5, 8 – 15
D7S820	7q11.21-22	AGAT*	215 – 247	6 – 14
D13S317	13q22-q31	AGAT*	165 – 197	7 – 15

* Repeat sequences represent all four possible permutations (e.g. AGAT is used for AGAT, GATA, ATAG or TAGA) [9]

RESULTS:

The results indicated that the three loci were in Hardy-Weinberg Equilibrium [13]. Table 2 shows the observed allele frequencies for the three STR loci of the population sample in NCD. Five alleles (allele 9 – 13) were identified for D16S539, five alleles (allele 8 – 12) for D7S820 and six alleles (allele 8, 9, 11 – 14) for D13S317. New alleles or Microvariant alleles were not observed in any of the STR loci.

The most frequent allele for D16S539 was allele 11 (observed 22, frequency 0.478), for D7S820 the most frequent was allele 12 (observed 13, frequency: 0.271), and for D13S317 most frequent was allele 8 (observed 18, frequency 0.360).

The most common frequency ranged from 0.360 (D13S317) to 0.478 (D16S539).

Table 3 shows the observed genotype frequencies of the 3 STR loci (D16S539, D7S820 and D13S317) as well as the homozygosity and heterozygosity of the genotypes of the population sample in NCD. The most frequent genotypes for the loci were, for D16S539 genotypes 11-11 (frequency 0.261) and 11-12 (frequency 0.261), for D7S820 genotypes 10-11 (frequency 0.167) and 12-12 (frequency 0.167), and for D13S317 genotype 8-12 (frequency 0.280). The observed Heterozygosity was highest in D13S317 (0.880).

The statistical parameters and forensic data for the three STR loci calculated for the population sample in NCD are presented in Table 4. The matching probability ranged from 0.104 (D7S820) to 0.176 (D16S539), the power of discrimination ranged from 0.824 (D16S539) to 0.896 (D7S820), polymorphic information

content ranged from 0.62 (D16S539) to 0.73 (D7S820 and D13S317), and the power of

exclusion range from 0.322 (D7S820) to 0.755 (D13S317).

Table 2: Observed allele frequencies of three STR loci of the population sample in NCD (frequency calculated as "Observed" out of "Total alleles")

Alleles	D16S539		D7S820		D13S317	
	Observed	Frequency	Observed	Frequency	Observed	Frequency
5		0.000				
6				0.000		
7				0.000		0.000
8		0.000	11	0.229	18	0.360
9	3	0.065	2	0.042	6	0.120
10	1	0.022	11	0.229		
11	22	0.478	11	0.229	11	0.220
12	11	0.239	13	0.271	11	0.220
13	9	0.196		0.000	3	0.060
14		0.000		0.000	1	0.020
15		0.000				0.000
Total	46	1.000	48	1.000	50	1.000

Table 3: Observed genotype frequencies for the STR loci of the population sample in NCD

Genotype Frequencies (observed in bracket)			
Genotypes	**D16S539**	**D7S820**	**D13S317**
8-8		0.083 (2)	0.080 (2)
8-9		0.042 (1)	0.080 (2)
8-10		0.083 (2)	
8-11		0.083 (2)	0.160 (4)
8-12		0.082 (2)	0.280 (7)
8-13			0.040 (1)
9-11	0.087 (2)	0.042 (1)	0.120 (3)
9-12			0.040 (1)
9-13	0.043 (1)		
10-10		0.083 (2)	
10-11		0.167 (4)	
10-12		0.042 (1)	
10-13	0.043 (1)		
11-11	0.261 (6)	0.042 (1)	0.040 (1)
11-12	0.261 (6)	0.083 (2)	0.040 (1)
11-13	0.087 (2)		
11-14			0.040 (1)
12-12	0.043 (1)	0.167 (4)	
12-13	0.130 (3)		0.080 (2)
13-13	0.043 (1)		
Total samples	1.000 (23)	1.000 (24)	1.000 (25)
Homozygotes	0.348 (8)	0.375 (9)	0.120 (3)
Heterozygotes	0.652 (15)	0.625 (15)	0.880 (22)

Table 4: Statistical parameters and forensic data for the three STR loci D16S539, D7S820 and D13S317 for the population sample in NCD

Statistical Parameters	D16S539	D7S820	D13S317
Probability of Match (PM)	0.176	0.104	0.146
Power of Exclusion (PE)	0.358	0.322	0.755
Power of Discrimination (PD)	0.824	0.896	0.854
Polymorphic Information Content (PIC)	0.62	0.73	0.73
Heterozygosity observed (Ho)	0.652	0.625	0.880
Typical Paternity Index (TPI)	1.44	1.33	4.17
Combined PD	0.9973		
Combined PE	0.9663		

DISCUSSION:

The combined allele distribution for the three STR loci in the present study ranged from 8 to 14, which is within the range (7 – 14) reported in other populations [4 – 6]. Allele 11 was the most common in D16S539, which support similar findings for other populations [4 – 6]. The observed allele range for locus D16S539 was from 9 to 13 (5 alleles), compared to allele range of 8 to 14 (7 alleles) reported for population in Banladeshi [4], Marmara region of Turkey [5] and Southern Italy [6].

The observed allele range for locus D7S820 was from 8 to 12 (5 alleles), compared to allele range of 7 to 13 (7 alleles) reported for population in Banladeshi [4], 7 to 14 (8 alleles) reported for Marmara region of Turkey [5] and 7 to 14 (8 alleles) for Southern Italy [6].

For locus D13S317 the observed allele range was 8, 9, 11 – 14 (6 alleles), compared to allele range of 7 to 14 (8 alleles) reported for population in Banladeshi [4], 8 to 14 (7 alleles) for Marmara region of Turkey [5] and 8 – 15 (8 alleles) for Southern Italy [6].

PIC values for the three loci were highly informative (PIC > 0.5). For forensic analysis the higher the PD of a locus, the more efficient it is in discriminating between members of the population [2 – 6]. The PD obtained for each locus can distinguish samples from different individuals with a probability or efficiency of 82.4% (D16S539), 89.6% (D7S820) and 85.4% (D13S317). The calculated combined PM for the three loci was 2.67 x 10-3, thus the combined PD was 0.99733. This implies that when used in combination, these loci can distinguish between samples from different individuals with an increased probability of 99.73 %.

Although the individual PE value for each of the locus is low, the calculated combined PE value (0.9363) is high, which indicates high degree of exclusionary power.

In conclusion, the data indicate that the three STR loci show high discriminating power, which suggests that they are useful for identity testing, forensics and for solving paternity cases among the population in NCD.

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CASE REPORT

NEED FOR HISTOPATHOLOGICAL EXAMINATION OF ODONTOMAS

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NEED FOR HISTOPATHOLOGICAL EXAMINATION OF ODONTOMAS

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ABSTRACT:

Odontomas are benign hamartomas which are often detected on routine radiographic examinations for other dental complaints. They are usually surgically removed but are rarely examined histopathologically. We report two cases with identical radiological features but different histopathological outcome, thus stressing the importance of histopathological examination of odontoma.

Key words: Odontoma, Compound odontoma, Ameloblastic fibro-odontoma, Radiological features, Histopathology.

INTRODUCTION:

Odontomas are the most common of the odontogenic tumors of the jaws. They are mixed tumors, consisting of both epithelial and mesenchymal cells that present a complete dental tissue differentiation (enamel, dentin, cementum and pulp) [1]. Odontomas are the most commonly encountered odontogenic tumors which account for 22% of all odontogenic tumors of the jaws [2]. It has been proposed that local traumas or infections may cause odontomas [2, 3]. We report two cases of odontomas, one occurring at a common site the other at an uncommon site. We also describe their histopathological features which are rarely reported.

CASE REPORT:

Case 1: A 22-year-old female patient reported to the dental clinic with complaints of irregularly

placed teeth in the front region of upper jaw. On clinical examination over-retained maxillary deciduous lateral incisor and canine were noticed. An intraoral periapical radiograph of the same region was made. The radiograph showed impacted maxillary permanent lateral and canine surrounded by normal crypt space.

A multiple radio-opaque globular mass resembling teeth were also observed obstructing the eruption of permanent lateral and canine. The radio-opaque masses were encircled by a radiolucent rim indicative of capsule (Figure 1).
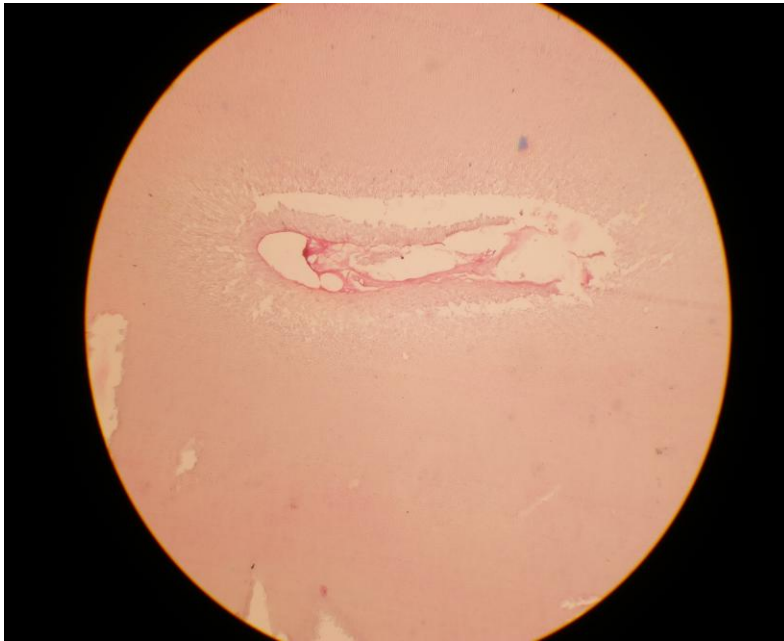
Figure 1: Intraoral periapical radiograph showing multiple teeth like radio-opaque masses and an impacted canine and lateral incisor.
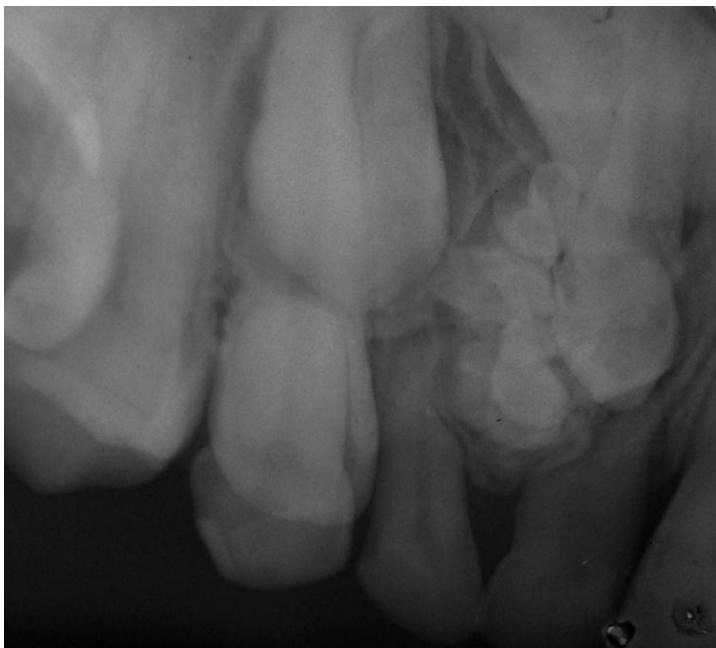
Based on the radiological findings and the site of occurrence a provisional diagnosis of compound odontoma was made. The deciduous lateral incisor and canine were extracted under local anesthetic and the

odontomas were excavated surgically. The surgical specimen was evaluated histopathologically. It revealed presence of calcified mass resembling dentine and a well differentiated pulp chamber (Figure 2).

Figure 2: Histopathological view showing presence of calcified mass resembling dentine and a well differentiated pulp chamber.

Case 2: A 27-year-old male patient reported to us with complaints of decayed tooth in the left back region of lower jaw since 6 months. The patient was clinically examined and an intraoral periapical radiograph was made. The radiograph showed grossly decayed lower first molar with inter-radicular radiolucency and periapical radio-opaque globular masses (Figure 3).

Based on the radiological findings and the site of occurrence a provisional diagnosis of complex odontoma was made. The molar was extracted under local anesthetic and the odontomas were excavated surgically. The post surgical intraoral periapical radiograph was made (Figure 4).

Histopathological examination revealed presence of calcified structures resembling dentine and abundant fibrous tissue interspersed with ameloblast-like epithelial islands (Figure 5). Based on these histopathological findings a final diagnosis of ameloblastic fibro-odontoma was made.

Figure 3: Intra oral periapical radiograph showing inter-radicular radiolucency and periapical radio-opaque globules.
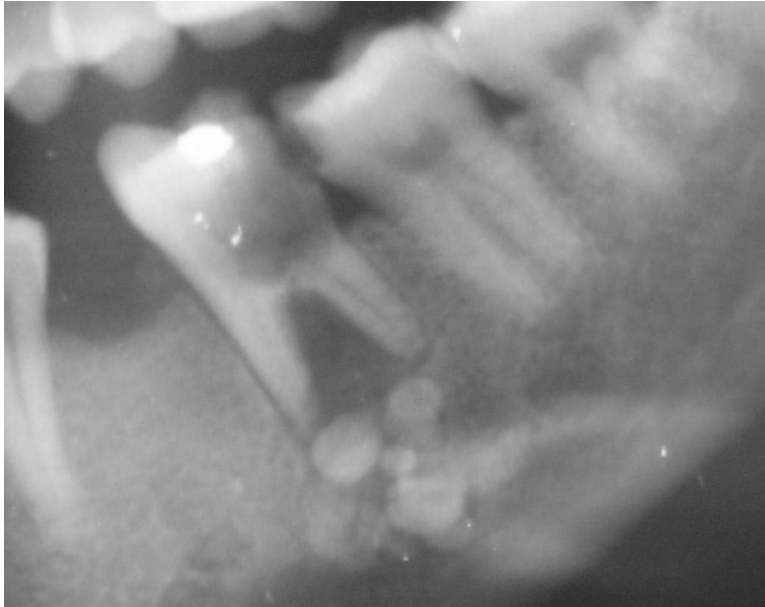

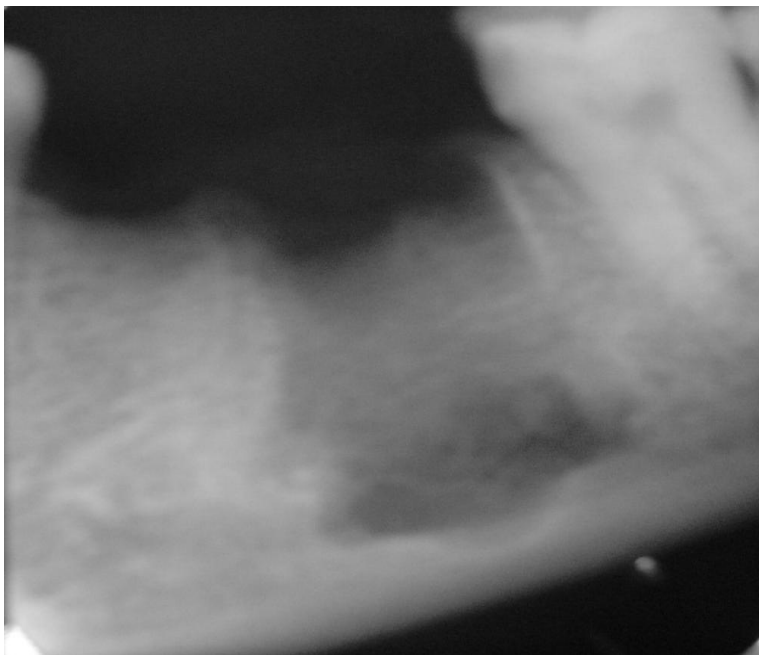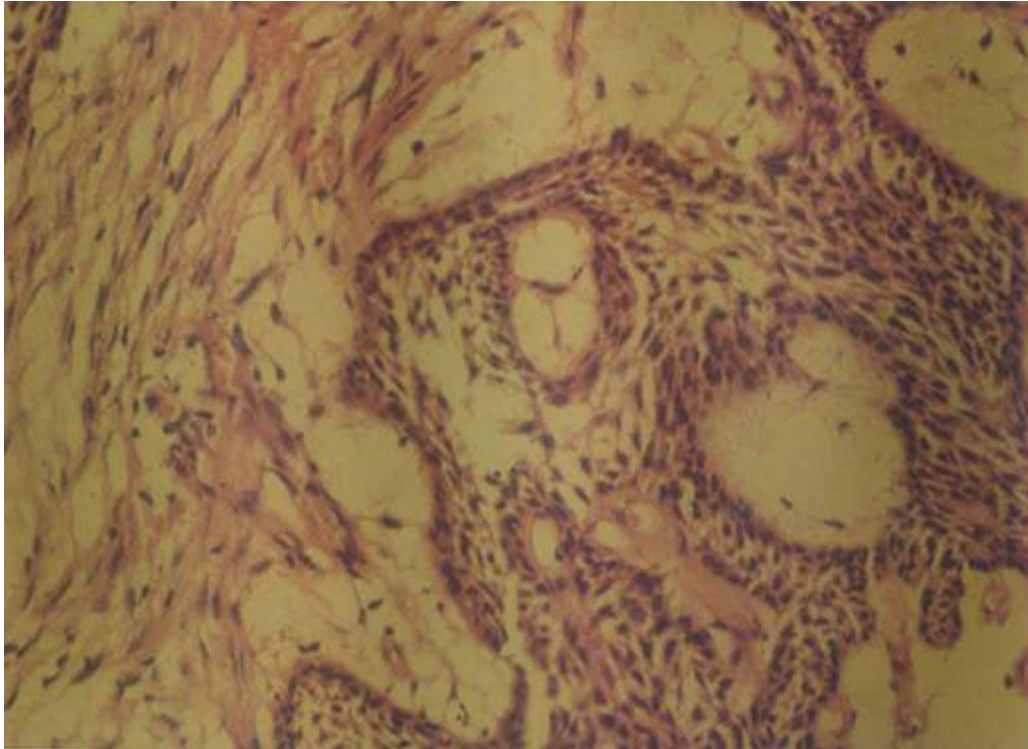Figure 4: Intra oral periapical radiograph showing post surgical site

Figure 5: Histopathological view showing presence of abundant fibrous tissue interspersed with ameloblast-like epithelial islands

DISCUSSION:

The term odontoma was first used in 1867 by Paul Broca. He defined the term odontoma as 'tumors formed by the overgrowth of transitory or complete dental tissues' [4]. Odontomas comprise of 22% of the odontogenic tumors occurring in the jaw [5]. Odontomas have no gender predilection and usually occur during the second decade of life [6]. Compound odontomas usually occur in the anterior maxilla more frequently on the right side [7]. The ameloblastic fibro-odontoma (AFO) is defined by WHO as a neoplasm composed of proliferating odontogenic epithelium embedded in a cellular ectomesenchymal tissue that resembles dental papilla, and with varying degrees of inductive change and dental hard tissue formation [8]. AFO is seen more commonly in males [9]. In our report odontoma was observed in female patient and AFO was seen in male patient. The common site of occurrence of AFO is mandibular posterior region [10]. Similar site of occurrence was observed in our case.

AFO and odonto-ameloblastomas show a great resemblance to common odontomas, especially in the radiographic examination. Therefore, it has been suggested that all

specimens should be subjected to histopathological examination [11].

In both cases the patients presented with similar radiographic features, however histopathological evaluation of the lesion was used to distinguish between odontoma and AFO. The relationship between odontoma and AFO was described by Philipsen et al [12]. They also stated that both these tumors develop along two separate lines, neoplastic and hamartomatous.

CONCLUSION:

We presented two cases with identical radiological features but different histopathological outcome. Our findings suggest the need for careful histopathological review of all surgically excised odontomas, to rule out the presence of tumor tissue.

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