SCREENING ANTIMALARIAL DRUGS FOR MODULATION OF AGGRESSIVE BEHAVIOUR IN *Drosophila melanogaster*

BY

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DECLARATION

To the best of my knowledge, I hereby declare that this research dissertation has not been submitted in full or in part to any other institution for any purpose. And that the views here on are my own and those of my supervisor, unless stated otherwise, and where such has been the case, acknowledgement or reference has been quoted.

Signed:----------

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Date: 1st November 2016

BPH/0008/123/14

APPROVAL

This research ~~proposal~~ Report has been submitted for consideration with my approval as the candidate's supervisor

---------- Date:--1st November 2016----------

PROF ADEDEJI AHMED

Supervisor

DEDICATION

This work is dedicated to my parents, my brothers (Savio, Martin and Tony), my sister Dorah and friends for their financial, emotional and academic support.

ACKNOLWEDEMENTS

I wish to acknowledge the invaluable guidance rendered to me by my supervisor, Prof. ADEDEJI Ahmed for his ideas, directions, patience to discuss and reading through this work, constructive criticism and suggestions.

I extend my gratitude to the Dean, School of Pharmacy for allocating us for research training with teachers who are willing to take us through, other staff members for constructive suggestion during our presentation of research plans, my classmates with whom I share progress, the pharmacy students' body and other colleagues who have contributed in a way to help in the proposal development.

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I wish to express my gratitude to my parents, brothers and sister who gave the financial support towards accomplishing this research.

Above all, I would like to thank the Almighty God for his guidance, provision and mercies throughout my life, and for helping me to get this point.

LIST OF ACRONYMS

IBR:	Institute of Biomedical Research
ACTS:	Artemisinin Based Combination Therapy
CNS:	Central Nervous System
WHO:	World Health Organization
GABA:	Gama Amino butyric acid
ANOVA:	Analysis of variance
cm:	Centimeters
ml:	Mililiters
mg:	Miligrams
Kg:	Kilogram

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ABSTRACT

Background: Aggressive behavior is crucial to survival and reproduction in organisms and this can be affected by environmental factors. Antimalarial are commonly used in medical practice, and it's possible that they would interact with modulators of aggression to influence behavior since some have been shown to cause neuro-damage. **Objective**: This was to evaluate common antimalarial drugs for modulation of aggressive behavior in Drosophila melanogaster model. **Materials and Methods**: Drosophila melanogaster specie (W118) was used in this study. Flies were divided into male and female and experiments were conducted on adult, middle and juvenile age groups. Flies were exposed to chloroquine (0.0025 mg/ml), quinine (0.000135 mg/ml), Fansidar (0.0025 mg/ml), Artesunate (0.0003 mg/ml), and Artemether lumefantrine (0.0003 mg/ml). These were later on exposed to neurotransmitter modulators i.e. octopamine (stimulatory-clonidine (1 mg/ml); inhibitory-promethazine (0.00025 mg/ml)), dopamine (stimulatory-L-dopa (0.001 mg/ml); inhibitor-haloperidol (0.0001 mg/ml), serotonin (stimulatory-fluoxetine (0.0002 mg/ml), inhibitory-cyproheptadine (0.00004 mg/ml)). Data was recorded in triplicate and analyzed in MS Excel. Information was presented in mean ± SEM and significance at 95% was considered. **Results and Discussion**: The study showed that Artesunate had the highest effects of aggression in male Drosophila melanogaster flies while Quinine and Chloroquine were associated with low effects and Artemether lumefantrine was associated with low level of aggression in female flies. These observations would have been due to their interaction with neurotransmitter release which is essential for aggressive behavior. Fansidar and Artemether-lumefantrine acted synergistic to octopaminergic stimulation in both males and females respectively. Artesunate antagonized actions of promethazine by leading to increased aggression especially in male flies. Fansidar and Artemether-lumefantrine acted synergistic to dopaminergic stimulation while Artesunate antagonized dopaminergic inhibition, showing that it plays a crucial role in aggression. Serotonin leads to decreased aggression and Fansidar showed antagonist activity in males while in females this was in Artemether Lumefantrine. Artesunate showed strong inhibitory activity on serotonin release, thus leading to increased aggression. In the age groups, aggression by Artesunate was highest in adult male and female flies and this raises major pharmaceutical concerns. In the juveniles, Fansidar and Artesunate showed significant aggression, thus showing implications in neural development. **Conclusion and Recommendation**: Molecular mechanism on actions of Artesunate and Fansidar on modulation of neurotransmitter release need to be investigated further to gain much clear insight.

CHAPTER ONE

1.0 INTRODUCTION.

1.1 Background

Aggressive behavior is widely present throughout the animal kingdom and is crucial to ensure survival and reproduction (Dierick & Greenspan, 2006). Aggressive actions are used to acquire territory, food or mates and in defense of the individual against predators (Adams, 2001). Two subtypes of aggression have been identified in humans: the controlled instrumental subtype and the reactive impulsive subtype (Vitiello & Stoff, 1997). Reactive aggression is considered to be more impulsive (It is usually associated with anger); whereas instrumental aggression is considered to be more purposeful and goal oriented. However, when a stressor e.g. drug, disease etc. is applied, it is not clear which one of the two subtype's human being would express.

Aggressive behavior may be modulated by a broad range of genetic and environmental factors e.g. drugs, gene mutations, climatic changes etc. Many of these factors have been studied on a variety of species but; the pathways by which they modulate that behavior are largely unknown (De Almeida, Ferrari, Parmigiani, & Miczek, 2005; Vitaro et al., 2015).

Human subjects are exposed to many factors. Given the situation around, drug is one of these factors and among those that the African population is exposed to frequently is antimalarial. A preliminary study in our laboratory that examined antimalarial drugs and behavioral effects showed some of the antimalarial presenting patterns suggestive of aggressive behavior. Therefore, it is the aim of this study to screen antimalarial drugs for modulation of aggressive behavior in drosophila melanogaster fly model.

Drosophila melanogaster, the fruit fly, has been a model organism for study of genetics in the 18th century (Milinkeviciute, Gentile, & Neely, 2012). Continuous works on the fly revealed that, although it has 400milliom years divergence from human, it conserves homologue genes with human up to 50% and has estimated 75% of disease related genes in humans with functional orthologs (Bier, 2005). Also, Drosophila, with its advanced set of molecular tools and its behavioral richness, has the potential to develop into a new model organism for the study of the neurobiology of aggression (Baier, Wittek, & Brembs, 2002). In addition, Drosophila melanogaster is a cheap animal model, easy to maintain and avails genetic manipulation techniques easy application (Liu et al., 2012) .

1.2 Problem statement

Inappropriate behavior as a result of poor judgment in our society today is a reflection of a person's mental state. Decisions and actions could be affected by stressor effect on the brain. Drugs despite use for alteration of disease condition interact with the body putting some strain on the system. Antimalarial drugs including Quinine, Artesunate, and Chloroqine etc. are a set of drugs frequently used in malaria endemic region that can modulate neurotransmitters in the body. These effects can include suppression or stimulation of the neurotransmitters; serotonin, dopamine, resulting in various aggressive behaviors. The behavioral modifications reported for Mefloquine including; restlessness, hallucinations, confusion, delirium, psychosis, violence and suicide attempts (Toovey, 2009), are suggestive of antimalarial drug action in this respect. Artemisinin based combination therapy (ACTS) which are most effective and commonly used antimalarial drugs have been associated with brain injury in laboratory animals (Cui & Su, 2009) and may ultimately influence the behavior that will present if same occur in human. These behavioral modifications may be due to modulation of aggression by antimalarial drugs.

1.3 Objectives.

1.3.1 Broad objective

1. To evaluate common antimalarial drugs for modulation of aggressive behavior in *Drosophila melanogaster* model.

1.3.2 Specific objectives.

1. To determine aggression behavioral changes in flies treated with antimalarial drugs.
2. To assess activities of the antimalarials on mediators of aggression.
3. To assess the relationship between age and antimalarial drugs induced aggressive behavior in treated flies.

1.4 Research questions/ Hypothesis

1. Do antimalarial drugs promote aggression behavior in drosophila melanogaster?
2. Does an antimalarial interfere with neurotransmitter path responsible for aggressive behavior?
3. Could these behaviors be age dependent?

1.5 Justification of the study

Aggression is a necessary phenomenon for survival and reproduction and may be positive or negative.

Several factors may contribute. The role played by drugs in this perspective and whether or not antimalarial contributes to it is not clear.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aggression

Aggressive behavior is widely present throughout the animal kingdom and is crucial to ensure survival and reproduction (Dierick & Greenspan, 2006). Aggressive actions are used to acquire territory, food, or mates and in defense of the individual against predators (Adams, 2001). Two sub types of aggression have been identified in humans; the controlled instrumental sub type and the reactive impulsive subtype (Vitiello & Stoff, 1997). Reactive aggression is considered to be more impulsive (it is usually associated with anger) whereas instrumental aggression is considered to be more purposeful and goal oriented.

Aggressive behavior may be modulated by a broad range of genetic, biologic and environmental factors. Many of these factors such as neurotransmitters, hormones, pheromones, sex and individual anatomical differences have been studied in a variety of species but, the pathways by which they modulate that behavior are largely unknown (De Almeida et al., 2005; Vitaro et al., 2015).

The aggression map is a complex interaction between genes, biological signals, neural circuits and the environment that influence the development and expression of aggressive behavior, (Nelson & Trainor, 2007). In the aforementioned, the relationship between biological signals and aggression is of great concern. Therefore, an establishment of this relationship will provide a better understanding of the contribution of biological signals to aggression in humans.

2.2 Biological signals and aggression

The evaluation of biological signaling molecules has provided additional clues about the neural circuits that are involved in complex social behaviors. Some of the important signaling molecules that have been linked to aggression include serotonin, dopamine, B-alanine, Gama amino butyric acid (GABA), monoamine Oxidase and nora-adrenaline, (Nelson, 2005) .

Specifically, serotonin hypo function may represent a biochemical trait that predisposes individuals to impulsive aggression, with dopamine hyper function contributing an additive fashion to the serotonergic deficit, (Seo, Patrick, & Kennealy, 2008). Disruption of the serotonergic system is a highly significant feature in predisposing aggression, (Alekseyenko et al., 2014). Generally, low 5-HT levels are associated with higher levels of impulsivity and aggressiveness, (Dolan, Anderson, & Deakin, 2001) and manipulations that lower 5-HT signals increase impulsivity and aggression, (Lesch & Merschdorf, 2000).

The role of dopamine in aggression has been elucidated in animal experiments. Animals can be conditioned to increase dopamine secretion in anticipation of aggressive interactions,

(Sasaki-Adams & Kelley, 2001) which suggests a connection with instrumental aggression. Antagonists of both the D_1 and D_2 receptors reduce aggression in male mice (Risbrough et al., 2006).

Drugs amongst other factors underlying aggression is another concern. Several types of drugs alter human behavior or mood and they include stimulants that excite the central nervous system (e.g. cocaine and amphetamine), depressants that induce progressive depression of the CNS , (for example alcohol and barbiturates), opiates that have morphine like reactions (e.g. morphine and codeine), Hallucinogens/Psychedelics that cause distortions in perception, cognition and mood (e.g. lysergic acid diethylamide (LSD) and mescaline and Marijuana), are examples (Pfaus et al., 2010) . The aforementioned drugs alter human behavior or mood in a short time period. Of kin interest are drugs that may alter human behavior in a long time period due to prolonged use. Antimalarial drugs, to which many in malaria disease endemic regions are exposed, are one of many possible candidates. Delving into the role of antimalarial drugs in eliciting aggression will

avail us with an evidence based understanding of the modulatory mechanisms of antimalarial drugs that result in aggression in humans.

2.3 Anti-malarias and aggression

The most prominent neuropsychiatric effects identified during the development of Mefloquine including vertigo, initially resembled those of cinchonism induced by quinine, (Janowsky et al., 2014). Amodiaquine which is also one of the anti-malarial has CNS side effects though they are less or milder than those from chloroquine therapy, (Nevill et al., 1994) . A study done by (R.C. et al., 2000) showed that chloroquine caused Parkinson's syndrome in a 5 year old and these symptoms rapidly declined when the drug was withdrawn. Plasmodium falciparum chloroquine resistance is a major cause of worldwide increases in malaria mortality and morbidity, (Sidhu, Verdier-Pinard, & Fidock, 2002). Psychiatric effects caused by Mefloquine include; anxiety, hallucinations, depression, unusual behavior and suicidal ideations, among others. Neurologic effects include dizziness, loss of balance, and tinnitus, bad dreams, anxiety,(Tran, Browning, & Dell, 2006). Gap junction channels, composed of proteins called connexins are involved in co-ordinated synchronization of neuronal activity, particularly of inhibitory interneurons found throughout the limbic system. At concentrations consistent with limbic accumulation, Mefloquine has been demonstrated to inhibit electrical coupling of neurons with effects on limbic inhibition and resultant mesolimbic dopaminergic tone, (Yamamoto & Suzuki, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 Area of study

This research took place in the institute of Biomedical research laboratory of Kampala International University, located in Ishaka, Bushenyi District.

3.2 Study design

The study design was experimental with control positive (P), Control negative (N) and the experimental group (D).

3.3 Materials.

3.3.1 Fly starch.

The wild (W1118) white strain *Drosophila melanogaster* was used for the study 12-hour dark cycle at room temperature prior to the experiment.

3.3.2 Chemicals and reagents:

Reagents included; Agar, yeast, wheat flour, apple juice media, water, glucose, nipagin, propionic acid, ethanol and ether. Chemicals included; Levodopa, haloperidol, clonidine, promethazine, fluoxetine and cyproheptadine.

3.3.3 Preparation of fly food.

Ingredients as shown above were dissolved in 1 liter of water and boiled extensively on a hot plate until all ingredients were dissolved. Propionic acid (a mold inhibitor) was added. The media was paired into 175ml bottles and it was allowed to solidify. A large drop of live baker's

yeast was added on the surface of the medium in each bottle. Each of the bottles was plugged with cotton wool.

3.3.4 Drugs:

Chloroquine, Quinine, Fansidar, Artesunate and Artemether /Lumefantrine.

3.3.5 Equipment.

Digital camera, brush, Petri dishes, microscope, plastic transparent vials, funnel, volumetric flasks, measuring cylinders, incubator. Refrigerators, micro pipettes, test tubes, stop clock, chromatographic paper, cotton wool, graduated tubes (30cm) long, thermometer and dark chamber.

3.4.0 Methods

3.4.1 Fly preparation for experiment.

Virgin flies raised in the lab in culture bottles were transferred to empty bottles 12 hours before the experiment.

A cotton plug estimated to be of the same size as the bottle neck was soaked with ether. The bottle containing the flies was gently tapped on the table such that the flies fell to the bottom and the cotton plug was quickly replaced by a plug with ether.

The cotton plug was removed soon after all flies were anesthetized. Using a microscope the flies were sorted according to sex, (male and female).

Flies of the same sex were placed in a vial using a brush with soft bristles to avoid injuring the flies.

Vials containing female flies were labeled F and those containing males were labeled M. For each experimental set up, a group of 8 vials each containing four vials with male flies and four vials with female flies were made for each of the 3 experimental setups, i.e., the control positive (P), control Negative (N) and the experimental group (D).The flies were starved for about 12 to 15 hours according to, (Dus, Min, Keene, Lee, & Suh, 2011).

3.4.3 Drug administration

A serial dilution to make concentration of the drug that is equivalent to the dose taken by human beings was made. Using a filter proper, a specific calculated amount of the drug was dispensed onto the filter paper ensuring that it is adequately wetted.

The starved flies were introduced into a vial containing the drug on filter paper. The flies were allowed to feed on the drug for 30-45 minutes.
Flies were observed directly using a camera, dish and computer for phenotypic aggression parameters in the first five days following treatment; at age 21-25 days and age 40-45 days in both sexes.

The parameters to be scored according to, (Zwarts, Versteven, & Callaerts, 2012) included; retreat, approach, wing threat, lunge, shove, thrust with a wing threat, head butt, fencing, chasing, holding, tussling and boxing where;

- ✓ Retreat refers to walking, flying or running away
- ✓ Approach refers to turning or walking toward the opponent
- ✓ Wing threat refers to raising one or both wings to a 45-90° angle toward opponent (< 1 min)
- ✓ Lunge refers to rearing up on hind legs and collapsing on the opponent
- ✓ Shove means thrusting torso towards the opponent with both legs extended without recoil
- ✓ Thrust with a wing means to thrust and lift one or both wings to a 45-90° angle (< 1 min)
- ✓ Head butt means to thrust the torso toward the opponent and strike the opponent with the head; usually followed by recoiling of the torso
- ✓ Fencing is to extend the leg and contact the opponent in a normal standing posture

- ✓ Chasing means to run after the opponent
- ✓ Holding is to grasp the opponent with forelegs and try to immobilize
- ✓ To tussle is to tumble over each other sometimes leaving the food surface and
- ✓ Boxing is to rear up on hind legs and strike the opponent with forelegs.

The above parameters were categorized into three groups as follows;

1. High aggression (Boxing, Tussling, Head butt, Lunge and Shove).
2. Medium aggression (Holding, Wing threat and Thrust with a wing).
3. Low aggression (Approaching, Chasing, Retreat and Fencing).

3.5.0 Pharmacological treatments.

Using a new set of flies, stimulation and inhibition of neurotransmitters serotonin, Octopamine and Dopamine was done as described by, (Baier et al., 2002).

3.5.1 for serotonin (5HT)

a. Flies were fed with 0.0002mg/ml of fluoxetine in Sucrose solution. This treatment produces high levels of serotonin ($5HT^{+}$).
b. Flies were also fed with 0.00004mg/ml Cyproheptadine in sucrose solution. This treatment produces low levels of serotonin ($5HT^{-}$)

3.5.2 for Dopamine

a. Flies were fed with 0.001mglml L-DOPA in sucrose solution. This treatment produces high levels of dopamine (DA^{+}).
b. Flies were also fed with 0.0001mg/ml Haloperidol in sucrose solution. This treatment produces low levels of dopamine (DA^{-})

3.5.3 for Octopamine

a. Flies were fed with 0.001µg/ml Clonidine in sucrose solution. This treatment produces high levels of octopamine.
b. Flies were also fed with 0.00025mg/ml Promethazine in sucrose solution. This treatment produces low levels of octopamine.

After 30-45 minutes, the flies were observed directly using a dish, camera and computer for phenotypic aggression parameters following treatment as shown above. Parameters were scored using tabulation.

3.6 Anti-malarial interference with Neurotransmitter pathways

A new set of flies was given antimalarial drug first as described in the first experiment above for 30 minutes -45 minutes. They were then allowed one hour for drug absorption to take place.

The same flies were then stimulated and inhibited for dopamine, Octopamine and serotonin as described in the second experiment above.

The interference of the antimalarial drugs with the dopaminergic, octopaminergic and serotonergic pathways was observed using the same aggression parameters as above and the mechanisms of modulation of the neurotransmitter pathways by these drugs was later discussed in the following chapters.

3.7 Expected outcomes

In this study, it was expected that anti-malarial drugs will modulate aggressive behavior in *Drosophila melanogaster*.

CHAPTER FOUR

4.0 RESEARCH RESULT FINDINGS

4.1 Behavioral changes in flies treated with antimalarials

The study showed that Artesunate had the highest effects of aggression in male *Drosophila melanogaster* flies while Quinine and Chloroquine were associated with low effects.

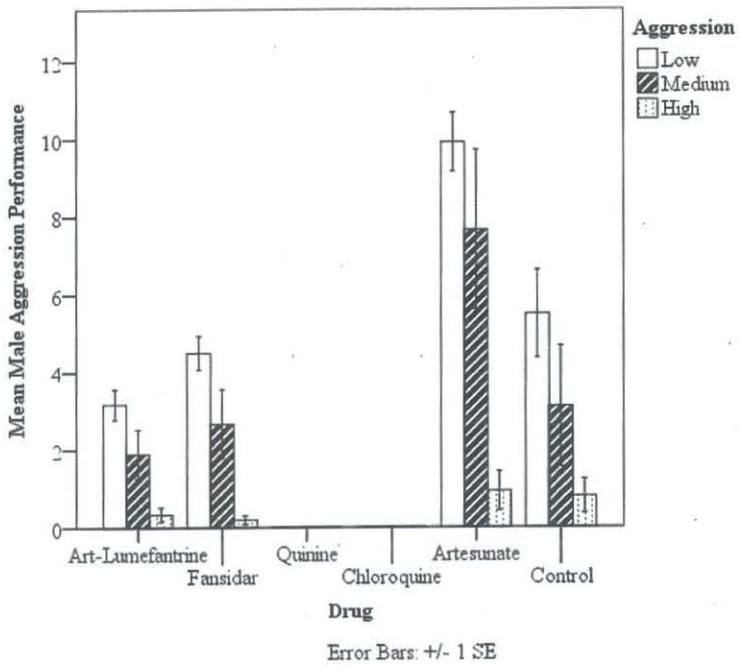


Figure 1: Showing behavior changes in male flies treated with antimalarial

Artesunate and Artemether Lumefantrine are associated with high aggression in female *Drosophila melanogaster* while moderate aggression was shown by Fansidar group.

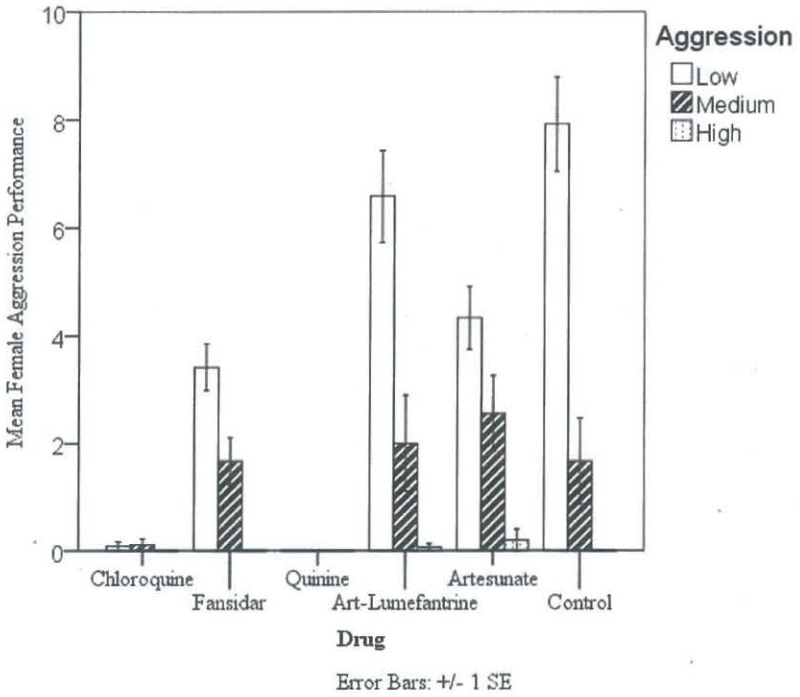


Figure 2: showing behavior changes in female flies treated with antimalarials

Drugs	Mean ± SEM Aggression performance	
	Male	Female
Art-Lumefantrine	1.67±0.5	2.72±0.42
Fansidar	2.25±0.5	1.56±0.42
Quinine	0.00±0.5*	0.00±0.42*
Chloroquine	0.00±0.5*	0.06±0.42*
Artesunate	5.61±0.5*	2.17±0.42
Control	2.94±0.5	3.06±0.42

Table 1: Showing group comparisons for behavioral changes in flies treated with antimalarials

KEY: * $P < 0.05$

The study showed an aggression performance of 1.67±0.5 and 2.72±0.42 in both males and females on Art-Lumef. Artesunate aggression was high in males than females at 5.61±0.5 and 2.17±0.42 and significant differences ($P < 0.05$) were seen in both Art-Lumef and Artesunate respectively as shown in **Table 1**.

4.2 Activities of antimalarials on mediators of aggression

4.2.1 FOR OCTOPAMINE

Action of Clonidine treatment (↑)

Artemether-lumefantrine and Artesunate showed high aggression behavior in male *drosophila melanogaster* while medium aggression was found to be highest in Fansidar group.

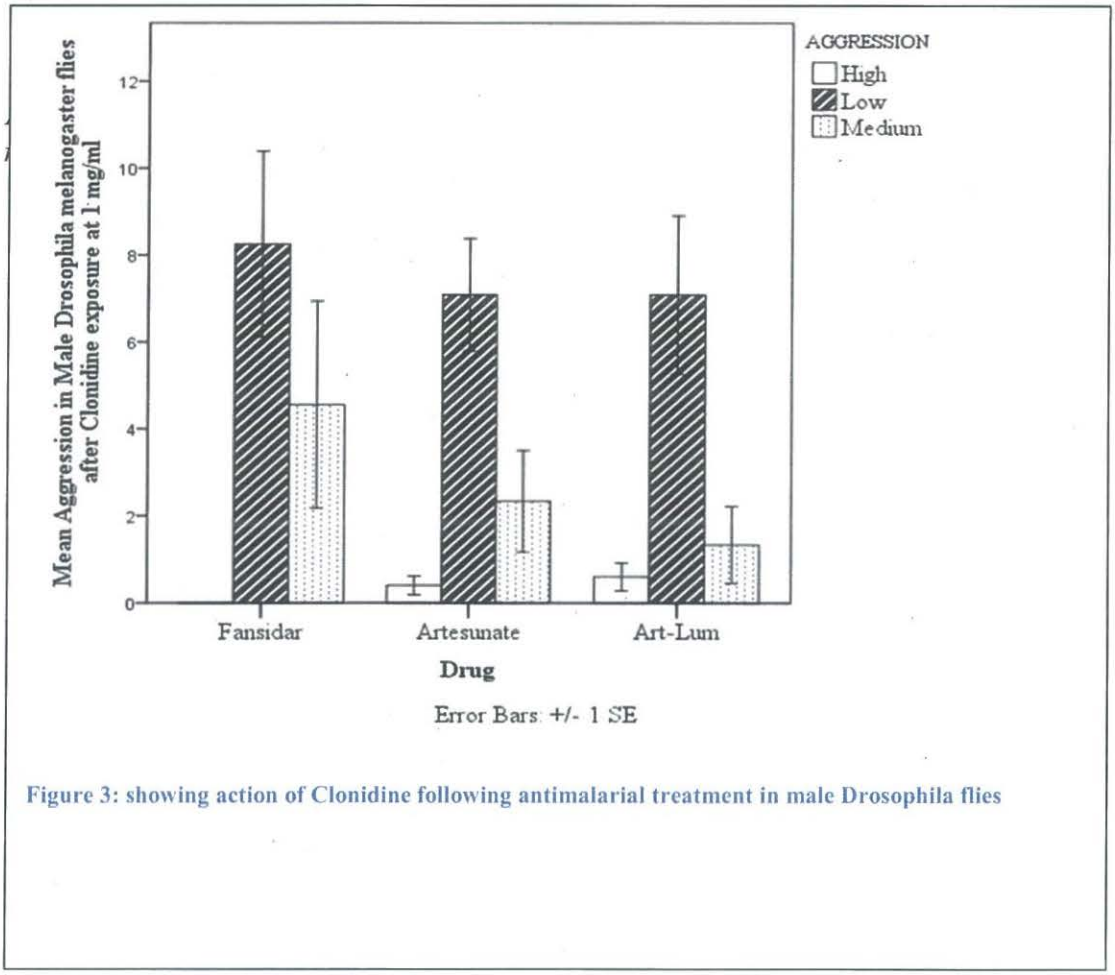


Figure 3: showing action of Clonidine following antimalarial treatment in male Drosophila flies

Artemether lumefantrine showed the highest aggression in female *Drosophila melanogaster* while Fansidar showed moderate aggression.

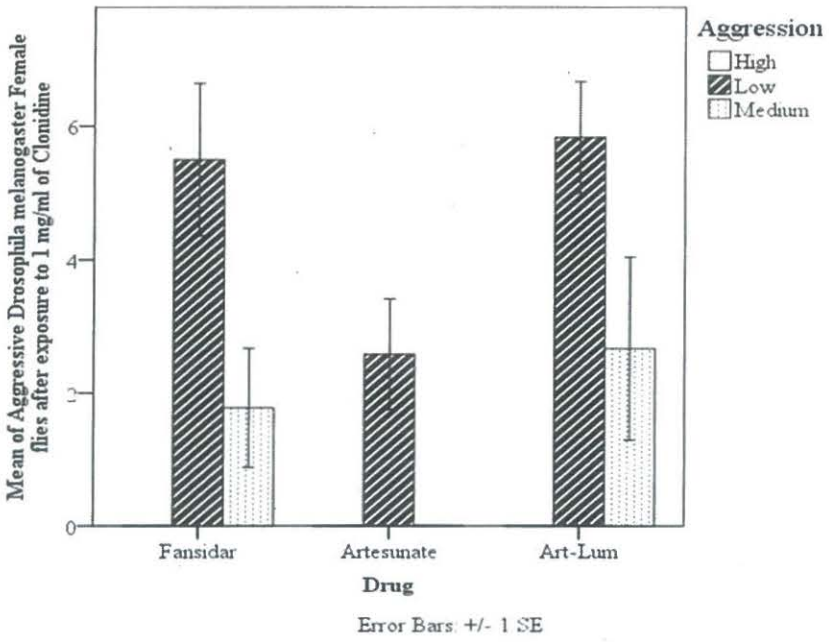


Figure 4: showing action of Clonidine following antimalarial treatment in female Drosophila flies

Action of promethazine treatment (↓)

Artesunate showed the highest aggression in male *Drosophila melanogaster* while Fansidar and Artemether lumefantrine showed no aggression at all.

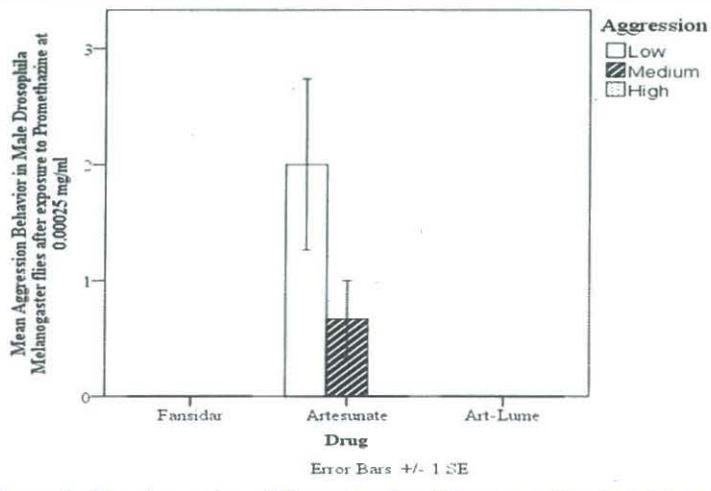


Figure 5: Showing action of Promethazine following antimalarial treatment in male Drosophila flies

KEY: Art-Lumef = Artemether Lumefantrine

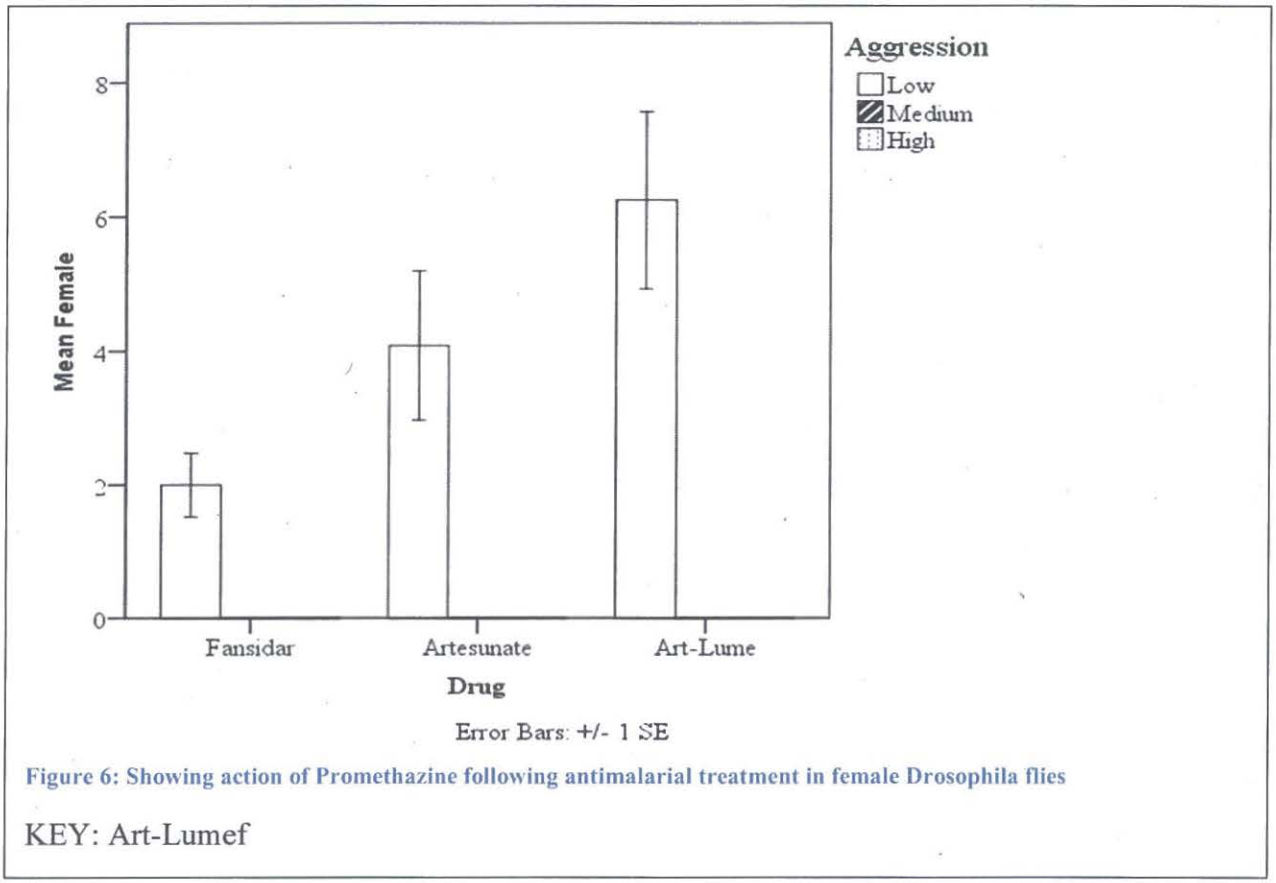


Figure 6: Showing action of Promethazine following antimalarial treatment in female Drosophila flies

KEY: Art-Lumef

4.2.2 FOR DOPAMINE

Action of L-DOPA treatment (↑)

Fansidar showed the highest aggression in male *Drosophila melanogaster* while Artesunate and Artemether lumefantrine showed moderate aggression.

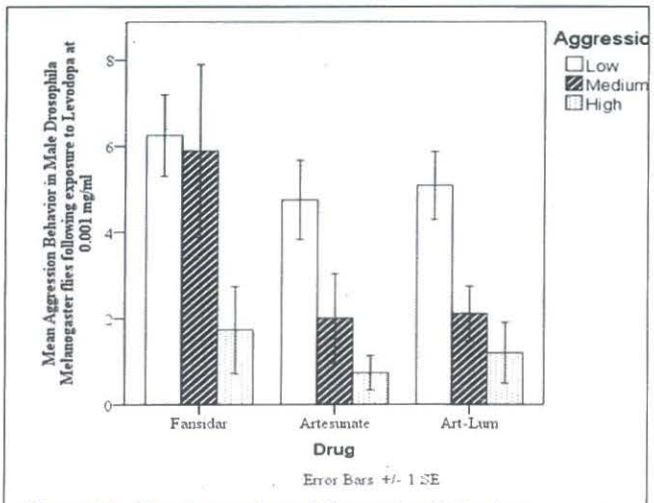


Figure 7: Showing action of Dopamine following antimalarial treatment in male Drosophila flies

Artemether Lumefantrine showed high aggression in female *Drosophila melanogaster* while aggression was found to be moderate in Fansidar group.

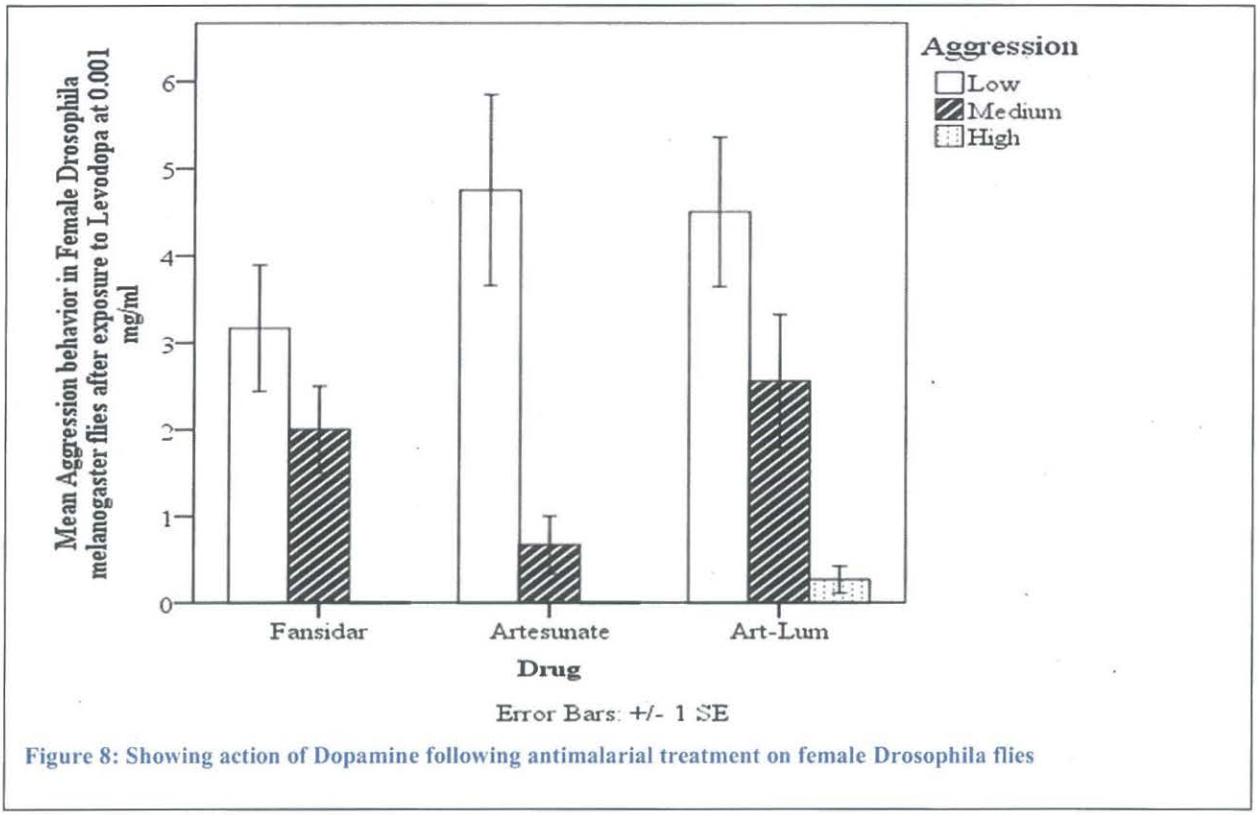


Figure 8: Showing action of Dopamine following antimalarial treatment on female Drosophila flies

Action of Haloperidol treatment (↓)

Medium aggression was found to be highest in Artemether Lumefantrine group in male *Drosophila melanogaster* while Artesunate showed moderate aggression.

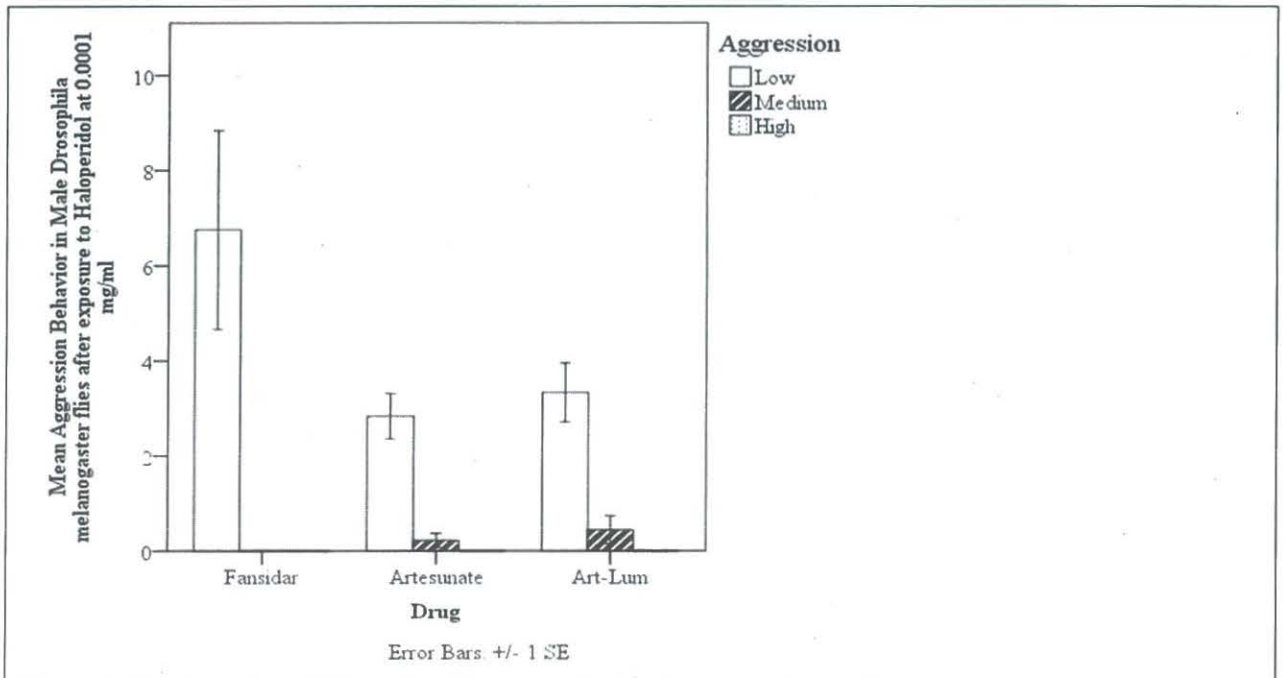


Figure 9: Showing action of Haloperidol following antimalarial treatment in male Drosophila flies

Artesunate showed medium aggression in female *Drosophila melanogaster* while low aggression was found to be highest in Fansidar group.

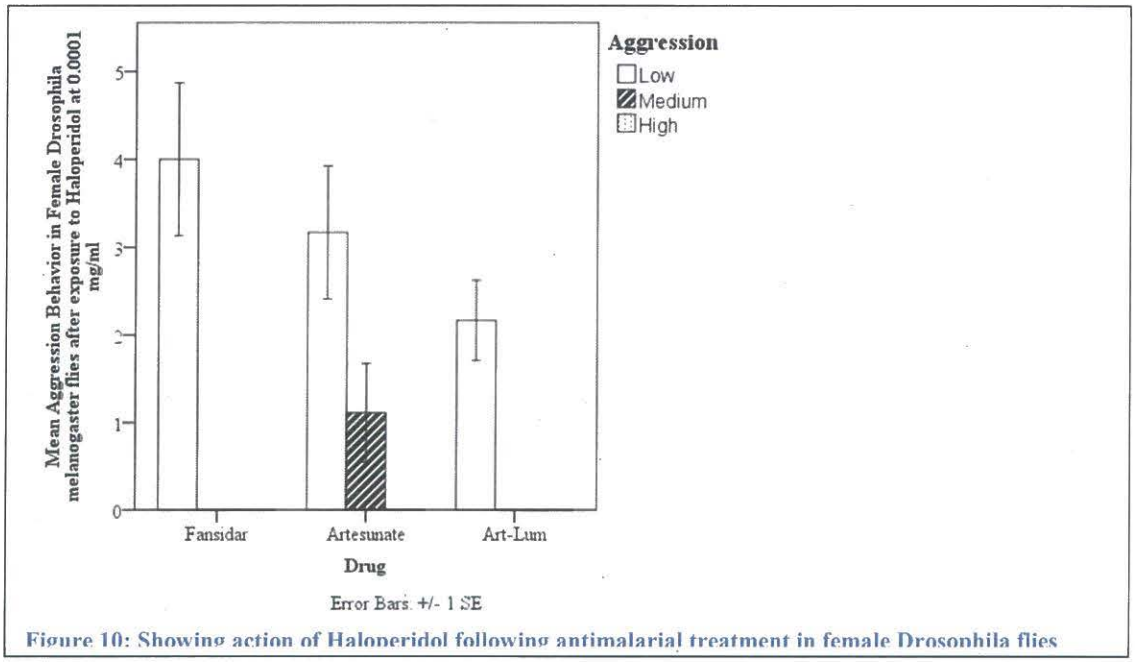


Figure 10: Showing action of Haloperidol following antimalarial treatment in female Drosophila flies

4.2.3 FOR SEROTONIN

Action of Fluoxetine treatment (↑)

Fansidar showed the highest aggression in male *Drosophila melanogaster* while moderate aggression was shown by Artesunate.

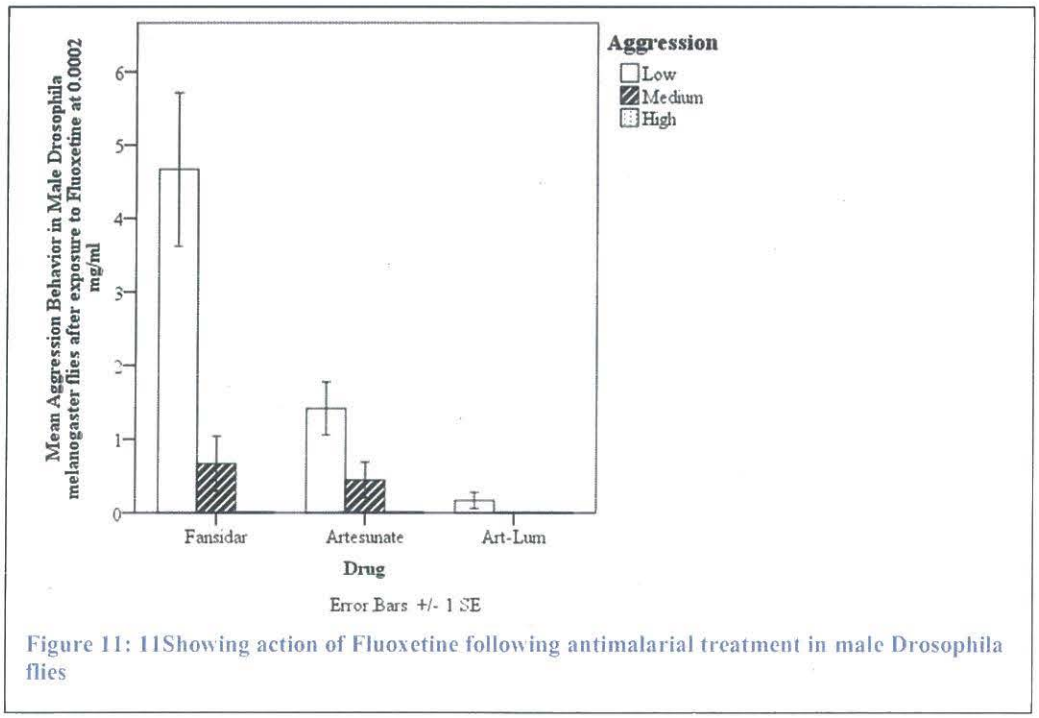


Figure 11: 11Showing action of Fluoxetine following antimalarial treatment in male Drosophila flies

Artemether Lumefantrine showed the highest aggression in female *Drosophila melanogaster* moderate aggression was shown by Artesunate.

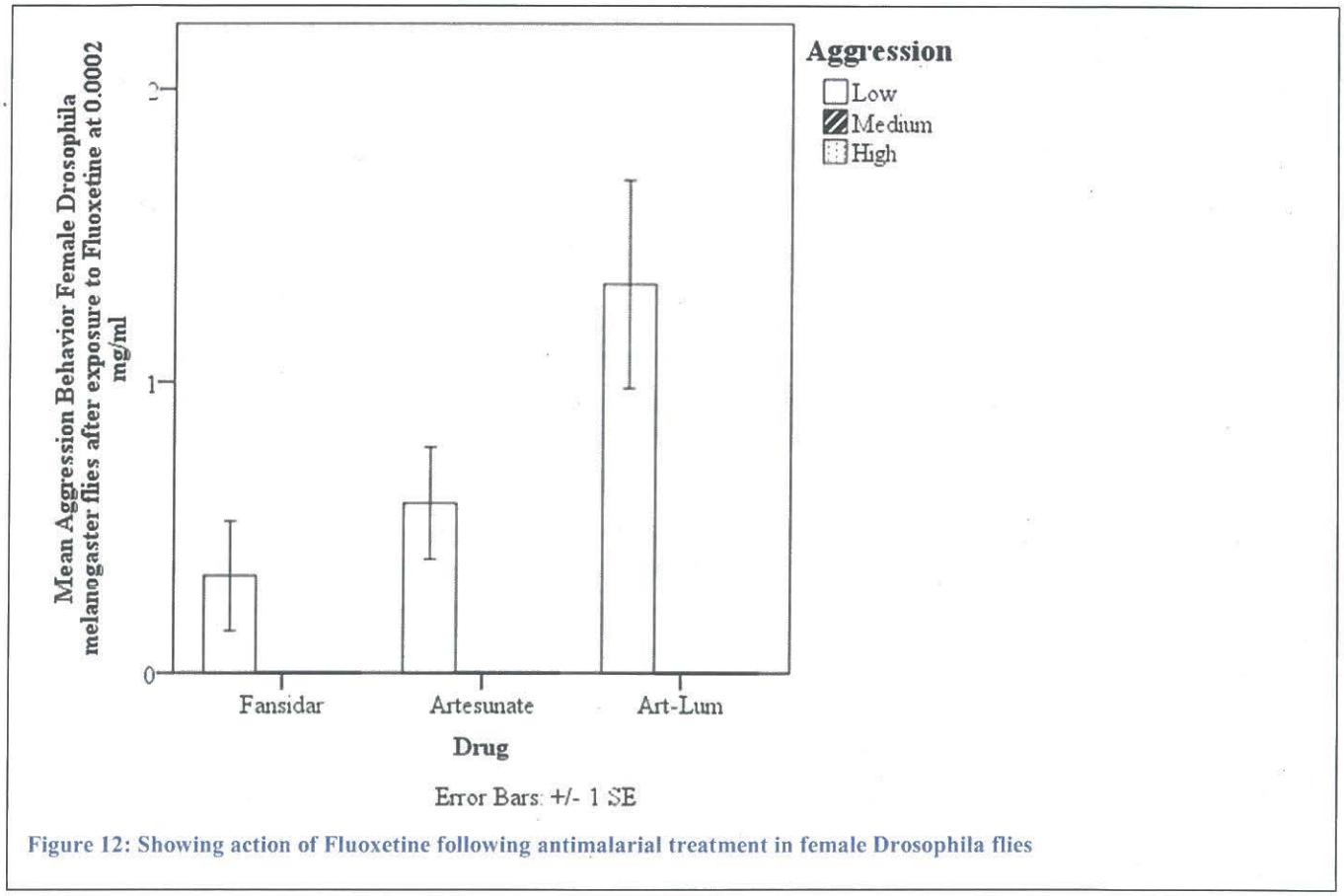
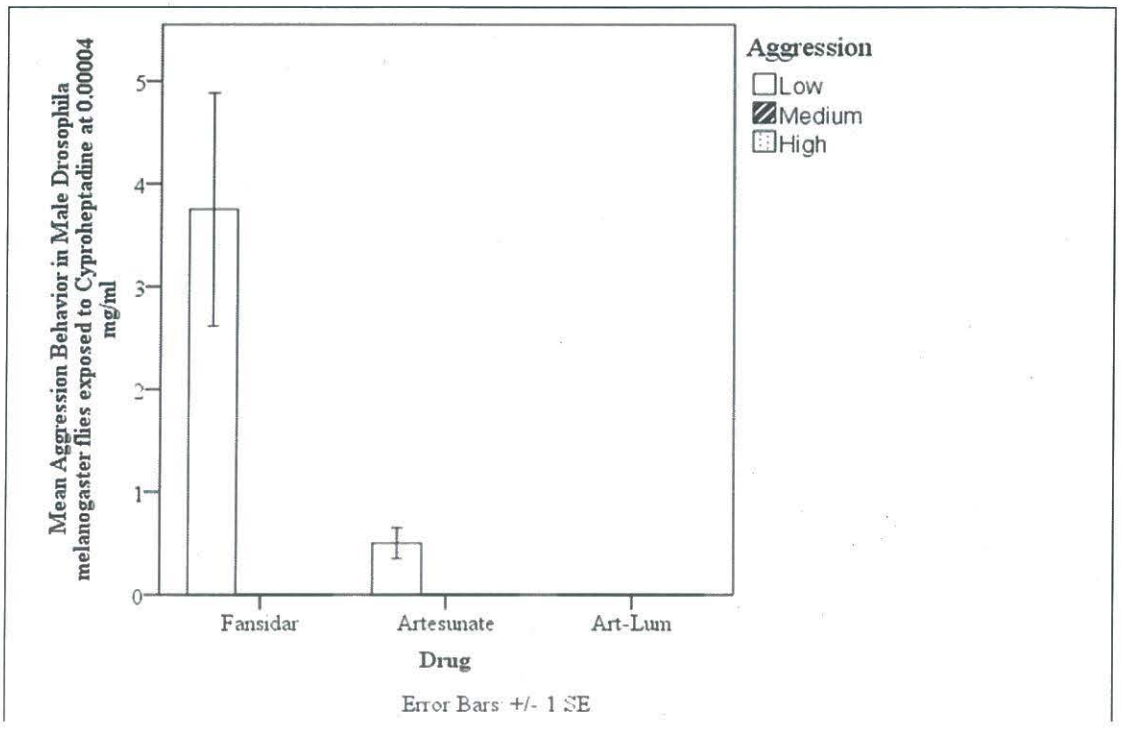


Figure 12: Showing action of Fluoxetine following antimalarial treatment in female Drosophila flies

Action of Cyproheptadine treatment (↓)

Fansidar showed the highest aggression in male *Drosophila melanogaster.*



Artesunate showed the highest medium aggression in female *Drosophila melanogaster* while low aggression was found to be highest in Fansidar group.

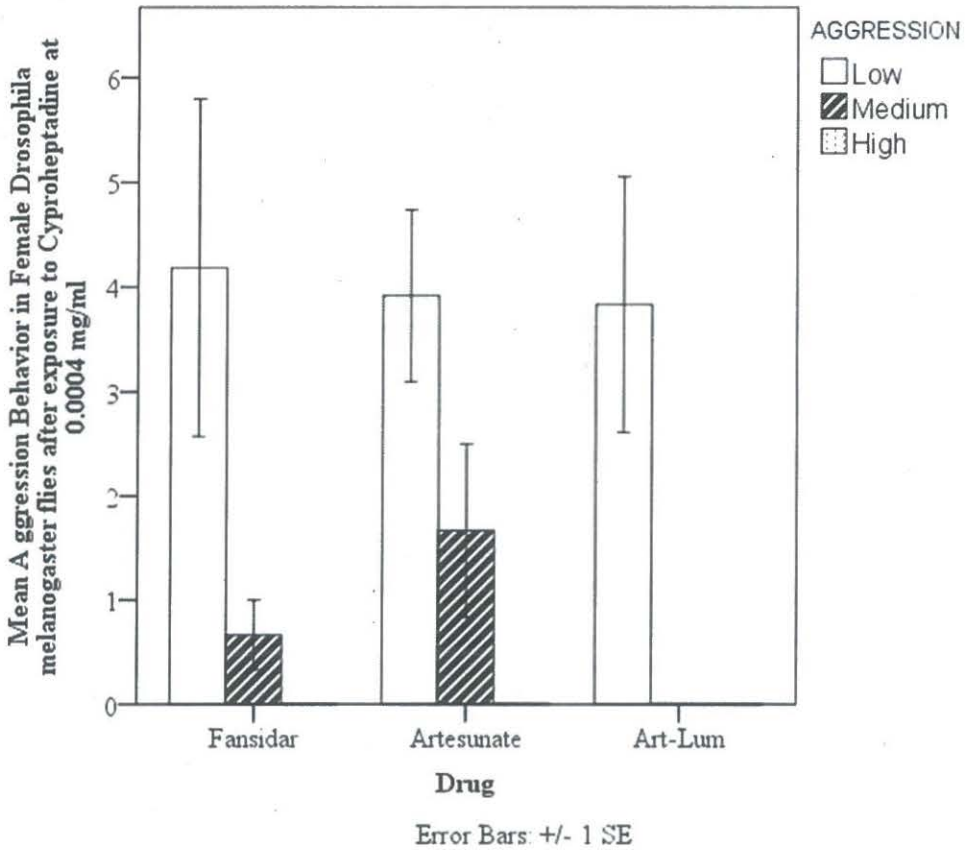


Figure 14: Showing action of Cyproheptadine following antimalarial treatment on female Drosophila flies

4.3 RELATIONSHIP BETWEEN AGE AND ANTIMALARIAL DRUG INDUCED AGGRESSIVE BEHAVIOR

Artesunate showed the highest aggression across all age groups in male *Drosophila melanogaster* with highest effects amongst 40 -45 days, while Fansidar showed moderate aggression in both 21-25 and 40-45 days.

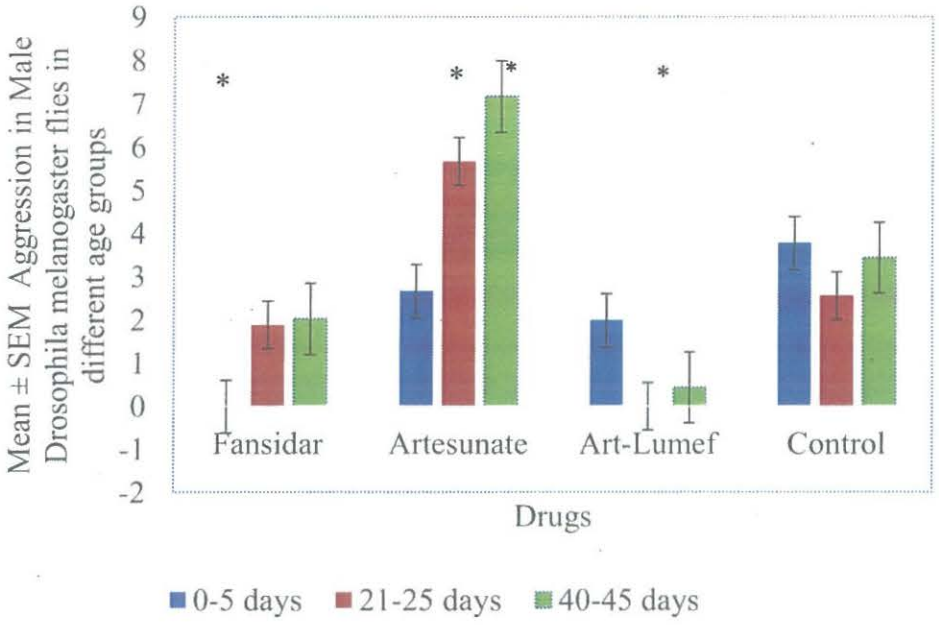
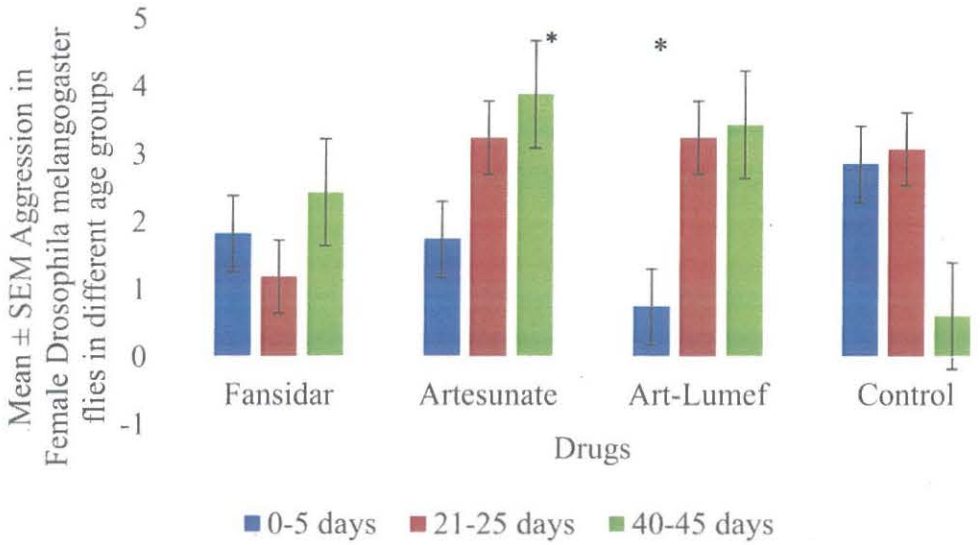


Figure 15: Showing effect of drug on age in male Drosophila flies

Artesunate showed the highest aggression for age group 40-45 days old female *Drosophila melanogaster*. Aggression for age group 21-25 in Artesunate equals that in Artemether-Lumefantrine and aggression for age group 0-5 in Fansidar equals that in Artesunate.



KEY: * Drug comparisons against the control ($P < 0.05$).

Figure 16: Showing effect of drug on age in female Drosophila flies

Drugs	Male	Female	Male	Female	Male	Female
	Mean ± SEM					
	0-5 days		21-25days		40-45days	
Fansidar	0.00±0.61*	1.83± 0.56	1.89±0.55	1.19±0.54	2.03±0.82	2.44±0.79
Artesunate	2.67±0.61	1.75±0.56	5.67±0.55*	3.25±0.54	7.17±0.82*	3.89±0.79*
Art-Lumef	2.00±0.61	0.75±0.56*	0.00±0.55*	3.25±0.54	0.44±0.82	3.44±0.79
Control	3.78±0.61	2.86±0.56	2.56±0.55	3.08±0.54	3.44±0.82	0.61±0.79

Table 2: Showing group comparisons on effect of drug on age in Drosophila flies

KEY: *In group comparisons against the control $P < 0.05$. Art-Lumef = Artemether Lumefantrine.

The group comparisons showed significant effects for Fansidar 0-5 days in male *Drosophila*, Artesunate 21-25 in male *Drosophila* and 40-45 days in both male and female *Drosophila* and Artemether lumefantrine 0-5 and 21-25 days in female and male *Drosophila* respectively as shown in **Table 2**.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Aggression in flies treated with antimalarials

The study showed that Artesunate had the highest effects of aggression in male *Drosophila melanogaster* flies while Quinine and Chloroquine were associated with low effects. Artesunate has been reported to have sedative effects, lower synchronization of movements which implies that the cerebral cortex and cerebellum of the brain play a crucial role in modulation of behavior (Zhao, 1985). In this study, Artesunate was able to increase aggression probably by increased activity of serotonin, GABA, glutamate, opioids, cholecystokinin, substance P, norepinephrine, dopamine, and acetylcholine have been shown to pay a crucial role in the modulation of aggressive behavior (Siegel, Roeling, Gregg, & Kruk, 1999). This is important since aggression in humans involves higher centers in the brain which are involved in cognitive function and this defines an individual's social world (Montagu, 1977). In humans, aggression has been shown to be dominant in both males and females depending on their background. This is because lessons learnt from previous exposure define behavior in man (Connor, Steingard, Anderson, & Melloni, 2003).

In addition, the study showed that female *Drosophila melanogaster* flies demonstrated a level of aggression which was quite different from that seen in the males. Precisely, Artemether lumefantrine was associated with low level of aggression, Artesunate moderate while Quinine didn't show any level of aggression. Furthermore, aggression was highly demonstrated in female flies (Table 1). Since Artemether-lumefantrine is actively used in the management of malaria, and has been associated with good safety level in use in the human population (Stover, King, & Robinson, 2012), it's important to re-evaluate the neurological effects of the agent since it has been shown to affect behavior in this current study. Further analysis showed significant effects ($P < 0.05$) in Quinine and Chloroquine in both male and female flies while Artesunate effects were only significant in the male group. Quinine and Chloroquine have been shown to have toxicological effects in myocardial tissue and these act through suppression of epinephrine (Thanacoody, 2016).

5.2 Activities of antimalarials on mediators of aggression

5.2.1 FOR OCTOPAMINE

Activity of Clonidine treatment (↑)

In this study, Fansidar displayed the highest aggression in male *Drosophila melanogaster.* Artesunate and Artemether lumefantrine showed moderate aggression. Octopamine functions as a neuromodulator, neurotransmitter, and neuro-hormone in insect nervous systems and plays a crucial role in sensory inputs, arousal, initiation, and maintenance of various rhythmic behaviors and complex behaviors such as learning and memory (Farooqui, 2007). Clonidine has been shown to play a crucial role in neurological behavior(Masala, Solari, Sollai, Crnjar, & Liscia, 2008), and octopaminergic receptors have been shown to be stimulated under clonidine which shows the role of clonidine in the stimulation of vertebrate alpha-adrenoreceptors which are closely related to the latter in the central nervous system (Evans, 1981). Sulfadoxine-Pyrimethamine usage in humans has been recommended and the need to revise the dosage recommended (Barnes et al., 2006), however its neurological effects raise major concerns. On the other hand, Artemether lumefantrine showed the highest aggression in female *Drosophila melanogaster* while Fansidar showed moderate aggression which showed the gender differences associated with the interactions with octopaminergic receptors in this study.

Action of promethazine treatment (↓)

Artesunate showed the highest aggression in male *Drosophila melanogaster* while Fansidar and Artemether lumefantrine showed no aggression at all. Promethazine has been associated with decreased aggression in humans (TREC Collaborative Group, 2003). This is a major concern since its abuse has been reported in human populations (Lynch, Shapiro, Coffa, Novak, & Kral, 2015). In this particular, the role of Artesunate in the modulation of aggression has been shown to be increased. This would probably be due to decreased activity of promethazine following exposure to antimalarials. On the other hand, Artemether lumefantrine showed the highest aggression in female *Drosophila melanogaster* while Artesunate showed moderate aggression, thus showing the importance of the drug in the female gender.

5.2.2 FOR DOPAMINE (da)

Action of L-DOPA treatment (↑)

Fansidar showed the highest aggression in male *Drosophila melanogaster* while Artesunate and Artemether lumefantrine showed moderate aggression. The dopamine D2 receptor (D2) has been implicated in aggressive behavior, showing its role in offensive behavior (Vukhac, Sankoorikal, & Wang, 2001). Abnormal behavior has been associated with increased activation of the dopaminergic system which is would be responsible for the high aggression behavior (Beiderbeck et al., 2012). Fansidar in this regard appears to have enhanced the release of the dopaminergic neurotransmitter thus showing the role of the drug in males. Furthermore, Artemether Lumefantrine showed high aggression in female *Drosophila melanogaster* while aggression was found to be moderate in Fansidar group.

Action of Haloperidol treatment (↓)

Haloperidol helps to calm situations of aggression thus showing its role in psychosis in humans (Powney, Adams, & Jones, 2012). Medium aggression was found to be highest in Artemether Lumefantrine group in male *Drosophila melanogaster* while Artesunate showed moderate aggression, which shows that its actions are antagonist to haloperidol. In the management of psychiatric behavior, haloperidol usage in combination with promethazine has been recommended (Huf, Alexander, & Allen, 2005), however modulator effects of Artesunate need to be considered seriously as demonstrated in this study. This is because Artesunate has been associated with medium aggression in female *Drosophila melanogaster* while low aggression was found to be highest in Fansidar group.

5.2.3 FOR SEROTONIN (5HT)

Action of Fluoxetine treatment (↑)

Fansidar showed the highest aggression in male *Drosophila melanogaster* while moderate aggression was shown by Artesunate. Fluoxetine has been shown to be associated with decreased aggressive behavior in man (Heiligenstein, Beasley, & Potvin, 1993). It has been associated with increased serotonin production thus making male fishes less aggressive which are associated

with increased aggressive behavior over territorial dominance and mating (Perreault, Semsar, & Godwin, 2003) and in dogs, the drug has been used to treat aggression and modulating behavior (Dodman et al., 1996). The study showed that Fansidar would probably possess the property of inhibiting the activity of serotonin in this regard especially in the male gender. Artemether Lumefantrine showed the highest aggression in female *Drosophila melanogaster* moderate aggression was shown by Artesunate. During aggression, serotonin (5-HT) remains the primary molecular determinant of inter-male and inter-female aggression, whereas other molecules appear to act indirectly through 5-HT signaling. Slight modulations in 5-HT levels, turnover, and metabolism, or in receptor subtype activation, density, and binding affinity affect aggression. Activation of specific 5-HT receptors evokes distinct, but highly interacting, second messenger systems and multiple effectors. Understanding the interactions between 5-HT receptor subtypes should lead to novel insights into the molecular mechanisms of aggression (Nelson & Chiavegatto, 2001).

Action of Cyproheptadine treatment (↓)

Fansidar showed the highest aggression in male *Drosophila melanogaster,* thus showing its role in the interaction in the inhibition of serotonin antagonists. Cyproheptadine is an antihistamine and serotonin antagonist used for control of CNS effects in humans that are associated with aggression (Meythaler, Roper, & Brunner, 2003; Strayhorn, 1998). Its usage has been recommended following serotonin medication overdose and reverses intoxication effects (McDaniel, 2001). On the other hand, Artesunate showed the highest medium aggression in female *Drosophila melanogaster* while low aggression was found to be highest in Fansidar group. These observations are important since cyproheptadine has also been reported to be responsible to fatalities in man (Hargrove & Molina, 2009). These findings show that antimalarials would be interfering with neurological behavior through an interplay of various neuro modulatory pathways involved in aggression (Nelson & Chiavegatto, 2001).

5.3 Effect of Drug on Age

Artesunate showed the highest aggression across all age groups in male *Drosophila melanogaster* with highest effects amongst 40 -45 days, while Fansidar showed moderate aggression in both 21-25 and 40-45 days. In a recent study involving young children, it was shown that Artesunate had no significant CNS effects in the population which is comparable with our findings (Ambler et al., 2009), however significant neurological activity exhibited through heightened aggression behavior was demonstrated in this study thus showing the need to study and understand effects of the drug in adults better. The study further on demonstrated that Artesunate led to highest aggression for age group 40-45 days old female drosophila melanogaster. Aggression for age group 21-25 in Artesunate equals that in Artemether-Lumefantrine and aggression for age group 0-5 in Fansidar equals that in Artesunate. Since memory and behavior tend to deteriorate with age in humans, it appears the older population is easily excitable thus leading to increased episodes of aggression than would be necessary, thus showing the need for cognitive control in these populations (Lindenberger, Marsiske, & Baltes, 2000). Statistical analysis showed significant effects for Fansidar 0-5 days in male *Drosophila*, Artesunate 21-25 in male *Drosophila* and 40-45 days in both male and female *Drosophila* and Artemether lumefantrine 0-5 and 21-25 days in female and male *Drosophila* respectively. This demonstrates the need to assess and effectively control the usage of these pharmaceutical agents effectively in the older population, since it has been associated with increased episodes of aggression.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

Antimalarial drugs especially Artesunate had significant effects on aggressive behavior through its interaction with specific neurotransmitters and neurons in the brain that are responsible for expression of aggressive behavior in these flies and antimalarials especially Chloroquine and Quinine reduced aggressive behavior. Since studies in laboratory animals show that ACTs cause brain damage, and Artesunate has shown increased effect on aggressive behavior in flies, the molecular mechanisms of these effects should be studied in depth using the available genetic tools in *Drosophila.* Once there is a better understanding of the action mechanism, then action can be taken about the use of these drugs in humans.

Clonidine and Levodopa agonists of Octopamine and Dopamine respectively increase aggressive behavior in *Drosophila* while Fluoxetine an agonist of Serotonin shows reduced aggressive behavior following antimalarial treatment. Promethazine, Haloperidol and Cyproheptadine antagonists of Octopamine, Dopamine and Serotonin respectively generally exhibit reduced aggressive behavior in *Drosophila melanogaster.* The interactions of antimalarial showed agonistic and antagonistic effects on neurotransmitter activity in this study and the need to establish molecular mechanisms still remain to be investigated. Among the antimalarials, Artesunate and Fansidar seem to have significant activity across the age groups, thus showing their relevance in a prospective study.

Since antimalarials have been demonstrated to affect neurological behavior through an interplay of various neurotransmitters in various age groups and sexes, there is need to revise the usage of antimalarials in patients with neurological complications. In addition, the precise pathway(s), through which they exert their effects, needs to be established for a clear understanding of the drug interactions at cellular level.

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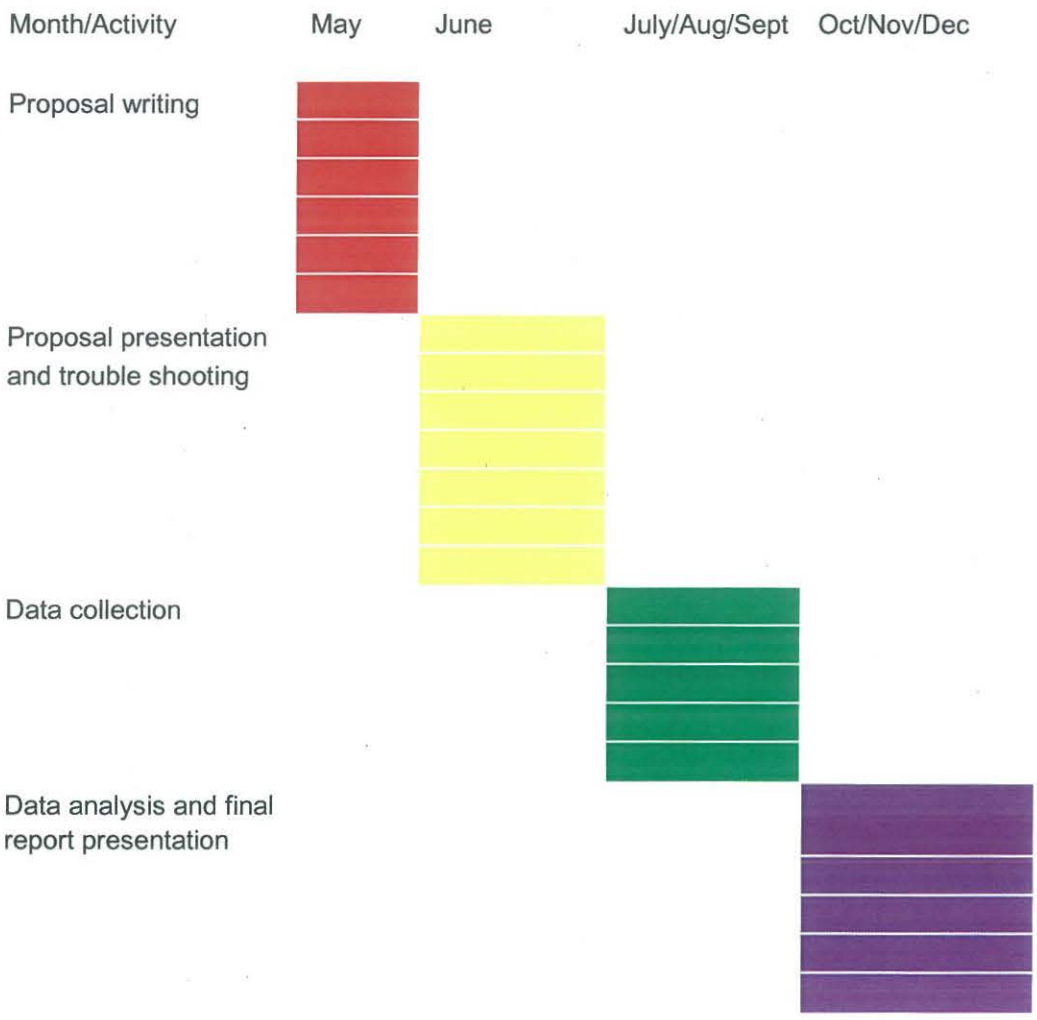
APPPENDICES

APPENDIX 1: TIME FRAME

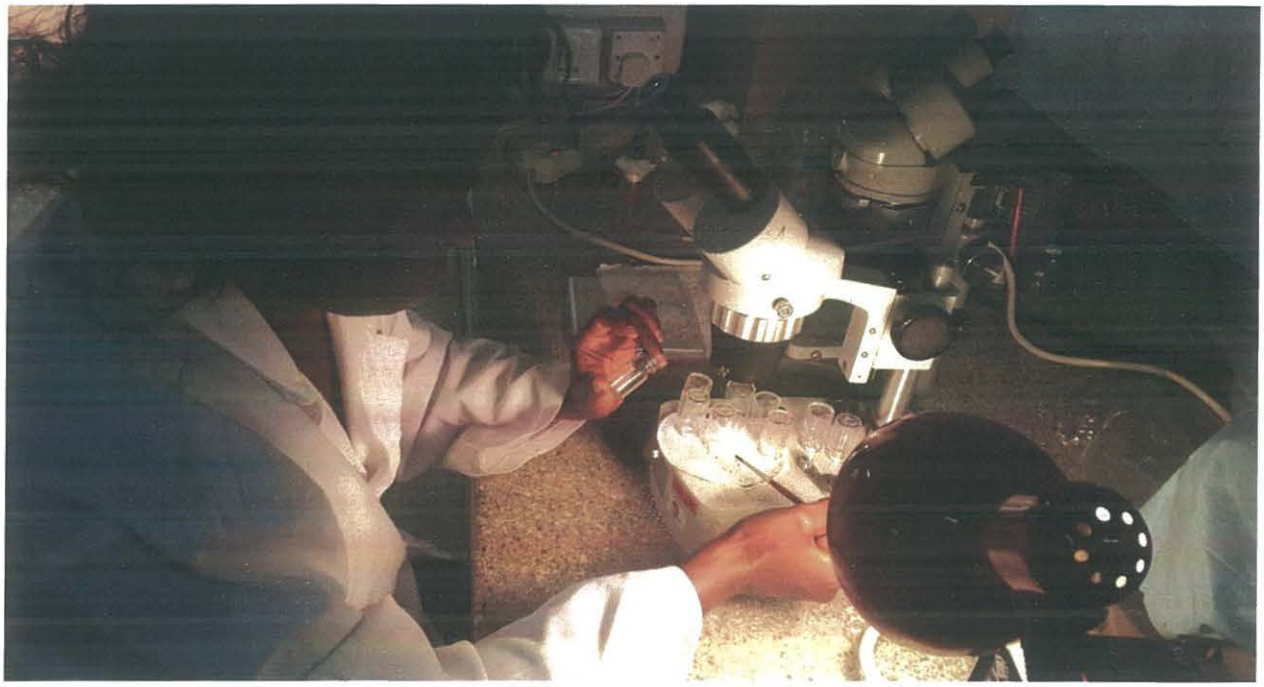


APPENDIX 2: BUDGET

ITEM	COST	TOTAL COST
Glucose	6,500	6,500
Yeast	3,000	6,000
Apple juice	10,000	10,000
Cornflower	7,000	14,000
Drugs (antimalarial)	100,000	100,000
Aluminum fool	3,000	3000
Internet	50,000	50,000
Cotton wool	10,500	10,500
Printing $ photocopying	125,000	125,000
TOTAL		300,000

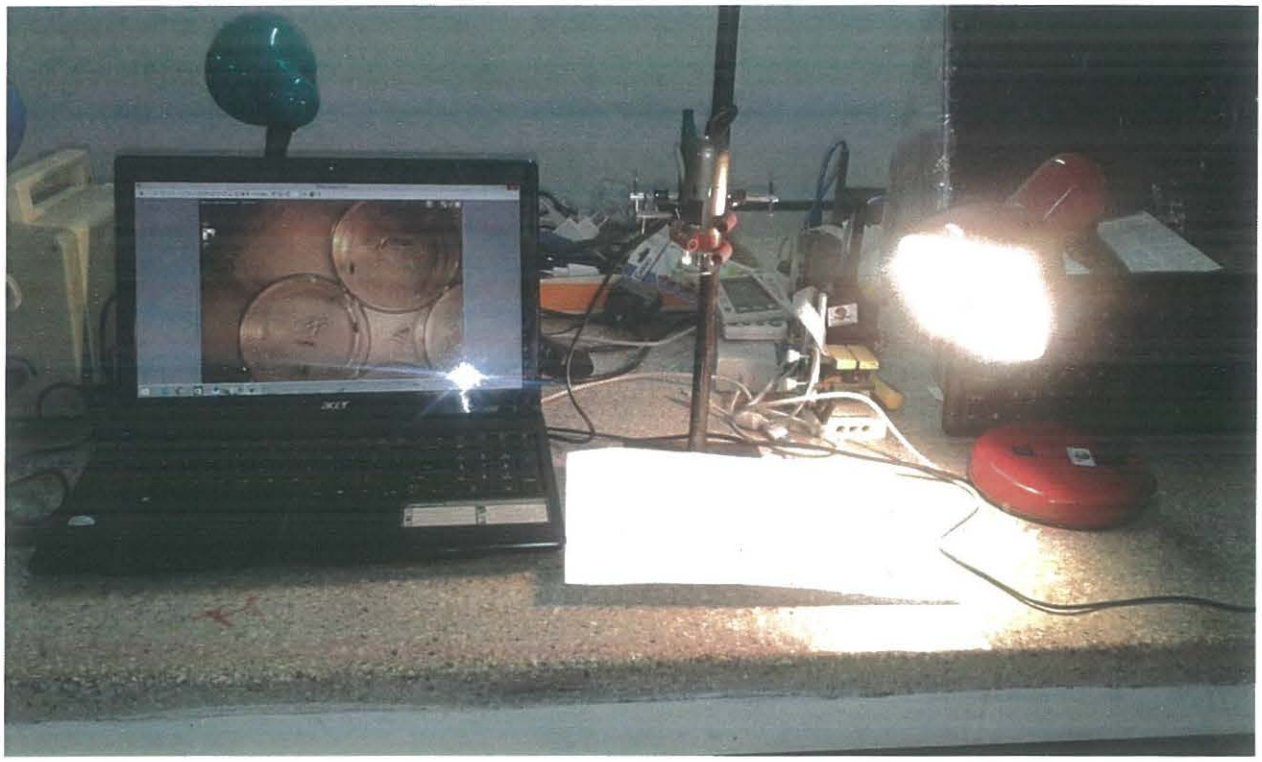
APPENDIX 3: BENCH WORK PICTURES

SEPERATING MALE AND FEMALE DROSOPHILA UNDER THE MICROSCOPE IN THE LAB



TRANSFERING FLY FOOD TO 175ML BOTTLES.

SET UP FOR VIDEO RECORDING IN THE LAB



SERIAL DILUTIONS OF ANTIMALARIALS AND NEUROTRANSMITTER MODULATORS