Official Publication of the Faculty of Basic Medical Sciences University of Ibadan, Nigeria

Arch. Bas. App. Med. 5 (2017) 29 – 36
www.archivesbamui.com

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Modulation of allostatic load by fluoxetine and ascorbic acid in stressed *Drosophila melanogaster*

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Abstract

Allostatic load from unabated stress condition may manifest in prolong irritation, physical exhaustion, memory loss, depressive-like behaviours and apoptosis. We investigate the role of fluoxetine and ascorbic acid to lower the threshold for allostatic load and reverse the effects of stressful experience. Wild –type strain of *Drosophila melanogaster* (w^{1118}), in three experimental groups were treated for 24h, 96h and 168h with fluoxetine (FT), ascorbic acid (AAT) and fluoxetine plus ascorbic acid (FAAT) before exposure to heat stress at 37°C. The exhaustion times- T_e and recovery times- T_{rc} were recorded. Motor and memory performance were assessed using climbing and cognition assays, and effects of the prolonged exposure to drug were monitored. The kinetics of disposition of the allostatic load effects in the flies were also determined. The T_e were significantly longer (P= 0.0003) and T_r significantly shorter (P= 0.00019) respectively in FT, AAT and FAAT groups than the control at 24h and 96h but not at 168h. The Performance Index (PI) of motor activity were significantly higher for the FAAT group (P= 0.03) compared to the FT and AAT after recovery from exhaustion. The area under the concentration-time curve ($AUC_{al\text{-}effect}$) was significantly lower and the clearance, $CL_{al\text{-}effects,}$ significantly higher with FAAT group. Learning Index (LI) in all treatment groups were higher compared to the untreated flies (P= 0.0006). Conclusively, fluoxetine, ascorbic acid and fluoxetine–ascorbic acid combination have potential to delay consequential effects of allostatic load and enhance clearance of stress effects, recovery and memory retrieval capacity in Drosophila. This may have implication in human stress management.

Keywords: allostasis, stress, *Drosophila melanogaster*, fluoxetine, ascorbic acid, motor, cognition

INTRODUCTION

Stress related processes account for causes of appreciable discrepancy in morbidity and mortality rates of diseases in human population (Adler et al., 1999, Cohen et al., 2007, Mc Ewen 2007; Gruenewald et al., 2006; Seeman et al., 1997; Borrell et al., 2007, Lewis et al., 2010). The risk for and resilience against ill health are influenced by coping responses from stressful life experiences and environment (Mc Ewen 2007; Mc Ewen 2010). Depending on the adaptive coping strategies and resources available to an individual, the real or perceived environmental demand of stress may create conditions of allostasis and allostatic load or overload (Selyl, 1956).

Allostasis is the dymanic regulatory process wherein homeostatic control is maintained by an active process of adaptation during exposure to physical and behavioural stressor (McEwen and Gianaros, 2010), and allostatic load, first described by McEwen and Stellar (1993), is the consequence of allodynamic regulatory wear and tear on the body and brain, promoting ill health and causing alteration in lifestyle (McEwen, 2007; McEwen, 2009; Shonkoff et al., 2009).

Almost all disease conditions or disorders that affect human induce a sort of stressful experience characterized by different physiological and biological responses. Malaria parasite and other infectious agents present pathophysiologic stress or allostatic demand on the infected, the outcome of which may be lethal, in cases where treatment is absent or failed to remove the cause (Cohen and Williamson, 1991; Raison & Miller, 2012; Mason, 1991; Perez et al., 2009; Konsman et al.,2002; Carabotti et al., 2015), Other debilitating disorders, including cancer, diabetes and associated complications, have notably shaped stressful experience in affected populations (Periano et al., 2012; Babbit et al., 2012;Clark et al., 2006, Kim et al., 2013). The stress responses in these conditions affect important brain regions including hippocampus, amygdala and prefrontal cortex (Mayer, 2000; McDonald, 1987; McDonald, Masagni &Guo, 1996; Petrovich et al., 2001; Kim et al., 2013) The autonomic nervous system and hypothalamo-pituitary-adrenal (HPA) axis are activated, leading to behavioural and physiological responses. The associated allostatic load may

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manifest as prolong or recurrent irritation, emotional draining, physical exhaustion, loss of memory, depressive-like behaviours, oxidative stress and apoptosis at organismal and cellular levels (McEwen, 2007; McEwen, 2011).

Most allostatic loads are product of unabated stress that outweigh the biological coping response. We hypothesized that certain chemicals or drugs may help to lower the threshold for allostatic load or increase recovery from exposure. In order to investigate the hypothesis, we select *Drosophila melanogaster*- a fly model known to have 75% homologues of genes related to diseases in human, and evaluate the activities of fluoxetine- a selective serotonin re uptake inhibitor antidepressant, and ascorbic acid- an antioxidant, administered prior to exposure of the flies to stress. There are evidences to show that the cellular effects of stress from both heat and oxidative stresses are similar (Bochner et al., 1984; Christman et al.,1985, Hass and Massaro., 1988; Morgan et al., 1986, Levinson et al.,1978; Lee et al., 1983; Fleming et al., 1992). Thus we model exposure to heat stress of Drosphila for allostatic load condition and evaluate the potential of fluoxetine and ascorbic acid to delay the threshold of stress effect and boost the compensatory efforts by the body to restore physiological system baseline.

MATERIALS AND METHODS

Fly strains: The wild –type strain of *Drosophila melanogaster* (w^{1118}) were used for the study. The flies were obtained from fly culture kept in the Institute of Biomedical Research (IBR) Laboratory, Ishaka, Uganda, at 22^0C-25^0C (on agar, yeast, dextrose and wheat flour), anaesthetized using triethylamine (fly nap) and transferred to a standard fly bottle containing dextrose medium. They were raised under a 12-hr light- dark cycle at 25ºC prior to experiment.

Experiments design: The flies were randomly divided into three different experimental groups; (a). Pre heat stress fluoxetine treated (FT) group (b). Pre heat stress ascorbic acid treated (AAT) group (c). Pre heat stress fluoxetine-ascorbic acid treated (FAAT) group. Each experiment has a control set up – Untreared Heat stress (UT) group. All flies were assessed for motor and memory performance using geotaxis/climbing and cognition (memory retrieval) assays. In the experiment, the flies were exposed to the drugs in food for 24h, 96h and 168h, and effects of the prolonged exposure were determined.

Drug treatment: In the experimental group, flies were fed on fluoxetine mixed with normal fly food for 24hrs. The drug was introduced in food recipe for a standard dose in human subject (10mg/kg body weight). Another group of flies were treated with 100mg (for 70kg man) dose equivalence of ascorbic acid. The flies used in the experiment were weighed and the average weight of 1mg body weight was used for the preparation of the dose of drugs. Treatments were conducted for 24, 96 and 168 hours in different groups before exposure to stress. Flies that were used for the motor and learning performance assays were pretreated for 24hours only.

Induction of heat stress: Flies were transferred into empty vials (10 flies per vial) and introduced into a preheated water bath at 37 ºC. They were plugged with cotton wool to avoid desiccation. The temperature in the vials was monitored using a thermometer to ensure harmonization with water bath temperature. The time of exhaustion or Exhaustion Time- T_e (the time between exposing healthy fly to heat and when the fly becomes completely immobilized and hit down from its normal negative geotaxis movements to the bottom of the vial) was recorded. The vial was then removed from the water bath and immediately put under room temperature to allow the flies to recover. The recovery time- T_{rc} (time interval obtained when the completely immobilized fly is placed at room temperature to the time of first movement that is sustained for at least 120seconds) were also recorded. A control experiment with flies fed on normal food but not exposed to heat (kept at room temperature) was set up.

The flies were allowed to rest for 45 minutes and then assessed for motor performance in climbing assay for 15 minutes. All flies were allowed to stay in food for two hours after the climbing assay was completed. The climbing assay was also conducted in the control group. The experiment was repeated three times for assessment of effects of the drugs.

Climbing assay; The climbing assay was conducted using the method of Bainton *et al.* (2000). A cylinder of 35cm length (1.5cm diameter), was used to assess the negative geotaxis potential of the flies using climbing assay technique. All flies exposed to stress and the control flies were evaluated in this experiment. The cylinder was plugged at the bottom end and separated into 3 areas; "Top", "Middle" and "Bottom" by clear and readable line with each of a distance of 10cm. The flies innate escape responses are assessed using the negative geotaxis characteristic of the Drosophila flies. A negative geotaxis is deemed to take place when flies ascend the wall of a cylinder after being tapped at its bottom. After 45secs flies located at each of the sections are recorded into scoring sheets. Each experiment was repeated three times to minimize trial error. The performance indices for all the flies in the different conditions were determine and recorded. The Performance index (PI) was determined by obtaining the ratio of the difference between the numbers of flies in the upper region (U) and the lower region (L) to the total number of flies (T);
PI= (U-L/T).

Cognition assay experiment: There were four experimental groups in the cognitive performance assay experiment with heat dish; normal flies for control (group A), flies treated with fluoxetine before training (group B), flies treated with ascorbic acid before training (group C), and Flies treated with fluoxetine and ascorbic acid before training (Group D). The treatment was for a duration of 24hours.The heat dish was designed to assess spatial memory in the flies with similar test principles as reported in previous studies (Wustmann and Heisenberg,1997; Zars 2000; Zars and Zars, 2006). Briefly, it comprised of a plastic bowl of 15cm diameter and 5 cm high with a lid cover that is hollow, 1cm deep, and can sit in the bowl from the top. This cover was marked with red spots spread all over the cover circumference. The dish top below the cover is marked with red tape. Hot water was poured in the cover sitting just above the red tape to make flies recognise that the red tape and beyond contains punishing condition (heat) and should avoid it. Before a fly was subjected to this learning, the wings were removed so that flying is restricted and allowed 12hrs to rest in food and acclimatize to being wingless before the experiment. Using hot water at a temperature of 50ºC quickly poured on the red spotted cover,

the fly was punished when it enters the red end. The flies in the groups A-D were allowed to move but trained to avoid a red coloured topmost part of the dish. Thereafter, they were introduced to the heat dish assay condition. The flies were taught to recognize the top of the dish was hot. Each time the flies get to the top, the dish was banged slightly and they could return to the bottom. This training was done repeatedly for 15 times with the temperature maintained at 50^{o}C. The test was carried out without the hot water in the red spotted cover sitting above the red tape. The flies were introduced and observed for those that will avoid (having recalled that the condition of the cover was hot and therefore avoided moving close) or those that will not avoid the hot cover and pass beyond the red tape. The counting was done for both control group (A) and the drug treated groups (B, C and D). All flies were observed for 24min and the Learning index (Li), measured as preference for selecting heat or no heat region, for each group was determined for the flies. PREF (no heat) = [number below red tape (no heat zone) – number above red tape (heat zone)] / total.

Kinetics of disposition of the allostatic load effects in the heat stressed flies: In order to examine the effects of drugs on the allostatic overload and restoration of allostasis in the heat stressed flies, the kinetic disposition of the effects were examined in a similar manner drug actions or parasite kinetics (Sowunmi *et al*., 2000) were studied. For each group of flies, allostatic overload effects (motor inactivation) mass were estimated and plotted against time. The areas under the curve of effects mass versus time ($AUC_{al\text{-}effect}$) were determined by a non-compartmental method using the computer programme *Turbo Ken* (designed by Clinical Pharmacology Group, University of Southampton, United Kingdom). Briefly, $AUC_{al\text{-}effects}$ was obtained, using the linear trapezoidal rule, from time zero (0 h, day 0) to the time of clearance of allostatic overload effects mass /min. The time it takes for the effects in either case to reduce to half were also calculated as $t_{1/2\ al\text{-}effects.}$ The unit is expressed per min. Also the effects mass cleared of the body per unit body volume were also determined as $CL_{al\text{-}effects..}$ The unit is expressed as effect mass/ µl body volume. The final allostatic overload effect at the time of clearance was assumed to be 0.001 effect mass/ µl body volume (a level assumed to be below physical observation).

Data analysis

All statistical analyses were performed with Paleontological Statistical Software Package for Education and Data Analysis (Past 3). Average values are expressed as mean ± SD. Comparison of the mean values in the different groups was done using ANOVA which were followed by planned pairwise comparisons with a Tukey correction. Unless stated otherwise, *n*= 10 in all experiments. Given the definition of the preference and PI scores, and given the fact that often these scores are not normally distributed, we opted for non-parametric statistics and display throughout. We used Kruskal–Wallis tests (K–W tests) for comparisons across multiple groups, followed in cases of significance by pair-wise comparisons with Mann–Whitney *U*-tests (M–W *U*-tests).

RESULTS

The heat stress induction was optimized at 37^{o}C in preheated water bath. Allostatic load is assumed developed when the flies became immobilized or lost motor system activity expressed as Time of Exhaustion, Te. Table 1 shows the different Te of flies pretreated with fluoxetine, ascorbic acid and fluoxetine plus ascorbic acid at 24, 96 and 168hours. The Time of Exhaustion, Te (mean + SE, range), for the pretreated groups at 24h [fluoxetine (20.0 ±1.0, 18-21) min ; ascorbic acid (22.0±2.6, 18-27)min and fluoxetine plus ascorbic acid (30 ± 1,7, 27-33) min] were significantly higher than the control (untreated heat stressed, UT, 12 ± 1.7min,range- 9-15), P= 0.001..

The Time of Exhaustion, T_e, in flies that were exposed to heat stress following pretreatment for 96h and 168h are shown in the Table. There were significant differences in the means compared to pretreatment at 24h. Turkey pairwise analysis showed that T_e for flies pretreated with ascorbic acid and fluoxetine plus ascorbic acid was significantly higher than control group (AATvs UT, P=0.02; FAAT vs UT, P=0.00079) except in group treated with fluoxetine (FT vs UT, P= 0.06). The recovery time from allostatic load was measured for different categories of the pretreated flies.

Table 1

Comparison of the Exhaustion Time (T_e) of flies pretreated with fluoxetine (F), Ascorbic acid (AA), fluoxetine plus ascorbic acid (FAA) for 24, 96 and 168h, and untreated control following exposure to heat stress

Parameter	Untreated control	Fluoxetine (F) treated group	Ascorbic Acid (AA) treated group	Fluoxetine plus Ascorbic Acid (FAA) treated group	P value
		T_e, 24h pretreatment (min)			
Mean ± SEM	12 ± 1.7	20 ± 1.0	22 ± 2.6	30 ± 1.7	0.001
range	9-15	18-21	18-27	27-33	
		T_e, 96h pretreatment (min)			
Mean ± SEM	12 ± 1.7	22 ± 1.7	22 ± 2.0	37 ± 2.6	0.00013
range	9-15	21- 24	18-24	33-42	
		T_e, 168h pretreatment (min)			0.00000005
Mean ± SEM	13.0 ± 2.0	38 ± 1.0	38 ± 1.0	47 ± 1.0	
range	9-15	36-39	35-39	45-48	

Table 2.
Comparison of the Recovery Time (T_{rc}) of flies pretreated with fluoxetine (F), Ascorbic acid (AA), fluoxetine plus ascorbic acid (FAA) for 24, 96 and 168h, and untreated control following exhaustion from exposure to heat stress

Parameter	Untreated control (UT)	Fluoxetine (F) treated group	Ascorbic Acid (AA) treated group	Fluoxetine plus Ascorbic Acid (FAA) treated group	P value
	T_r, 24h pretreatment (min)				
Mean ± SEM	28 ± 2.0	15 ± 1.7	14 ± 1.0	11 ± 1.0	0.00019
range	24-30	12-18	12-15	9-12	
	T_r, 96h pretreatment (min)				
Mean ± SEM	27 ± 1.7	13 ± 1.0	12 ± 1.7	11 ± 1.0	0.0001
range	24-30	12-15	9-15	9-12	
	T_r, 168h pretreatment (min)				
Mean ± SEM	29 ± 1.0	34 ± 1.0	44 ± 1.0	44 ± 1.0	0.00001
range	24-30	33-36	42-45	42-45	

The Recovery time- Tr (mean + SE, range) are shown in Table 2 for the pretreated groups at 24h [fluoxetine (15.0 +1.7, 12-18)min ; ascorbic acid (14.0+ 1.0, 12-15)min and fluoxetine plus ascorbic acid (11 + 1.0, 9- 12) min] were significantly shorter that the control (Untreated, UT, 28 + 2.0 min, range-24-30), P= 0.00019. The Tr in flies that were also exposed to heat stress following pretreatment at 96h and 168h are also presented in Table 2. Turkey pairwise analysis showed that Tr for flies pretreated with fluoxetine, ascorbic acid and fluoxetine plus ascorbic acid were significantly shorter than control group, (FT vs UT, P= 0.0014; AATvs UT, P=0.009; FAAT vs UT, P=0.00038). However, the pairwise analysis showed no significant difference between flies pretreatment with fluoxetine and those with ascorbic acid (P= 0.96) or fluoxetine plus ascorbic acid (P=0.30).

Climbing assay findings

The climbing assay results are presented in Figure 2A-B. The motor activity was examined in the flies pre treatment with fluoxtine (FT), ascorbic acid (AAT) and fluoxetine plus ascorbic acid (FAAT) before exposure to heat stress and after recovery from the allostatic load effect. There was no significant difference in Performance Indices (P= 0.55) across treatment groups; [FAAT group (0.63 ± 0.09, range 0.48-0.79), FT (0.69 ± 0.01, range= 0.68-0.70) and AAT (0.69 ± 0.01, range= 0.67-0.71), UT group (0.73 ± 0.03, range)] before exposure to stress. Following exposure to heat stress, significantly higher PI (P= 0.005) was obtained from the FAAT group (0.71 + 0.09, range 0.58-0.89), FT (0.59 ± 0.05, range= 0.48-0.67) and AAT (0.59 ± 0.05, range= 0.49-0.66) compared to UT group (0.27 ± 0.001, range = 0.26-0.29) after recovery from exhaustion induced by the stress.

Kinetics of disposition of the allostatic overload effect mass in the heat stressed flies

Table 1 showed the kinetic of disposition of effect mass of the different drugs on the allostatic overload defined by the area under the effect time curve, half live of effects and the clearance of effects from the pretreated and untreated stressed flies. There were significant differences between the pretreated flies compared to untreated flies. The $AUC_{al\text{-}effect}$ was significantly lower and the clearance, $CL_{al\text{-}effects,}$ significantly higher with fluoxetine plus ascorbic acid pretreated flies compared to fluoxetine or ascorbic acid pretreated flies and the untreated heat stressed flies respectively.

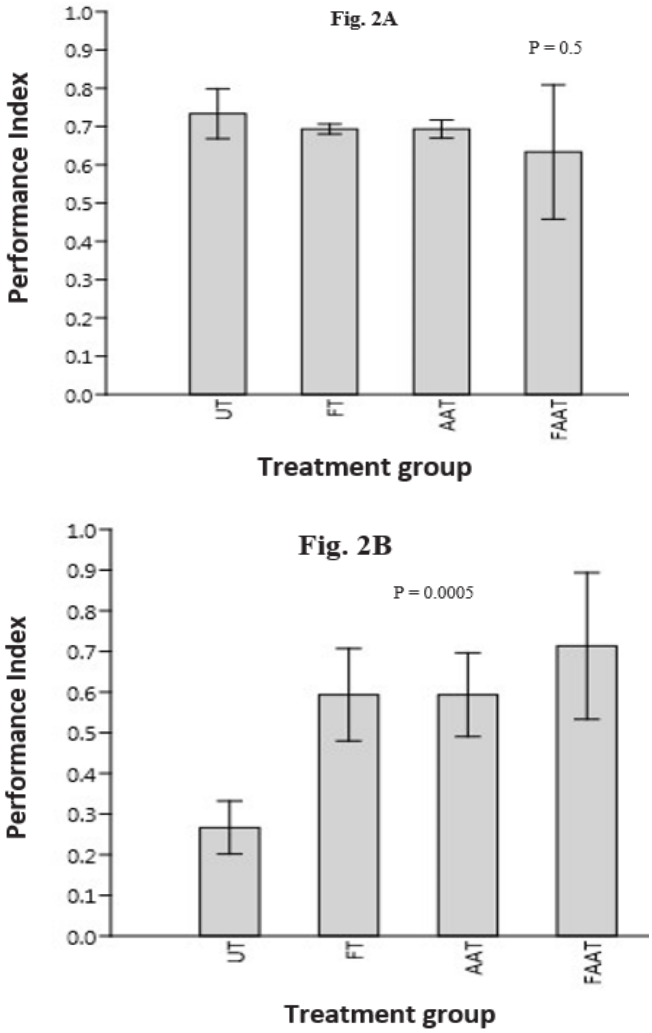


Figure 2A-B:
Performance Index plot of pre treated flies before (A) and after (B) exposure to heat stress (UT- Untreated group, FT- Pre heat stress fluoxetine treated group, AAT- Pre heat stress ascorbic acid treated group, FAAT -Pre heat stress fluoxetine plus ascorbic acid treated group).

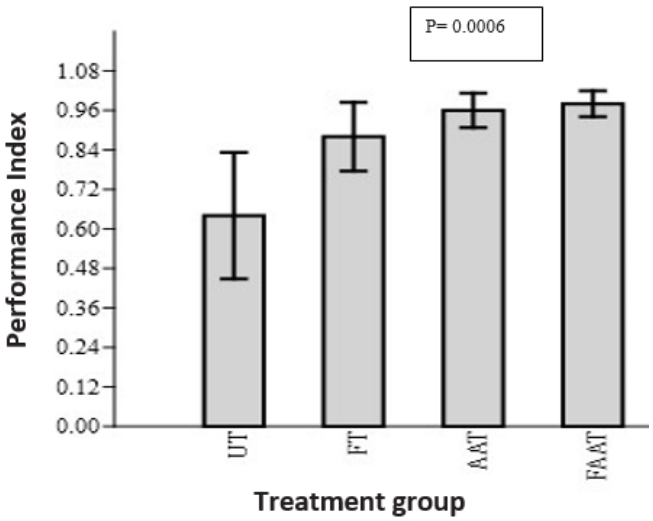
Table 3.
Kinetics of disposition of allostetic load effect mass on recovery of the motor activity in the drosophila flies exposed to heat stress

	Untreated	Fluoxetine Treated	Ascorbic acid Treated	Fluoxetine plus ascorbic acid Treated	P value
$AUC_{al\text{-}effect}$ (effect mass/ min)	189.8 ±2.5	78.2 ±5.8	85.9± 5.8	66.9 ±3.5	0.0001
$t_{1/2\ al\text{-}effects}$ (mins)	0.54 ±0.07	0.51± 0.05	0.47 ±0.05	0.47 ±0.05	0.336
$CL_{al\text{-}effects}$ (effect mass/ ul/min)	0.05± 0.005	0.13 ±0.01	0.12 ±0.01	0.14 ±0.05	0.0004

Figure 3.
Learning Index plot of pre treated flies following heat dish cognition experiment (*UT*- untreated group, *FT*- Pre heat stress fluoxetine treated group,*AAT*- Pre heat stress ascorbic acid treated group, *FAAT* -Pre heat stress fluoxetine plus ascorbic acid treated group). *$AUC_{al\text{-}effect}$ - the areas under the curve of effects mass versus time; $t_{1/2\ al\text{-}effects}$ - the time it takes for the effects in either case to reduce to half; $CL_{al\text{-}effects}$ - the effects mass cleared of the body per unit body volume.*

However the half life of the effects mass, $t_{1/2\ al\text{-}effects}$, though lower with fluoxetine plus ascorbic acid , and ascorbic acid only treated flies, was similar in the groups (P= 0.336).

Findings from Cognition assay heat dish experiment
Figure 3. shows the Learning Index (LI) of the different groups of pre-treated flies and the untreated control when exposed to the cognitive assay with heat dish experiment. The LIs for flies pre treated with fluoxetine (0.88 ± 0.5, range 0.6-1.0), ascorbic acid (0.96 ± 0.02, range 0.8-1.0), or fluoxetine plus ascorbic acid (0.98 ± 0.02, range 0.8-1.0) showed significantly higher memory retrieval capacity (P= 0.0006) compared to the untreated flies (0.68 ± 0.02, range 0.8-1.0)



DISCUSSION

The cost incurred to maintain normal physiology or reverse physiological stress may sometimes be over bearing on human biological system and may cause complete collapse (a state of ill health). Preventing allostatic load may help in quick recovery of stressed body system physiology arising from diseases or other environmental factors.

In the present study, we modeled a condition of uncontrollable stress bearing in *Drosophila melanogaster* from heat stress exposure following treatment with antidepressant (fluoxetine) and an antioxidant (ascorbic acid). These drugs were used as probes for subjective condition relative to depression and oxidative stress based on their known pharmacology.

The findings in this study showed that *Drosophila melanogaster* treated with fluoxetine and or ascorbic acid has delay to reach exhaustion from exposure to heat stress compared to those without the drugs. This suggests that the heat stress may have created disturbance in physiological functions mediated by serotonin neurotransmitter and or reactive oxygen species (ROS)- a cause of oxidative injury, or other processes that are yet unclear. These two conditions are pathologic signals of several disease conditions, for example depression, in human (Ng et al., 2008).

The biochemical changes associated with this disturbance constitute cellular effects with response that may initiate antioxidant enzyme activities, production of heat shock protein and thermotolerance (Morgan et al., 1986, Hass et al., 1988, Lee et al., 1983) for homeostatic regulation. However, persistent generation of heat stress or the oxidative injury would lead to allostatic load (McEwen and Gianaros, 2010; McEwen, 2007; McEwen, 2009; Shonkoff et al., 2009). The delay observed in the manifestation of the allostatic load suggests the valuable role of these drugs to interfere with the biochemical and metabolic processes and prolong allostasis.

Interestingly, this delay in the manifestation of allostetic load appears to be dependent on the duration of exposure of the flies to treatment drugs. A pronounced delay was seen in those treated with the drugs for 168hrs compared to those for 96 and 24 hours respectively. The reason for this is not clear from the present study; however a possible explanation is the ability of these drugs, given their mechanism of actions and other reported clinical values, to sustain serotonin in the neural transmission, alleviate mood, and scavenge the free radicals during stress.

Ascorbic acid has been reported to decrease mood disorder, reduce psychological abnormalities in hypovitaminosis and augment sub effective dose of fluoxetine in patients (Evans-older et al., 2010; Brody, 2002; Kennedy et al.,2010; Gosney et al., 2008). This may explain the significant effect of the combination of fluoxetine and ascorbic acid in the present study. Similar observations have been reported in studies involving depressed patients who received fluoxetine treatment and ascorbic acid (Amr et al., 2013)

Conversely, the recovery time from allostatic load, expressed physically as exhaustion and immobility of the flies

following heat exposure, were significantly influenced by the treatment drugs. Except for the 168hours drug treated group, recovery times of those treated with fluoxetine and ascorbic acid or the combination were significantly twice lower than those untreated. There are two possible explanations for this observation- (i) Although there was delay depletion of serotonin in neuronal system, mood regulatory pathway, and oxidative injury due to actions of drugs, there are other biochemical engagements of the drug molecules at cellular level, augmenting or promoting resilience that resulted in quick recovery. This may be by mobilizing glucose, suppressing protein breakdown and enhanced wakefulness center activity in the brain, and (ii.) the exposure possibly turned on the antioxidant enzyme activities, production of protective heat shock protein and thermotolerance such that a quick recovery is achieved. The reason(s) for the poor recovery in the groups treated with the drugs for 168 hours remains unclear. Changes in the way fluoxetine works when administered for acute and chronic duration in mice have been reported. A stabilization of serotonergic modulation at the central synaptic transmission has been implicated for attenuation of hyperactivity behavior in mice under chronic exposure to fluoxetine (Kobayashi et al., 2011). Could fluoxetine have in 168 hours in our Drosophila tend towards the serotonergic modulation pattern reported in mice under chronic administration, andwhat biochemical process supports the observed quick recovery are not clear from this study? More studies are required to establish the cellular basis of these observations.

The kinetic disposition of the allostatic load effects, presumably a mass of effect,was described with similar phenomenon to that with that for drug kinetics and as applied to parasite clearance in peripheral blood to measure effects of antimnalarial drugs (Sowunmi et al., 2000). The load effects mass is assumed similar to a bolus of drug molecule injected in a *Drosophila melanogaster* fly. The heat exposure built up a mass of effects running in the whole organism to a peak when the physiological activities are paralyzed and fly became inactive. At the point of withdrawal from source of stress, the mass effects start to wear out in a similar manner to drug clearance. The kinetic parameters measured (AUC al-effects, t1/2 al-effects and CL al-effects.) showed that there were significant differences in the area under the time curve of allostatic load effects and clearance of mass effects of the allostatic load condition in the different groups treated with fluoxetine, ascorbic acid and combination of the two. However, the time for the effect to reduce to half its original loading mass was not significantly different across the drug treated flies and the untreated group. Fluoxetine plus ascorbic, . acid treated groups had significantly shorter AUC al-effects and higher CL al-effects .This outcome further mimic the observed variation in the recovery times of the different groups of flies, treated and untreated, suggesting that the drugs are capable of hastening recovery, The process by which this is done is not clear in the present study. It is possible that a biological process that washes off the burden of the stress in initiated.

In order to assess the physiological effects of the allostatic load, we evaluated motor system and cognition potential of the flies. The performance indices, obtained from climbing assay in the Drosophila, an evaluation of the physiological ability to sustain negative geotaxis motor program, before and after heat stress indicate that the treatment with drugs enhanced better motor function recovery and performance. It is possible that there are protective effects on muscle and brain activities during stress due to the presence of the fluoxetin and ascorbic acid molecules in these flies. In an earlier study (Jee et al., 2012), fluoxetine was reported to prevent the breakdown of the tight junction integrity in endothelial cells of blood vessel and reduced the expression of inflammatory mediators after injury, and ascorbic acid has been shown to have antidepressant-like effect in animals with reactive oxygen species (ROS) induce depressive-like behavior (Kobayachi et al., 2008). Since biochemical alteration that leads to immobility or motor function loss, following exposure to heat stress, may involve disturbance in calcium alteration and thermoprotective response (Barclay and Robertson, 2002; McKieman, 2013; Lehmann et al., 2013), it is not unlikely that the drugs act to minimize the heat effects on this process through an unknown mechanism. The loss of motor activity signals and release of neurotransmitter are synonymous and calcium plays vital role, If treated Drosophila had quick restoration of motor function, the calcium supply to enhance motor activation may have resulted from intracellular store supply (Ryglewski et al., 2007) in process of recovery. Additional probable explanation may be that the availability of serotonin, due to block of uptake receptor by fluoxetine or its combination with ascorbic acid, at cellular level, increases evoked motor unit activity that innervates body wall muscle fibers as observed by Dasari and Cooper (2004). This may allow for possible quick muscle reactivation after potential flaccid experience.

The evaluation of context retrieval after learning post heat stress is enhanced in treated flies. It presupposes that similar protective measures are experienced by learning pathway in the brain or against possible brain oxidative process that can lead to some cognition problems (Morgana et al., 2011; Fontella et al., 2005; Lucca et al.,2009; Madrigal et al., 2001). More studies may be required to elucidate this observation.

Conclusively, fluoxetine, ascorbic acid and fluoxetine–ascorbic acid combination have potential to delay consequential effects of allostatic load and enhance quick recovery, clearance of stress effects and memory retrieval capacity in Drosophila. Many studies are required to elucidate more of the involvement of the drugs and their combination, especially in respect of physiological cellular responses to heat exposure in Drosophila and potential for drugs to ameliorate allostatic load as observed in the present study. This may have implication in management of stress in human as he lives in environment of continuous perturbation from different kind of stressors. A sub therapeutic- prophylactic- use of drugs, such as fluoxetine and ascorbic acid, may be beneficial.

Acknowledgement

We acknowledge the technical support, contribution and constructive criticism of Ms. Helen Kinyi, Mrs. Ajagun-Ogunleye Mulkah, Ms. Kembabazi Stella, and Mr. Sanusi Jega Ahmed during the period of the study.

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