

The Effects of *Toxocara canis* Infection With and Without Red light on the Levels of Melatonin Hormone and Cytokines Peripheral Blood of Albino Rat

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Summary

Present study was carried during period between August 2010 and February 2011 . A total of 26 stray dogs were examined to detection of *T.canis* infection in deferent area of Hilla city . Experimental infection performed in albino male rats and subdivided into five groups these were, control negative and positive group, red negative and positive group and positive blindness group. The rat was killed and their serum were evaluated for cytokine (IL-1 β and IL-6) and melatonin hormone.

The present study showed the significant differences in melatonin level in types and time of of *T.canis* infection and red light exposure and intercept between them (F=12.357, P=0.05, F=4.247,P<0.001). The present study aimed to show the effect of interleukin (hormone like peptide) especially IL-1 β and IL-6, the result IL- β don't showed significant differences between group (time* type) whereas post hoc analysis showed the significantly decreasing in types of groups in IL-1 β concentration. This study also showed significant variation for time, type and intercept between them in interleukin-6 level (F=971.98, P<0.001; F=621.33, P=< 0.001 and F=1.33.26, P< 0.001 respectively).

INTRODUCTION

Toxocara canis is a common round worm found in the small intestines of doges, however, *T.canis* can also infect other animals. This parasite was classified by Soulsby (1968) in order Ascaroidea, family Ascaridae, which has a world-wide distribution. Several field and laboratory studies showed that the infection of parasite (protozoa, nematodes, cestodes and arthropods) can caused the changes in endocrine-immune system and finally alteration of behavioral factors. In recent years much attention has been devoted to the possible interaction between melatonin and the immune system.

The wavelengths of light induce an eye-brain-mediated-hormonal and immune response depends upon the transmission properties of eye of the particular species. In lower animals, uv-B (280-320), uv-A (320-400) and visible light may be transmitted to the retina because these wavelengths are not filtered by their cornea or lens. Therefore, both uv and visible light might induce can an eye-brain-mediated response. In adult primates, including humans, the cornea cuts out all light below 295 nm, while the lens filter out light between 295-400 nm, so that only visible light (400-700 nm) reaches the primate retina (Reme *et al.*, 1991). The aim of the present work to investigate the *Toxocara canis* infection as well as combined *Toxocara canis* – red light exposure on levels of hormones(melatonin) and hormone like peptide (IL-1 β and IL-6) in peripheral blood of white rat and interaction between them.

MATERIALS AND METHODS

Stray dog collection

A total of 26 stray dogs were collected in Hilla city and examined in this study. Dogs were shoted at close range in the neck just behind the skull, Euthanasia was chosen over shooting for reason of expediency.

Extraction and embryonation of *T. canis* larvae

The proportion and separated of embryonated eggs from pregnant worms according to Desavangy (1975).

Experimental animals

Male albino rats were used at 8-12 weeks of age, where allowed to adapted for 2-3 weeks in a temperature 25 °C and humidity 50 \pm . The animals caged in cage at 60 \times 50 \times 60 cm². The investigation conformed with small mammals manual published by UK (2004).

Methods of intubation

Experimental infections of rat were carried out by oral intubation of larvae directly into the stomach. The eggs (2000) were suspended in 2 ml of distilled water after washing them three times and then intubed into the stomach .

Anesthesia

The rats were anesthetized with ketamine (100 mg/Kg) intramuscular (I.M) (small mammal manual).

Toxocara canis infection and combined *T.canis* red light

Three kinds of stress were used in present study these where: Red light, Blindness and parasitic infection (*Toxocara canis*) . The animal randomly assigned and grouping into red light group (n=20), red light infected group (n=20), infected group (n=20), Blindness infected group (n=20). Animals that were not stressed are considered as control group (n=20). This groups killed on days 3, 7, 14, 28 and 56 from stress, also the same procedure were carried out but in hours 3, 6, 12 & 24 and all of them killed at 8 a clock.

Hormonal and Immunological assay procedure

To determine the rat plasma melatonin, interleukin-1 β and interleukin-6 the quantitative sandwich enzyme immunoassay technique were used according to manufacturing company(Cusabio Biotech co., LTD., eBioscience, USA).

Statistics

Data were analyzed using a general factorial ANOVA. Between groups differences were analyzed Post-hoc using multiple F-test and significance levels. A level of P<0.05 was accepted as statistically significant. Pearson correlation (2-tailed) assay also used in this study.

RESULTS

Intestinal infection of *T. canis* in stray dogs

Our study showed the percentage of infection with *Toxocara canis* in stray dogs reach to 3.84% (1 from 26)

Table (1): percentage of infection in stray dogs

Infection rate in stray dogs	No.
Tested	26
Positive	1
Negative	25
Percentage of infection	3.83

Table (2): Effects of light and parasitic treatment on changes overtime in mean \pm S.D of melatonin levels (pg/ml).

Group	3 h	6 h	12 h	24 h	3 day	7 day	14 day	28 day	56 day
Red (+)	581.2 \pm 3.13	485.5 \pm 13.13	514.8 \pm 73.07	377.6 \pm 100.86	560.3 \pm 43.45	474.3 \pm 33.20	477.3 \pm 66.95	427.3 \pm 53.48	412.7 \pm 60.01
Red (-)	526.7 \pm 39.81	504.4 \pm 2.76	490.9 \pm 11.23	644.9 \pm 9.25	297.3 \pm 7.24	408.1 \pm 7.24	491.0 \pm 46.45	512.1 \pm 35.32	618.3 \pm 34.13
Control (-)	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50	510 \pm 44.50
Blind (+)	567.4 \pm 3.69	503.0 \pm 2.04	458.7 \pm 32.28	528.8 \pm 63.36	502.3 \pm 21.22	469.5 \pm 56.47	526.3 \pm 10.21	534.5 \pm 9.60	534.5 \pm 9.60
Control (+)	544.3 \pm 20.10	484.6 \pm 68.79	485.7 \pm 54.57	532.1 \pm 29.68	624.4 \pm 66.85	478.1 \pm 32.60	436.6 \pm 85.14	389.3 \pm 32.14	539.8 \pm 104.1

L.S.D (type \times time) =74.903

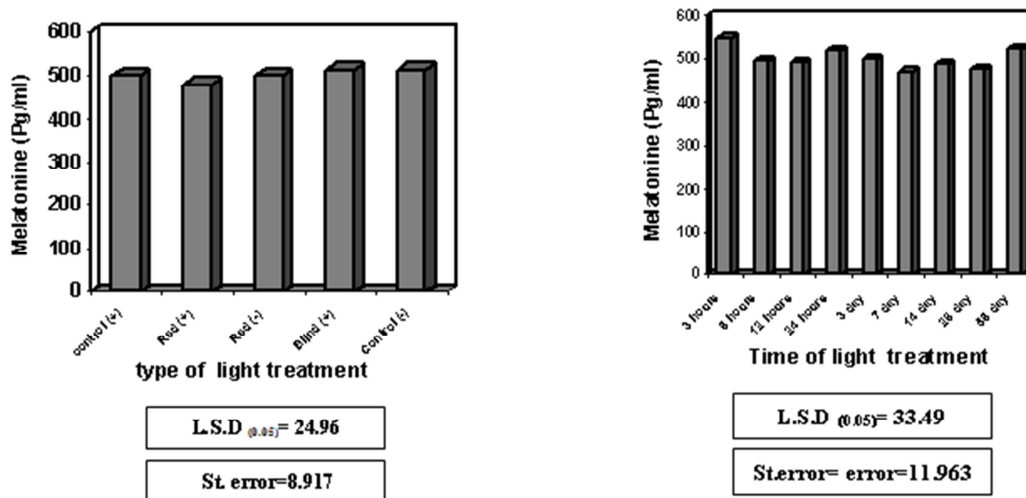


Figure (1): Effect of total type and time of parasite and light treatment on change overtime in mean \pm S.E of melatonin levels(pg/ml).

Table (3): Effects of light and parasitic treatment on changes overtime in mean \pm S.D on Interlukin-1 β concentration (pg/ml).

Group	3 h	6 h	12 h	24 h	3 day	7 day	14 day	28 day	56 day
Red (+)	19.93 \pm 1.0	19.6 \pm 0.65	19.2 \pm 0.17	18.80 \pm 0.70	18.33 \pm 1.79	16.63 \pm 0.90	15.36 \pm 1.87	12.80 \pm 0.91	17.73 \pm 1.05
Red (-)	23.60 \pm 0.52	18.03 \pm 0.23	20.4 \pm 0.51	16.86 \pm 1.09	18.33 \pm 0.25	15.03 \pm 0.65	17.86 \pm 1.70	14.53 \pm 0.75	17.03 \pm 0.57
Control (-)	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12	41.70 \pm 21.12
Blind (+)	15.40 \pm 1.30	21.16 \pm 4.72	19.03 \pm 1.95	19.23 \pm 3.05	14.53 \pm 0.11	15.30 \pm 0.51	15.16 \pm 0.15	13.83 \pm 2.36	13.83 \pm 2.36
Control (+)	17.53 \pm 1.19	17.46 \pm 1.01	18.60 \pm 0.78	17.36 \pm 0.46	17.53 \pm 2.65	16.03 \pm 1.05	17.40 \pm 0.36	16.56 \pm 0.46	14.0 \pm 1.0

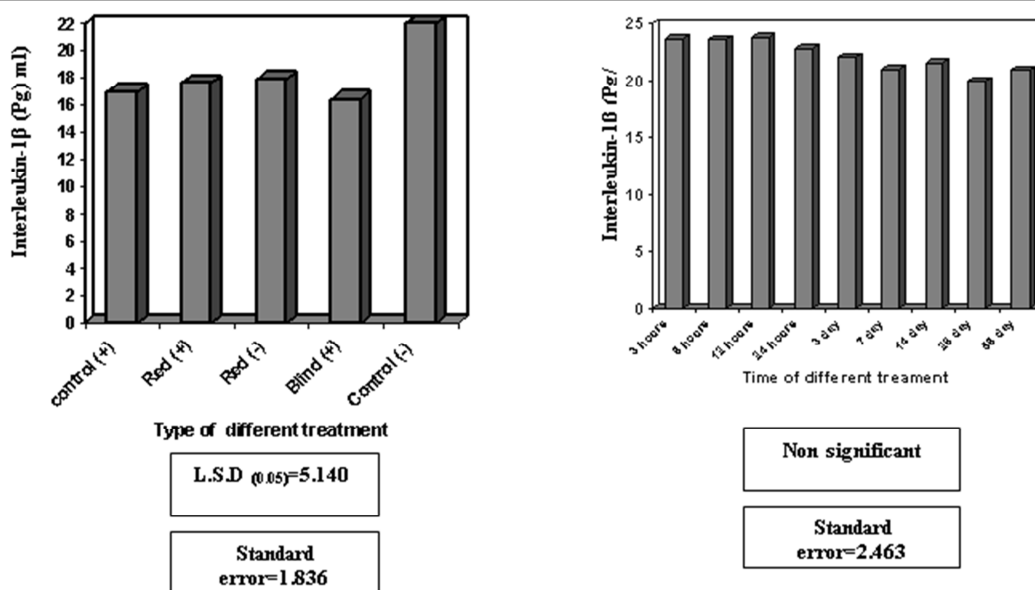


Figure (2): Effect of the total time and type of parasite and light treatment on change overtime in mean \pm S.E of interleukin-1 β level (pg/ml)

Table (4): Effects of light and parasitic treatment on changes overtime in mean±S.D on Interlukin-6 concentration (Pg/ml).

Group	3 h	6 h	12 h	24 h	3 day	7 day	14 day	28 day	56 day
Red (+)	113.26 ±23.26	96.73 ±7.55	91.5 ±3.5	80.33 ±1.52	76 ±13.43	105.93 ±24.61	95.83 ±23.03	83.26 ±9.64	76.56 ±2.75
Red (-)	120.26 ±35.24	84.56 ±11.57	66.8 ±2.16	81.86 ±3.82	72.43 ±2.5	85.03 ±13.4	72.1 ±1.65	77.26 ±1.6	126 ±2.64
Control (-)	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32	96.06 ±3.32
Blind (+)	76.73 ±0.8	88.63 ±2.44	121.16 ±16.34	110 ±23.06	94.36 ±3.15	98.133 ±7.68	82.56 ±3.12	104 ±7.21	104 ±7.21
Control (+)	83.36 ±19.17	72 ±6	90.13 ±0.2	99.6 ±20.49	65.7 ±3.65	113.53 ±13.08	69.06 ±12.97	83.73 ±11.63	72.26 ±2.82

L.S.D (type×time)=18.66

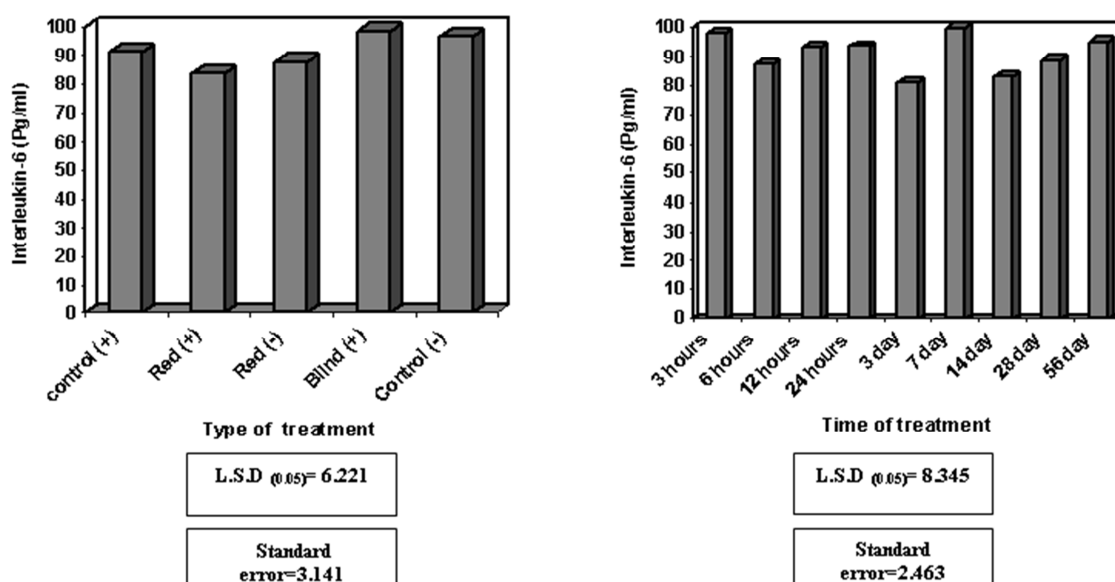


Figure (3): Effect of the total time and type of parasite and light treatment on change overtime in mean ±S.E of interlukin-6 level(pg/ml).

Plasma melatonin following exposure *T.canis* with and without red light

Unianova by type of stress and time revealed significant main effect of types of stress, time and type×time (F=12.357, P=0.05, F=4.247, P<0.0001 and F=6.275, P<0.001 respectively). Post hoc analysis revealed a significant effect of toxocora treatment on plasma melatonin in rat after 3 day (624.4 pg/ml) and in 28 day (389 pg/ml) as compare with control negative (510 pg/ml) and other time of control positive where the L.S.D for time×type was 74.903. Melatonin concentration in red light and treatment by Toxocara was reduce significantly in 24 hour, 28 day and 56 day (377.66, 427 and 412 pg/ml) respectively as compared with control or with other time groups. Rat plasma melatonin that exposure to the red light only revealed a peak concentration at 24 hour after exposure (644.966 pg/ml) and decrease significantly in 3 day (297.33 pg/ml). Melatonin concentration also showed increasing by increase the red light exposure from three day to the 56 day (297.33, 408.13, 491.06, 512.13 and 618.36 pg/ml). Unianova indicated that there are no any effect of blindness and Toxocara infection in the change of melatonin concentration (Table-1). The multiple comparisons between the type group also showed the significant differences by using L.S.D. the results revealed differences between red positive group with blindness positive group and control negative group. Multiple comparison between

homogenous subsets (time×time) also showed multiple significant differences if applied the L.S.D value with another subjected times .

There were significant effects of the total time on the changes in means±SE (Figure-1). The data also showed the significant effect of types as a total on melatonin concentration where the significant differences occur between red positive and control negative group and between red positive and blindness group only (Figure-1)

Plasma Interlukin-1 β following exposure *T.canis* and red light exposure.

Unianova showed a significant differences for type (F=35.663, P<0.005), whereas there are no significantly for time and type×time (F=0.331, P>0.952; F=0.098, P>1.00).

Post hoc tests revealed significant differences between types group as compare with control negative, in all control animals the mean Interlukin-1 β was 41.7 pg/ml, but decreased as rang from 23 to 13 pg/ml in all types group after different kinds of stress.

The intercept between time groups the factorial with C.R.D analysis did not have any effect on serum Interlukin-1 β levels after all stress (Figure-3).

The present study showed there are no effect of the time as total on the interlukin-1 β , whereas the types of stress (as total) showed the effects on the IL-1 β concentration especially between the control negative group with other series stress groups (Figure-3).

Plasma Interlukin-6 after exposure red light & *T. canis* treatment

A significant differences was revealed between types, times and intercept type×time (F=971.98, P=0.001; F=621.33, P=0.001 and F=133.26, P=0.001 respectively). Post hoc test for types×time in red positive (Toxocara infected with red exposure) revealed the mean interlukin-6 its peak in 3 hour after infection (113.26 pg/ml) and decrease by times from 80-90 pg/ml except the 7th day (105 pg/ml). In opposite of this in animals that infected and exposure to the control positive the plasma interlukin-6 rat decrease in 3 hour of infection and increase until reach to 113 pg/ml in 7 day after exposure. Red light exposure only showed two peak in 3 hour and 56 day after exposure (120.26 and 126 pg/ml), whereas the other time groups did not reach to the 85 pg/ml.

In blindness groups the plasma interlukin-6 rats increased in 12, 24 hour, 28, 56 day after infection (121, 110, 104, 104 respectively) as compare with other time blindness groups, between the types alone and times alone the post hoc tests also showed the significant differences see (Table-2).

Plasma interleukin-6 concentration also showed the significant differences between types and time of groups (as total) at LSD_(0.05) for time= 8.345 (Figure-2).

DISCUSSION

Melatonin and wavelength

Our data suggest that the continuous red light exposure at wavelength 654 nm from three day to 56 day was significantly decreased in melatonin concentration (Table-1).

Also, the same finding with some different was found in red light positive group (377.66, 427 and 412 pg/ml for 24 hour, 28 day and 56 day respectively). The numerous studies showing that exposure to the short wavelength in interval 470-525 nm has the most robust melatonin suppression effects. Poeggeler *et al.* (1995) showed that melatonin declined linearly in albino rats in different intensities of red light (600-690 nm) during the middle of night. The high intensities of red light was as effective as white light (780 nm) to suppression of NAT activity and melatonin concentration, this result was agree with our data where the red light (654 nm) exposure showed the declined the melatonin secretion in albino rat.

Honma *et al.* (1992) also showed the effects of light on the pineal and plasma melatonin in Wistar and long-Evans rats at two different times. The green light pulse (520 nm) given at 24.00 h suppressed the pineal and plasma melatonin to the day-time level for at least 2 h while the red light (660 nm) at same time of the day suppressed melatonin only transiently and did not suppress the plasma melatonin at all. These study confirm our data that the melatonin effected by the wavelength and the circadian phase, where the red light (654 nm) suppress melatonin and the loss of visual blindness group don't effected by light loss. Since Berson and co-worker (cited by Chellappa *et al.*, 2011) detected intrinsic photosensitive retinal ganglion cell (IPRGC) in mammalian retina, it began to emerge that the eye play a dual role in detecting light for a range of behavioral and physiological responses a part from the classical visual response. This melanopsin-containing (IPRGC) have specialized non-visual retino-hypothalamic tract which provides direct neuronal connections to suprachiasmatic nucleus as well as direct and indirect (via SCN) projection to the brain area. This cell are most sensitive to short-wavelength (blue) light 480 nm and the absorption spectrum of the melanopsin is distinct from the absorption spectra for the rods or cones. Circadian phase resulting and alerting effect of light are also short-wavelength sensitive in humans, this suggestion the novel non-rod and non-cone photoreceptor system primarily mediated a wide range of non-visual effect of light (Lockley *et al.*, 2007).

Our results also supported this findings, in blindness group have approximately the same concentration (458±32, 567±3.69), because we used physical blindness by closed the eyelids thus the melanoepsin don't work. Jean-Louis and co-workers (2005) suggest that the ophthalmic and illumination factor might have an additive

effect on the timing acrophage of melatonin excretion (6-sulphatory-melatonin). Factors that may contribute to these individual differences include light exposure history, iris color race, gender and age. Light history has been shown to influence to light- induce melatonin suppression and these findings have recently been extended to circadian phase shifting (Canton *et al.*, 2009).

The present investigation also showed that there is no significant effect on parasite treatment on circadian melatonin rhythm on blindness group as compare with control positive and control negative. This result can may be illustrate the role of high level of melatonin (in blind rats) to attenuated the parasite migration to the brain and effected on brain tissues .

Melatonin and *T.canis* with and without red light exposure.

According to recent experimental findings, stress-related events are also related with to melatonin alterations in animals and humans. For example, repeated maternal separation and deprivation caused low blood melatonin levels and a significant negative correlation between blood melatonin levels and spatial memory performance in both male and female a descent rats, which suggest an association between melatonin production and neurodevelopment (Vysal *et al.*, 2005). Physical-immobilization stares in laboratory rats led to a significant increase of pineal melatonin level, psychosocial stress also may induce a robust increase of melatonin metabolite 6-sulffatoxy melatonin in subordinate animal (Bob and Fedor-Freybergh, 2008).

In human, stress may cause sleep disturbances such as in somina, and reduce of pineal melatonin secretion that is often present in depressed patients on the other hand the melatonin receptors are also present in regions that participated in stress response, such as the hippocampus and the adrenal gland. Bob and Fedor-Freyberh (2008) showed that the pineal gland expresses a high density of glucocorticoid receptors, suggest that the gland may be a target site for glucocorticoid damage during stress and it is similar as other regions, such as the hippocampus, which is highly sensitive to stress and prolonged glucocorticoid secretion during chronic stress may have deleterious effect to the pineal gland. Many studies showed that the decreased melatonin levels in patients with depressive disorder were reported although melatonin increase has also been documented (Pacchierotti *et al.*, 2001). Typical melatonin alteration have also been found in schizophrenia and suggest that diminished melatonin secretion may be associated with the pathophysiology of subgroup of schizophrenic patients, and that a subnormal plasma melatonin level may be a marker of a subgroup of schizophrenic patients (Bob and Fedor-Freybergh, 2008). Our investigation showed that stress-related events are also related to the melatonin alterations in albino rats especially in red light groups where the plasma melatonin decreased significantly until reach 297.33 ± 7.24 after 3 day of red light exposure and 377.66 ± 100.86 in the positive red light group after 24 hours of red light and parasite treatment. The melatonin increased significantly after 24 hours and 56 day of red light exposure and after 3 day of parasite treatment where reached to 624.43 ± 66.85 as compare with control and other time related and untreated groups.

Our data in agreement with previous findings that says the melatonin is decreasing and increasing with the stress. Where our results showed the time related melatonin level decreased or increased in different type of stress. These data suggest that the pineal gland may be significantly affected by stress and in other hand suggest that the melatonin alteration represent an important neuroendocrinologic marker of psychopathological dysfunction.

Our new hypothesis, we can say that the alteration of melatonin concentration is considered as attempts of the host for dealing with the stress (Shift to right), for example, in parasite treatment group the plasma melatonin is increased significantly after 3 days of infection whereas decrease step by step until reach significant value (389.33 ± 32.14) after 28 day of infection, finally reach to resemble control value in 56 day (539.83 ± 104.1). These data may illustrate the host attempts to reach the normal value. This also gives us the hormonal mapping for dealing with stress and titration dose or concentration in different time for healing. This is hypothesis may be applied for red positive and red negative groups, where there are many differences between host response to the different type of stress. For example melatonin concentration were 377.33, 644.96 and 624.43 for red positive, red negative and control positive groups respectively after 24 hours of stress, the melatonin concentration in different sort of stress indicated that this differences may be correlated with host responses but not to the parasite infection or light exposure. On the other hand, we can hypothesize the opposite , where the alteration of hormones concentrations are parasite attempts to change the physiological host for example to decrease melatonin concentration that its responsible for immune elevation and activation of precursors of macrophage and its interleukins. The exposure to environmental (external) or physiological (internal) stress may be have a positive or negative effect on parasites infection and this depend upon the kind of stress (external and internal), time of stress, family history for exposure of history, adaptive for stress or not and classify this stress (acute or chronic). The psychological stress also may lead to changing for host behavior this is may be suitable for parasite to complete life cycle or for reproduction and reverse is true. Melatonin concentration, for example, is dark light rhythm harmony, the wavelength decreasing or increasing or light exposure increasing lead to conversion of the melatonin concentration and many physiological, immunological and hormonal parameter that may be causing

the parasite spread or recede go back in the host. On the other hand, this parameter may change the most characteristic feature for host, for example, the free-running and fast walking is main feature of rodents, the reduce of melatonin can cause the change the bodily movement (previously mention) and this more benefit for predator, finally the parasite complete its life cycle. These strategies for reactions and response of reactions are interchangeable from stress and host on the other hand, and the hormonal variation may be describe or depicted this strategies in the host.

Interlukin-6 after parasite treatment and red light exposure

Studies in rodents indicate that a variety of severe stressors, such as foot shock, physical restraint and open field exposure, stimulate increases in plasma IL-6 concentrations (Steptoe *et al.*, 2001). Our results showed the increasing interlukin-6 after three hour of exposure red light, where the red light group showed the two peaks of plasma IL-6 concentration in 3 hours (120.26 ± 35.24) and in 56 day after exposure (126 ± 2.64). The lowest plasma IL-6 concentration in 12 hours after red light exposure where reached to 66.8 ± 2.16 (Table-4). Red light positive group showed increasing in the beginning and decrease step by step until reach to 76 ± 13.4 pg/ml in 3 day after exposure and elevated to 105.93 pg/ml in 7 day after exposure and also decreasing by progressive the time until reach 76.56 in the 56 day of exposure. The control positive group in contrast showed decreasing in plasma IL-6 concentration in different times after parasite treatment where the IL-6 concentration reach to 65.7 ± 3.65 pg/ml in 3 day after treatment (Table-4). In blindness positive group the IL-6 concentration is decrease at beginning (76.73 ± 0.8) and increase significantly in 12 hour of treatment (121.16 ± 16.34) as compare with negative control and time blindness groups. As a total our data has been showed the significant differences between types except between control positive group and red negative group, red (+) and red (-) and finally between control (+) and blindness (+) with control negative group (Figure-7). Time as total also showed significant differences at $L.S.D=8.345$. Change in plasma IL-6 concentration (Figure-2). Our data has revealed variation in IL-6 concentration overtime (in different stress). The present study showed that the external stress resemble by red light at 654 nm only caused to elevated the interlukin-6 concentration at 3 hours of exposure and begin to decreasing in other time except 56 day after infection, whereas the companion with internal stress (parasite treatment) the interleukin concentration become different if we compare with other same time group in different stress. These also can be applied between the control and blindness positive group. This variation also indicated firstly that the IL-6 was so related with psychological stress and secondly our investigation by series of experimental showed the IL-6 changing overtime of stress, making it conceivable idea that the IL-6 affected by the duration and type of the stressors.

Here we also can hypothesize that the increasing and decreasing of IL-6 concentration illustrate the recovery mapping or the way map by which the host reaches to the normal condition. The interlukin-6 concentration variability in dark-light stress group also correlated with melatonin secretion because the last its secretion rhythmically by light-dark exposure and its very sensitive to the wavelength as previously mentions. The melatonin affected in macrophage activation thus may be the IL-6 increasing or decreasing is depending upon the variation of the melatonin secretion. On the other hand may be found the interaction or balance between the cytokine (IL-6) and hormone (melatonin) to deal with the stress, when the melatonin is elevated the interlukin-6 decreasing and revers is true. The number of stress also can cause un stabilizing in cytokine secretion as compare with one stress alone. This can be added to the factor that effected on immune suppression or elevation by stress.

There are many studies coincided with our result that improve the relationship between stress disorder and elevated or decreasing of IL-6 according the type of stress. Maes *et al.* (1999) showed serum IL-6 concentration significant higher in post-traumatic stress disorder patient than in normal volunteers. Goebel *et al.* (2000) the *in vitro* production of the IL-6 and TNF- α was differentially affected depending on the type of stimulus.

Zhu *et al.* (1996) suggested that the cold water stress (5 min. for 4 day) increasing the IL-6 concentration in mice by LPS as compare with treatment with LPS only. Lemay *et al.* (1990) study, where the exposure rats to the open field caused to increase to IL-6. Bharosay *et al.* (2011) found the significant correlation between stroke scoring and serum IL-6 in the patient at the time and after 7day after admission. Friedman and Hero (2010) showed the inversely associated between IL-6, CRP and fibrinogen with both income and education. On the other hand regression analyses showed that the plasma interlukin-6 level were lower in women scoring high on positive relation- ships, whereas IL-6R level were lower in women scoring on purpose in life (Friedman *et al.*, 2007). Carpenter *et al.* (2010) showed the association between plasma IL-6 response to acute stress. Meyer *et al.* (2011) were showed the highly significantly association between the cognitive-affective symptoms of depression and elevated serum levels of interlukin-6.

Our finding revealed that the red light causes the IL-6 secretion in three hours after exposure as compare with control and blindness positive groups. The result of our investigation can be use red light for short period (such as twelve hours) for light therapy, where in our results the red light causes the interlukin-6

significantly inhibition as compare with control negative group and 3 hours, 6 hours red light groups. According to our result may be used the alteration of interleukin-6 as biomarker for stress indicator, this conclusion in agreement with many studies that used IL-6 for psychological biomarker.

Interlukin-1 β after parasite treatment and red light exposure

Our data disagreement with previously study (Bomum *et al.*, 2008; Nguyen *et al.*, 1998; Blandin *et al.*, 2006; Deak *et al.*, 2003 and 2005), where all of these studies showed positive relationship between IL-1 β and stress, in different area of brain. This is may because the different between plasma and brain IL-1 β detection thus the differences have to be occur. Also the continuously exposure for stress in our study have to be concerned for this differences.

Although, the findings are inconsistent with these previous study that suggest the different stress cause increasing the IL-1 β . In other hand, there are no any studies showed the interaction between our study stress (red light, blindness, toxocara infection) with IL-1 β . Our samples provided sufficient power to detect relatively small indicator for red light exposure, blindness and *Toxocara* treatment to decreasing the interleukin-1 β in different times.

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