Original Research Article

Effect of Direct Sunlight on Plasma Vitamin-D and Histo-pathology of Some Selected Organs of Mice

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ABSTRACT

An experiment was done with a view to observing the effect of direct sunlight on body weight plasma vitamin-D and histo-pathology of skin, liver and kidney. A total of 15 apparently healthy mice aged between 40 and 45 days with an average body weight of 28.8 ± 0.57 gm were used in the study. Mice were randomly divided into 3 equal groups. They were fed on normal mice pellet throughout the experimental period of 70 days. One group of mice was kept inside the room throughout the entire period of experiment and was considered control (Group A) whereas remaining two groups (B and C) were exposed to sunlight every other day in the morning. Between these two groups, A was exposed to sunlight for 1 hr and the B group was for 1.5 hr. Body weight was recorded on the first day, on 35th day and on the last day of the experiment. Blood and tissue samples (skin, liver and kidney) were collected at the end of the experiment by sacrificing the mice. The body weight was found significantly ($p< 0.01$) higher in all mice with the advancement of experiment and highest weight was recorded at the end of experiment. The plasma vitamin-D was altered in the mice exposed to sunlight compared to control. The mice exposed to sunlight daily for 1.5 hour exhibited much lower ($p< 0.05$) vitamin-D in their plasma compared to control group of mice. In histological investigation, skin did not show any alteration irrespective of group but venous congestion in liver and kidney was recorded in both the treated groups which is thought to be due to heat stress prevailing in the area of experiment.

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Comment [A3]: Gender of the mice to be specified

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Key words: Mice, sunlight, Vitamin-D, blood.

1. INTRODUCTION

Vitamin-D is a hormone like fat soluble nutrient plays a wide range of functions. Without vitamin-D, the body cannot absorb calcium and phosphorus accurately. It is found in broad range

in sunlight. Vitamin-D is produced in skin exposed to sunshine (Rathish and Arun, 2012). Out of this, it is also found in dietary factors such as cod liver oil, fortified milk, egg yolk, yogurt-cheese and margarine etc. It supports in the improvement of heart and blood vessels, maintains cholesterol level in blood and plasma. Over this it prevents colon, prostate, and breast cancers. It has also effects on esophagus and lymphatic system. The researcher reported that sunlight has beneficial effects on cardiovascular disease independently of vitamin-D (Weller, 2016). Other researchers reported vitamin-D_3 on survival and the host's immune response in experimental bacterial meningoencephalitis in mice after intra cerebral *E. coli* infection (Marjia, 2015). To produce different systemic vitamin- D_3 concentrations, mice received a low, standard, or high dietary vitamin- D_3 supplementation. Epidemiological evidence suggests that vitamin-D deficiency is associated with anemia. Stimulation of eryptosis may limit lifespan of circulating erythrocytes and thus cause anemia in mice (Lang *et al*., 2015). However, little is known about the effect of vitamin-D deficiency on host pulmonary defense to *Aspergillus fumigatus* (*A. fumigatus*) (Pei *et al.,* 2014). Experimental autoimmune encephalomyelitis development was markedly suppressed in mice lacking the vitamin-D receptor and partially suppressed in vitamin-D insufficient mice. The absence of either of the two key hydroxylase neither inhibits nor enhances the development of experimental autoimmune encephalomyelitis (Wang *et al.,* 2012). The ultraviolet radiation in sunlight has both positive and negative health effects, as it is both a principal source of vitamin-D_3 and a mutagen. A dietary supplement can supply vitamin-D without this mutagenic effect, but bypasses natural mechanisms that would prevent overdoses of vitamin-D generated internally from sunlight. Vitamin-D has a wide range of positive health effects, which include strengthening tissues and possibly inhibiting the growth of some cancers. UV exposure also has positive effects on endorphin levels, and possibly for protection against multiple sclerosis. Visible sunlight to the eyes gives health benefits through its association with the timing of melatonin synthesis, maintenance of normal and robust circadian rhythms, and reduced risk of seasonal affective disorder. But long-term sunlight exposure is known to be associated with the development of skin cancer, skin aging, immune suppression, and eye diseases such as cataracts and macular degeneration. Short-term over-exposure is also the cause of

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sunburn, snow blindness, and solar retinopathy. UV rays, and therefore sunlight and sunlamps, are the only listed carcinogens that are known to have health benefits, and a number of public health organizations state that there needs to be a balance between the risks of having too much sunlight or too little. There is a general consensus that sunburn should always be avoided. In Bangladesh, there is no published report with plasma vitamin-D in mice no matter how long they are exposed to direct sunlight in the open field or on the roof of the building. Therefore, the proposed study has been designed using the mice as the model animal with the following objectives: evaluate the effects of direct sunlight exposure on plasma vitamin-D concentration in healthy mice, and observe the effects of direct sunlight exposure on healthy tissue morphological changes of skin, liver and kidney in mice.

2. MATERIALS AND METHODS

The study was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The experiment was carried out from February 2016 to May 2016 for a period of 70 days.

2.1 Animals

Fourty Swiss Albino Mice (*Mus musculus*) were collected from Pharmacy Department, Jahangir Nagar University, Savar, Dhaka-1342, Bangladesh. The body weight of mice was approximately 27.0 gm and the age was between 40 and 45 days. Mice were supplied with broiler feed. Pellet was supplied at least at a dose of 15 gm/mice/day for entire period of the experiment. Feed and water were provided *ad-libitum* during the experimental period.

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Comment [A7]: Gender of the mice to be specified

2.2 Experimental Design

A total of 15 mice were randomly selected from 40 mice after 7 days acclimation for the present experiment. Mice were divided into 3 equal groups (A, B & C) containing 5 mice in each group. Group A was fed on standard broiler pellet throughout the experimental period and was considered as control group. The remaining groups were considered as treated groups (B and C). Mice of group B & C were directly exposed to sunlight for 1 and 1.5 hours, respectively. The experiment was conducted as per the following layout (Fig. 1).

Comment [A8]: 5 in each group. Is it statistically acceptable?

2.3 Collection of Blood and Plasma

At the end of the experiment blood samples were collected directly from heart of anesthetized mice. Before applying anesthesia the mice were kept on fasting overnight. Then the mice were placed inside the desiccators containing cotton soaked with chloroform. After being completely anaesthetized, the abdominal and thoracic cavity was surgically opened and the heart area was pointed. Blood was collected directly from the heart. About 1-1.5 ml blood was drawn from each mouse. Blood was stored in the test tube containing anticoagulant (3.8% sodium citrate).

Test tube containing blood with anticoagulant was placed in ice for 30 minutes. Plasma was separated from unclotted blood by centrifuge at 3000 rpm for 20 minutes and again for 10 minutes. Then supernatant was collected in eppendorf tube by micro-pippette and stored in refrigerator at -20°C until use for biochemical test.

2.4 Collection of Target Tissues for Histo-pathological Analysis

The target organs (skin, liver, kidney) were collected irrespective of sexes as soon as possible and fixed with 10% formalin (pH 7.4) for histological analysis.

2.5 Light Microscopy

Paraffin embedded blocks of skin, liver and kidney specimens were cut at a thickness of 5 µm to obtain semi-serial sections and stained with Hematoxylin-Eosin (HE) to examine the histo-pathological characteristics of these structures. Briefly, for the routine HE staining, at first the sections were deparaffinized by several changes of xylene for 15 minutes in each step. Then rehydration was carried out by a series of descending grades of alcohol (100%, 100%, 95%, 80%, 70%). In each step the incubation time was 20 minutes. Running tap water was used to wash off the slides for 10 minutes. Then the slides were rinsed with distilled water. After that, the tissue sections were stained with hematoxylin solution for 15 minutes. The slides were rinsed into distilled water again. Then the sections were immersed in eosin solution for 2 minutes. The dehydration process was done with a series of ascending grades of alcohol (70%, 80%, 95%, 100%, 100%, 100%). The incubation time was 20 minutes. Finally, several changes of xylene

were used to clean the slides for 20 minutes in each change.Finally, a cover slip was mounted over the tissues samples on the slide, using optical grade non-aqueous mounting medium DPX.

The detailed histo-pathological study was done by using a light microscope. Necessary photographs were taken with Olympus BX 51 photographic light microscope and placed for better illustration of the result in the Department of Anatomy and Histology, BAU, Mymensingh.

2.6 Statistical Analysis

Data were presented as mean ± SE (Standard Error). All the collected data were analyzed using Statistical Package for the Social Sciences (SPSS; version 11), *P*-value below 0.05 considered statistically signifi

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3. RESULTS AND DISCUSSION

3.1 Effects of Sunlight Exposure on Body Weight

The body weight changes are shown in Table 1. On day 1, day 35 and 70 body weight of mice kept as control was (28.8 ± 0.5) gm, (35.2 ± 0.9) gm and (41.4 ± 1.0) gm, respectively. On the other hand, mice of group-B showed (28.0 ± 0.7) gm, (36.9 ± 1.3) gm and (41.2 ± 1.1) gm on day 1, day 35 and 70, respectively and group-C, showed (27.6 ± 0.7) gm, (35.4 ± 1.9) and (41.0 ± 1.5) gm, respectively. These data indicate that the body weight of mice in all groups increased with the advancement of time. In each group, the body weight gain was significant ($P < 0.01$). But the rates of increasement among all mice groups were very similar ($P < 0.01$). There was no effect of sunlight in mice group B and C but the weight was upgrading gradually. The body weight of the control group and the experimental groups were similar. When the experiment was set up the body weight of mice of each group was similar on day 1, but on day 35 and day 70 body weights was gradually increasing from the beginning. The study resulted that the body weight of healthy mice was increased for a certain period of time and after 70 days the increased body weight was more or less similar of all experimental groups. A study suggested that exposure to moderate amounts of sunshine may slow the development of obesity and shining UV light at overfed mice slowed their weight gain (Geldenhuys *et al*, 2014). The direct sunlight-derived ultraviolet radiation (UVR) increases normal body weight (Geldenhuys *et al*, 2014) and the finding in this

Comment [A10]: The increase in body weight can be an effect of normal growth.

study fully agree with this statement. Moreover, the body weights of mice that were fed diets with low, recommended or high levels of vitamin-D_3 for 16 weeks did not differ (Nadine *et al.*, 2012) and the results of this present study highly agree with these findings.

3.2 Concentration of Plasma Vitamin-D Exposed to Sunlight in Mice

Table 2 show the effects of sunlight exposure and plasma vitamin-D in mice. Group A used as a control which was non-exposed to sunlight containing plasma vitamin-D (39.8 ± 1.1) nmol/L. The treated mice of group B exposed to direct sunlight daily for 1 hour had plasma vitamin-D (40.6 ± 8.0) nmol/L. But the treated mice of group C exposed to direct sunlight daily for 1.5 hours showed (24.8 ± 2.7) nmol/L plasma vitamin-D.

The direct sunlight in group B and C caused a significant ($P< 0.05$) increase of plasma vitamin-D in mice than in the mice of control group (A). Among the sunlight treated mice, the Vitamin-D value was significant ($P< 0.05$) in group C lower (24.8 ± 2.7) nmol/L compared to mice of group B (40.6 ± 8.0) nmol/L vitamin-D. Vitamin-D deficient diet induced significant reductions in plasma vitamin D ($P< 0.001$) (Ellam *et al.*, 2014) and the finding in this study partially agreed with this statement.

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Comment [A12]: How do you explain this?

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3.3 Histo-pathology of Liver

There was a morphological alteration such as congestion in the central vein present in control group (Fig. 2a). There were no degeneration or necrotic changes were observed in the control group like other treated groups. In the group B, the liver showed congestion in the central vein (Fig. 2b). In the group C, the liver parenchyma showed diffuse lymphocytic infiltration (Fig. 2c) extending from portal areas. The histology of liver showed the degenerative changes of parenchymal cells and central vein congestion but there were no morphological changes of all sunlight exposure groups including control groups. These findings have similarity in rat where enlarged and dilated hepatic vein along with no degeneration of hepatocyte with and excessive hepatic cell necrosis (Shweta *et al.* 2013; Altunkaynak and Ozbek, 2009). Liver degenerations

might be mitigated by supplementing vitamin-D (Shweta *et al*. 2013). These changes are found in sunlight exposed mice.

3.4 Histo-pathology of Kidney

The kidneys of exposed mice of all groups showed morphological alteration is likely to the control group (Fig.3a). The kidneys among the control and treated groups, showed mild to large amount of congestion. Changes increased with time frame of sunlight exposure. In the group B, kidneys parenchyma revealed congestion and degeneration (Fig.3b). In the group C, kidneys parenchyma showed demarkated congestion and diffuse lymphocytic infiltration (Fig.3c)

which is a major circulating form and 1,25-dihydroxyvitamin-D which is the biologically active form respectively (Alexandra *et al.* 2013). 1, 25-dihydroxyvitamin-D plays an important role in regulating calcium and phosphate metabolism for maintenance of metabolic functions and for skeletal health. The cause of histological alteration in mice of all groups is beyond scientific explanation but is may be due to heat stress excited during the time of experiment.

3.5 Histo-pathology of Skin

The skin of exposed mice of all groups showed no morphological alteration in comparison to the control group (Fig. 4a-c). There were no histological changes of skin in the time of sunlight exposure and non exposure groups. During exposure to sunlight 7 dehydrocholesterol in the skin absorbs UV B radiation and is converted to previtamin-D_3 which in turn isomerizes into vitamin-D_3. Previtamin-D_3 and vitamin-D_3 also absorb UV radiation and are converted into a variety of photoproducts some of which have unique biologic properties. Topical 1,25-dihydroxyvitamin-D (1,25D) and other vitamin-D compounds have been shown to protect skin from damage by ultraviolet radiation in a process that requires the vitamin-D receptor (Hill *et al*. 2014).

The mole rats were exposed to UVC radiation for a significant period and the rats were found to have changes in the skin histology (Mustufa *et al.,* 2004). In the present study, since the exposure to sunlight only 70 days (1-1.5 hours daily), this was unable to induce any change in skin histological pattern.

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4. CONCLUSION

The findings of the present study is not uniform and is little difficult to explain in making a good interpretation. Plasma vitamin-D is synthesized in the body and may improve several systems. The results of the present study indicated that there was no effect of sunlight in body weight of mice. It has also been revealed that sunlight exposure causes alteration in the vitamin-D concentration of plasma and histo-pathological changes in some vital organs. The present study is a primary overview on the use of sunlight to produce vitamin-D in plasma. There were many limitations during this study such as environmental condition, heat stress and duration of sunlight exposure on mice.

Comment [A15]: There are no useful conclusions drawn from the experiment. The study may be repeated by controlling the environmental conditions.

5. REFERENCES

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Figure 1. Layout of the experiment

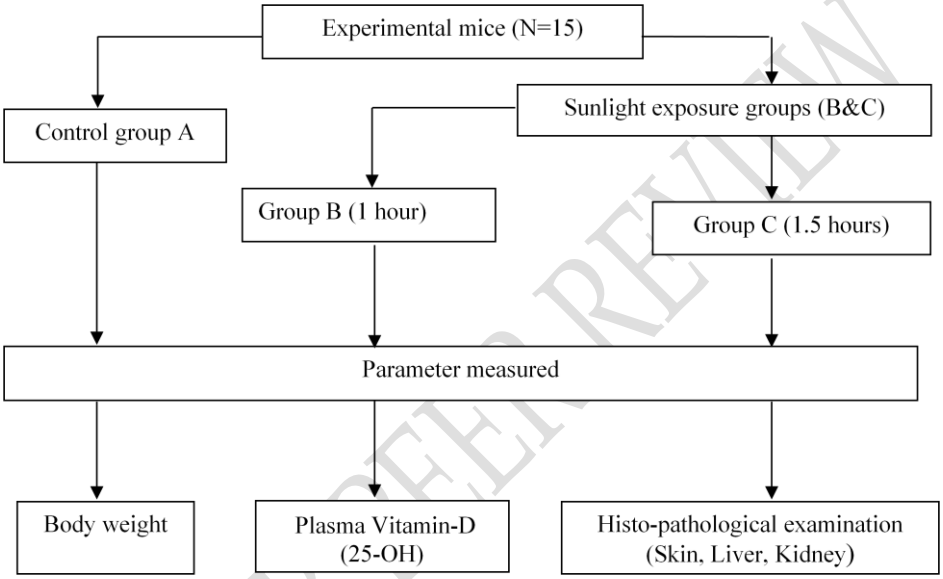

Figure 2. Histo-pathology of liver

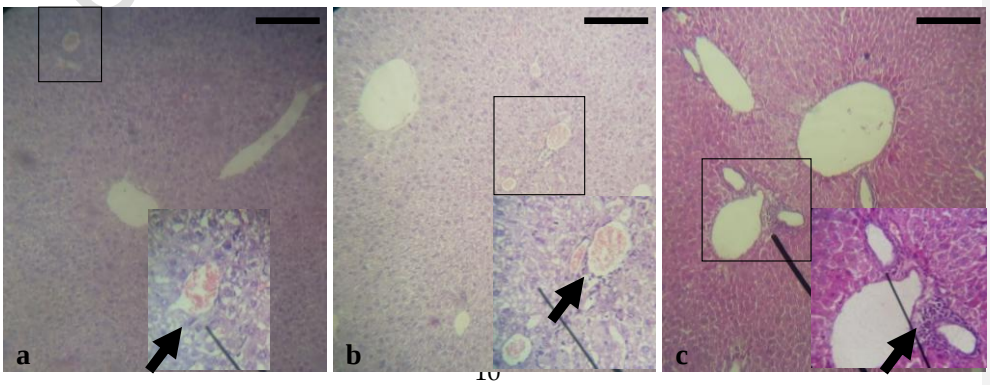

Figure 2. Histo-pathology of liver of control group A of mice (a) and sunlight exposed group B and C of mice (b and c). Group A and B shows congestion (black arrow in Fig. a and b) of the central vein. Group C showed diffuse lymphocytic aggregation (black arrow in Fig. c) in portal area. HE staining (10X) and bars 100µm.

Figure 3. Histo-pathology of kidney

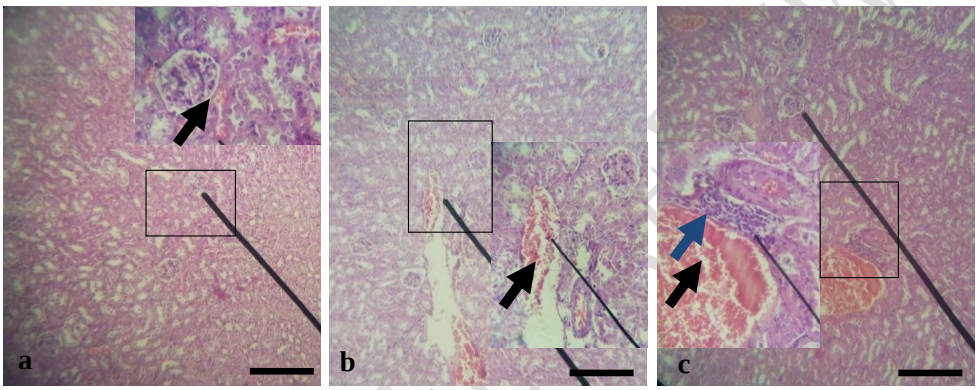

Figure 3. Histo-pathology of kidney of control group A (Fig. 3a) and sunlight exposed group B and C of mice (Fig. 3b and c). Group A and B show congestion (black arrow in Fig. 3a and b) in the kidney. Group C shows congestion (black arrow in Fig. 3c) and diffuse lymphocytic infiltration (blue arrow in Fig. 3c). HE staining (10X) and bars 100µm.

Figure 4. Histo-pathology of skin

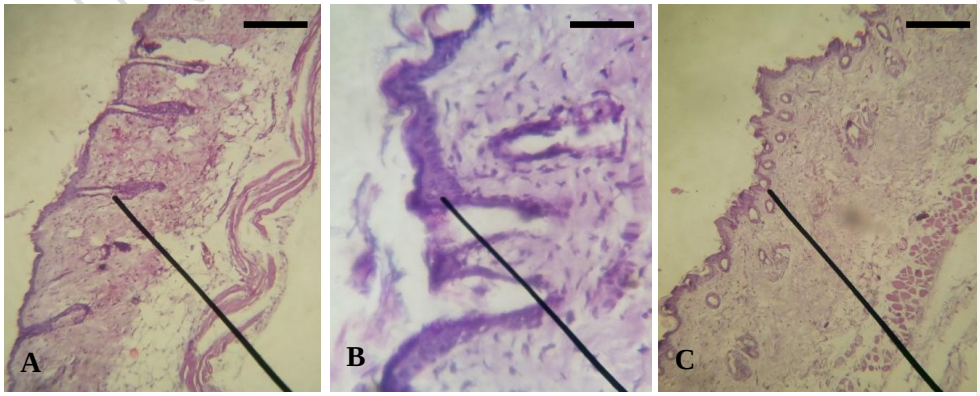

Figure 4. Histo-pathology of skin of control group A (Fig. 4a) and sunlight exposed group B and C of mice (Fig. 4b and c). All groups show normal histo-morphology of skin. HE staining (10X) and bars 100µm.

Table 1: Effects of sunlight exposure on body weight (gm)

Groups	1st day	35th day	70th day
Group-A (Control)	28.8 ± 0.5	35.2 ± 0.9	41.4 ± 1.0
Group-B (1hr)	28.0 ± 0.7	36.9 ± 1.3	41.2 ± 1.1
Group-C (1.5 hr)	27.6 ± 0.7	35.4 ± 1.9	41.0 ± 1.5

Values above represent mean ± standard deviation of 5 samples per group (n=5)

Table 2. Concentration of plasma vitamin-D exposed to sunlight in mice

Groups	Plasma vitamin-D (nmol/L)	*P*- value
Group-A (Control)	39.8 ± 1.1	
Group-B (1 hr)	40.6 ± 8.0	0.001***
Group-C (1.5 hr)	24.8 ± 2.7	0.001***

Values above represent mean ± standard deviation of 5 samples per group (n=5)

***significant at $p < 0.01$