EFFECT OF MARINE CYANOBACTERIA ON CIGARETTE SMOKE EXPOSED MICE -A PRELIMINARY REPORT

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Abstract

There is sound evidence that cigarette smoking represents a major risk factor causing oxidative stress leads to cancer at various site and many other chronic degenerative diseases. To have an understanding about this we studied the biochemical, immunological and haematological changes accompanying cigarette smoke induced stress in mice and this in turn is followed by treatment with *Oscillatoria willei* extract. The overall results revealed some stress related pathological changes in the mice model exposed to cigarette smoke. Interestingly some of the cigarette smoke induced stress changes were brought back to normal when *Oscillatoria willei* extract was given to the cigarette smoke exposed mice.

Keywords: *Oscillatoria willei*, cigarette smoke

I. INTRODUCTION

Tobacco smoking is being practiced in the form of cigar biddies etc to a minor extend in certain regions only (3) and these tobacco smoke is a human lung carcinogen, as well as also a major risk factor for oral, pharyngeal, colo rectal and oesophageal cancers (4, 6). Epidemiological studies suggested that about 1.2 to 1.4% of risk for passive smokers (1). The chemical constituents of tobacco are Acetaldehyde, Acetone, Acrolein, Acrylonitrile, Ammonia, Benzene, Benzo (a) pyrene, Bicyclohexyl, Crotonaldehyde, Cyclopentane, Cyclohexane, Hydrazine, Hydrogencyanide, Methylamine, Methyl Chloride, Methylpyrazines, Tar, Trimethylamine, Urethane and Vinylchloride etc. (7).. These chemicals elevate the level of reactive oxygen species (ROS) which leads to cell damage and malfunction through the free radical mediated decomposition of vital molecules such as DNA, proteins and lipids (2&8). Increased expression of Cytochrom P_{450} isozymes, Cytochalasin B and other drug metabolizing enzymes may influence the carcinogenicity of these substances (10).

Kiecolt-Glaser and Ronald Glaser (11) have reported lower natural killer cells (NK cells) in the smokers. The whole body exposure of BDF1 mice to mainstream cigarette smoke resulted in an evident increase of mononuclear normochromatic erythrocytes in peripheral blood (12). D'Agostini (13) observed a decrease in the macrophage count in mice exposed to cigarette smoke and that too the pulmonary alveolar macrophage undergoing apoptosis.

The potential uses of Marine Cyanobacteria are being given new attention to the World over, in view of their importance in agriculture (14, 15 & 16), industry (17), environmental pollution abatement (18&19) and pharmaceutical markets (20, 21, 22 & 23).

The antitumor activity of cryptophycin produced by *Nostoc* sp in human tumor xenograft models were explored (25).

Having gathered the basic information on cigarette smoke induced stress on animal models & human beings and the bioactive potentials of cyanobacteria, this preliminary study was undertaken by us at NFMC, Department of Microbiology, Bharathidasan University, Tiruchirappalli – 620 024.

II. MATERIALS AND METHODS

a. Organism chosen: The marine cyanobacterium *Oscillatoria willei* was obtained from the germplasm collection of the National Facility for Marine Cyanobacteria (NFMC), Department of Microbiology, Tiruchirappalli – 620 024, India. The organism was cultured in ASN III N^+ media in an auxenic condition (26) and incubated at 1,500 lux. in the germplasm of the facility and used after 15-20 days incubation.

b. Extraction Procedure: The weighed wet mass was ground in a pestle and mortar with 100% alcohol (distilled) to obtain ethanolic extract. Crude extract was used for the study.

c. Experimental animal chosen for this study: Swiss albino male mice were chosen for this study, were obtained from the animal house of Dept. of Microbiology, Bharathidasan University, Tiruchirappalli – 620 024. The animals were fed with freshly prepared feed containing milk and wheat powder & salt to taste and tap water *ad libitum.*

d. Study Design: The selected mice were divided into 4 groups containing 5 animals each. The control animals (C1) were not exposed to cigarette smoke and next set of control animals (C2) received PBS pH 7.2 from 16th day to 30th day of the study period. Animals of the experimental group (T1) were exposed to cigarette smoke for fifteen (15) days continuously, whereas the next test group (T2) was exposed to cigarette smoke for fifteen days continuously followed by *Oscillatoria willei* extract for next fifteen (15) days (16th – 30th day). C1 acted as the control for T1 and C2 acted as the control for T2.

e. Exposure to cigarette smoke: Whole body exposure to main stream cigarette smoke was obtained by using commercial cigarette (Scissors ™). The cigarette was allowed to burn in the cage with test group of animals (T1 & T2). Cigarette smoke induced stress was given to the test groups T1 and T2 by burning one Scissors ™ cigarette per day per cage having 5 animals. This was given for 15 days continuously. The cages were closed while exposing the animals to cigarette smoke. After treatment the test group animals were allowed to inhale fresh air.

f. Treatment dose: Crude *Oscillatoria willei* extract was given intraperitoneally (IP) at a concentration of 25μg/day/animal for 15 days continuously to test group animals (T2) after cigarette smoke exposure i.e. from 16th day to 30th day. The C2 animals were given with PBS pH 7.2 25 μl/day/animal from 16th to 30th day, as PBS pH 7.2 happened to be the suspending medium of crude extract of *Oscillatoria willei* extract.

g. Analysis- The animals were watched regularly for their general health condition, feed intake, water intake, excretion quantity and whole bodyweight . After the study period (15 days & 30 days) the animals were analyzed for hematological & immunological parameters. At the time of dissection the haematological parameters like bleeding time, clotting time, total count of erythrocytes (RBC) & leukocytes (WBC), differential count and hemoglobin content were seen. Total count of RBC and WBC were made using Neubauer's chamber according to Thomson & Inwood (1976) (27) and hemoglobin content was determined adopting the method of Oser (1965) (28). Gravimetric analysis of the various organs, primary lymphoid organs and secondary lymphoid tissues were seen by making use of an electronic balance (Sartorius). Immunoreactive cell count was also done with the help of hemocytometer and microscope. The treatment groups were analysed by Mean±Standard Error.

III. RESULTS

There were no observable changes phenotypically like hair falling, itching etc. Test groups were on par with the control group animals as far as the general health condition and body weight was concerned (Table 1) (fig.1).

There was a reduction in feed consumption of T2 group of animals and the reduction was roughly around 1% only (fig.2). The intake of water was found to be doubled in T1 compared to the control C1 and C2. Though there was an increase in the intake of water in T2, it was lower than that of T1 (fig.3). Contradictory to the water intake there was a drastic change in the quantity of excreta of mice between control groups & test groups and the decrease was greater in test groups T1 and T2 (fig. 4).

The time interval between the prick time (the bleeding start) and the bleeding stop is the bleeding time. Here in this study the bleeding time was decreased more than 50% in test groups T1 and T2 compared to the control groups C1 and C2. Roughly around 60% decrease was noted in the bleeding time of T2 compared to control C2. But the difference between the test groups T1 and T2 was around 1% only (fig. 5). On the other hand the clotting time of T1 was almost equal to the control groups C1 and C2. The clotting time of T2 was lower than that of control C1, C2 and T1 (fig. 6).

WBC count was found to be increased in T1 and T2 compared to the control C1 and C2 (fig. 7). Fig. 8 showed an increase in the RBC count of T1 than that of control C1 and C2, whereas the RBC count of T2 was equal to control C2 and lower than T1. There was an increase in neutrophil count (10%) in T1 than the control groups C1. Lymphocyte count was decreased in the test groups T1 and T2. Monocyte & lymphocyte count of T2 was enhanced compared to T1 showing the recovery from the cigarette smoke induced stress in mice (fig. 9a, 9b, 9c). Though there was a change in the hemoglobin level of all the animals the difference was not statistically significant (fig. 10).

The gravimetric analysis revealed 40% reduction in liver and 25% reduction in kidney between T2 & C2. But the immune system of both the test groups showed an increased weight than the control groups C1 and C2 (Table 2).

The gravimetric analysis of primary lymphoid organ showed difference in T1 and T2 compared to control. The secondary lymphoid tissue showed an enhancement in the gravimetry of T1 and T2 saying that the animals has responded to the treatment, smoke as well as smoke followed by cyanobacterial extract (Table 3). The immunoreactive cells count of thymus was decreased in test groups T1 and T2 compared to the control C1. Contradictory to this the cell count of various lymphnodes studied showed increased cell count in T1 & T2 than that of control group C1 (Table 4).

Table 1. Phenotypic Changes – General Health Condition

OBSERVATION	RESULTS
Color change	No change
Hair Falling	No change
Any other	No change

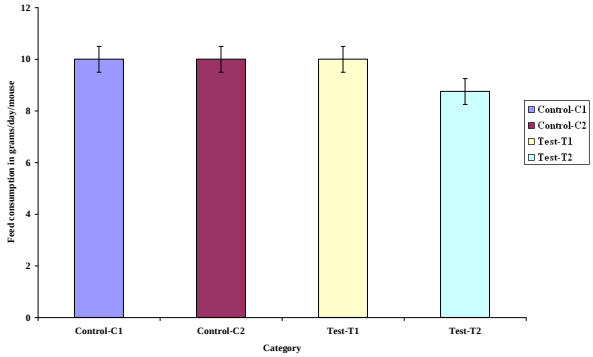

Fig.1. Morphometric Analysis

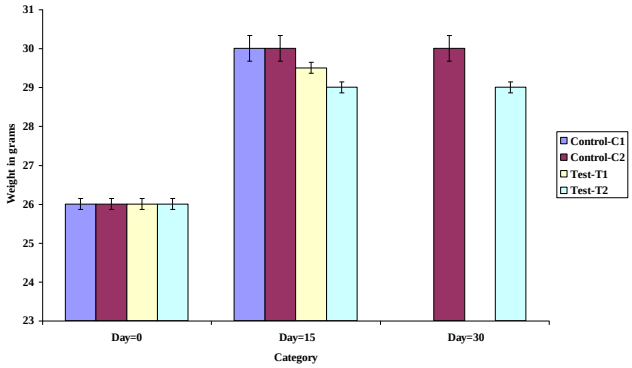

Fig.2. Feed Consumption

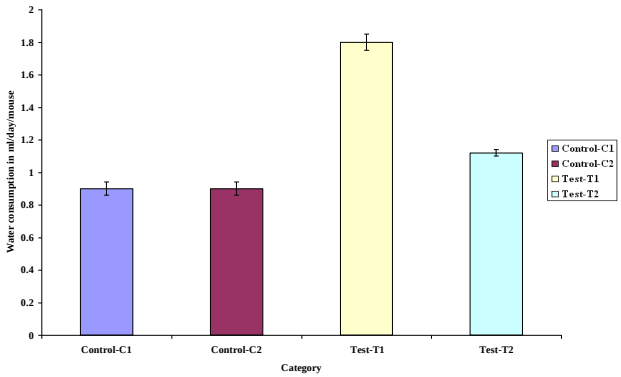

Fig.3. Water Intake

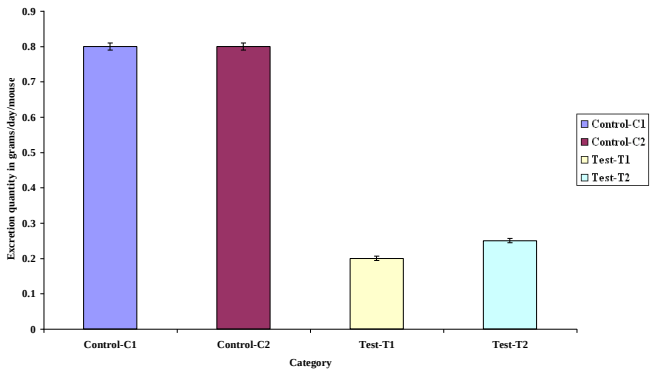

Fig.4. Quantity of Excreta

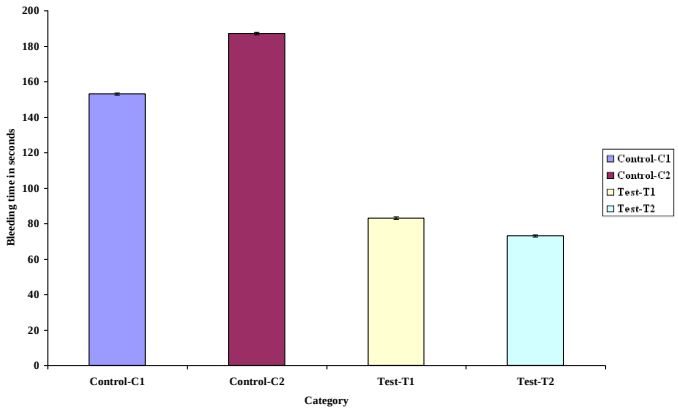

Fig.5. Bleed Time

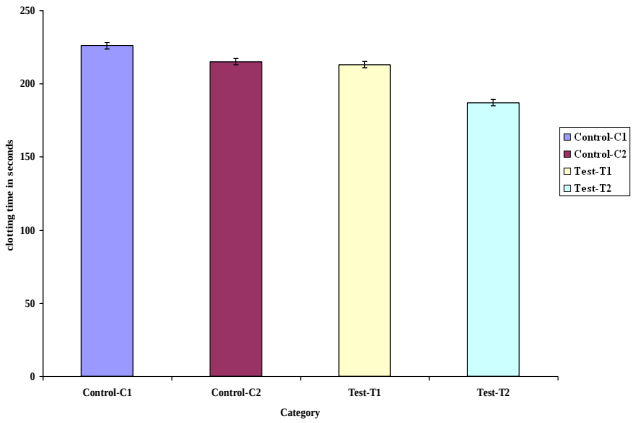

Fig.6. Clotting Time

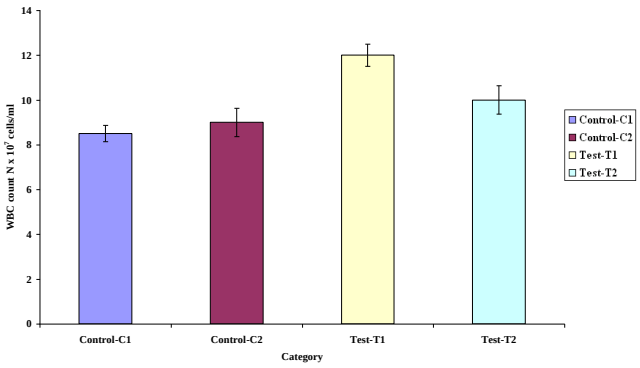

Fig.7. Total WBC Count

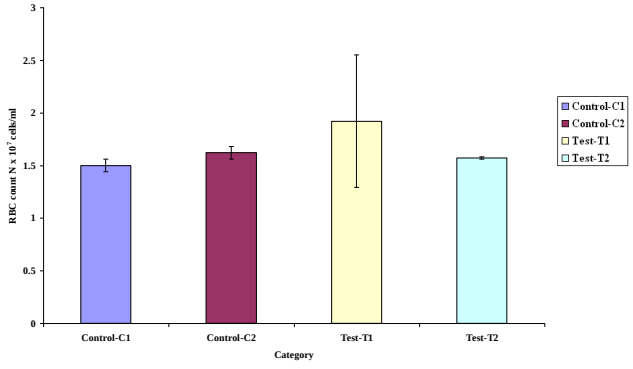

Fig.8. Total RBC Count

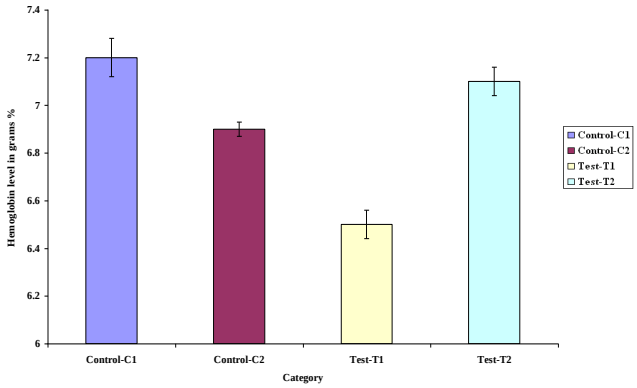

Fig.9. Differential Count

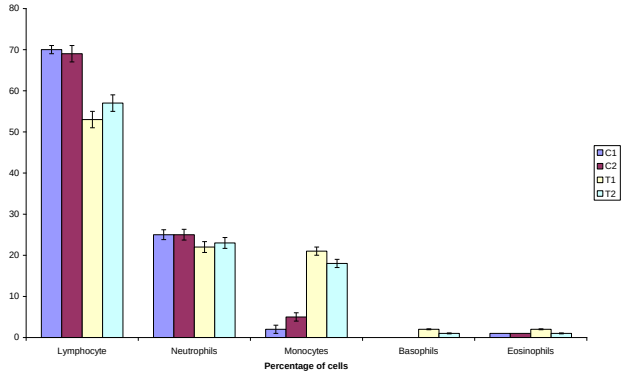

Fig.10. Haemoglobin Level

Table 2: **Gravimetric Analysis of Organ/System Weights**

S. No	Organs/System	Weight in gms.			
		Control (C1)	Control (C2)	Test (T1)	Test (T2)
1.	Liver	1.425±0.057	2.071±0.054	1.261±0.054	1.383±0.063
2.	Lungs	0.175±0.006	0.235±0.002	0.252±0.006	0.175±0.003
3.	Kidney	0.354±0.016	0.426±0.006	0.322±0.002	0.305±0.002
4.	Spleen	0.082±0.006	0.062±0.002	0.071±0.001	0.062±0.002
5.	Immune system	0.128±0.002	0.125±0.006	0.147±0.006	0.133±0.001

Table 3: Gravimetric Analysis of Immune System (Weight in mg)

S. No	Organs/Tissues	Weight in mg.			
		Control (C1)	Control (C2)	Test (T1)	Test (T2)
1.	Thymus	25.6±0.457	24±0.245	28.5±0.480	24±0.362
2.	Spleen	72.6±0.006	61.5±0.480	70.5±0.480	61.5±0.630
3.	Inguinal lymphnode	13.4±0.002	14±0.006	18±0.006	19±0.003
4.	Auxiliary lymphnode	8.4±0.006	14.5±0.006	16±0.002	17.5±0.002
5.	Perivertiberal lymphnode	7.6±0.002	11±0.002	13.5±0.003	10.5±0.001

Table 4: Immunoreactive Cells Count (no. of cells x 10^7/mm^3/ml)

S. No	Test group	Primary Lymphoid Organ	Secondary Lymphoid Tissues			
		Thymus	Spleen	Inguinal lymphnode	Auxiliary lymphnode	Perivertiberal lymphnode
1.	Control C1	40±2.4	20±0.63	2.3±0.57	1.3±0.06	1.4±0.06
2.	Control C2	40±4.8	21.0±0.57	6±0.57	8±0.06	6±0.05
3.	Test T1	37±4.9	37±0.49	11±0.63	10±0.05	8±0.03
4.	Test T2	16±2.4	20±0.36	10±0.48	10±0.04	11±0.02

IV. DISCUSSION

The present study was aimed to find out the effect of *Oscillatoria willei* extract on modulation induced by cigarette smoke in *in - vivo* system of mouse model There was an observable change in the body weight of the animals, which was contradictory to the results of D'Agostini *et al.,* (13) and Witschi *et al.,* (29) who have reported the no body weight change in the rat and mice models respectively. On the other hand this is similar to the report of Ashwani Koul (30) and Chunliu et al., (31) where they had done the same on the ferrets and mice models respectively.

The feed consumption of the smoke exposed mice were similar to the control, Ashwani Koul (30) also found the same. The feed consumption of the T2 group animals was less which may be due to the treatment of *Oscillatoria willei* extract. There was an increase in the

intake of water by the cigarette smoke exposed animals which was deviating to the report of Ashwani koul (30). The cigarette smoke would have induced the thirstiness of the smoke exposed mouse. Interestingly, there was a decrease in the intake of water by T2 compared to T1 but it was higher than control groups C1 & C2. The probable reason could be the effect of *Oscillatoria willei* (i.e.) extract. The quantity of excretory material of both T1 and T2 were drastically decreased in this study could be again due to change in physiology by cigarette smoke. *Oscillatoria willei* has no effect on the recovery of smoke induced changes.

The increase in bleeding time of the T1 was observed in this study which may be due to the effect of the chemical constituents of cigarette smoke on the clotting mechanism of the mice. Roughly around 60% decrease was noted in the bleeding time of T2 compared to C2, the difference was around 1% only compared to T1. This again says that *Oscillatoria willei* extract does not help in bringing back the changes induced by cigarette smoke.

The effect mounted in the bleeding time was not reflected in the clotting time. There was not much variation in the clotting time between T1 and C, but it was lowered in T2 group compared to T1 and C2.

There was an increase in WBC count of T1 & T2 compared to control C1 & C2. The increase in WBC count in T1 indicates an impact of the smoke on the defense mechanism of the smoke exposed mice. This is in line with the results shown by Felix and Mary Ko (32). Though there was an increase in WBC count of T2 compare to Control (C1& C2) it was decreased when compared to T1. This may be due to the action of *Oscillatoria willei* extract on the defense mechanism of the mice. Sundararaman et al., (33) also reported similar result with 9 other marine cyanobacterial extract. Earlier on the effect of marine cyanobacteria on rat conducted in this lab also showed similar kinds of results (34,35 & 36) Baojiang et al., (37) also reported that polysaccharide of *Spirulina* can improve both specific and nonspecific mechanism of immune response.

It was observed that there was an increase in the RBC count of T1 in this study. Balansky et al., (12) also reported increase in peripheral blood erythrocytes in cigarette smoke exposed rats and the difference was statistically significant, which reached the maximum of 2.1 fold increase over control for five week cigarette smoke exposure. Liu et al., (5) also showed an increased angiogenesis in the mice having colistic while exposing to cigarette smoke. Though there was a decrease in the RBC count of T2 compared to T1 it was similar to C1, showing that the extract had effect on changes caused by cigarette smoke thereby bringing back the pathological effect of cigarette smoke on RBC to normal.

There was a significant increase in neutrophil and monocyte count of T1 compared to control, this may be due to the effect of the chemical constitutes of cigarette smoke (30) & (38). The neutrophil count was decreased by 9% on T2 compared to T1 which was closer to the count of C1, showing the capability of *Oscillatoria willei* extract on recovering it from the cigarette smoke effect. On the other hand the *Oscillatoria willei* extract have no effect on recovering the monocyte count where it was increased in T1 and T2 when compared to C. This indicates that there is a specific & non specific immune response.

Gravimetric analysis of liver and kidney showed reduction in both the test groups T1 and T2 compared to C1. Even though the weight of liver of T2 was lesser than C1 it was higher than T1, reflecting that *O. willei* extract has the ability to recover from stress introduced changes by cigarette smoke, which in turn was reflected in the gravimetry of liver.

Gravimetry of immune system was higher in both the test groups T1 and T2 than the control C1, but the weight of immune system of T2 was lesser than T1, this showed the efficiency of *O. willei* extract on recovery of mice model from the effect of cigarette smoke on immune system.

Increase in weight of thymus was seen in T1 compared to control C1, indicates that the compounds of cigarette smoke acts on thymus & causes megaly of thymus. It was reverted back to normal in *O. willei* extract given animals (T2) and the toxic effect of cigarette smoke was a temporary one. Increased weight of lymphnodes were observed in both the test groups (T1 and T2), because both the cigarette smoke & *O. willei* extract was recognized as antigen by the immune system and processed.

There was a significant reduction in immunoreactive cells count in thymus & spleen in both T1 & T2, showed the impact of chemicals in cigarette smoke on thymus & spleen. *O. willei* extract has no effect the immunoreactive cells count as in T1.

Immunoreactive cells count of lymphoid tissues (lymphnodes) of both T1 & T2 showed a significant increase in count. This may be due to the chemicals constituents of cigarette smoke and *O. willei* extract.

From this preliminary work it was somewhat clear that the extract of *O. willei* has the ability to recover the mice model from stress induced changes on the hematology & immunology by cigarette smoke.

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