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Screening of Antineoplastic Activity of *Oscillatoria Annae* against Diethyl Nitrosamine Induced Cancer in Rats

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Abstract

Ethanolic extract of Oscillatoria annae (EEOA) was studied for the antineoplastic activity by in vivo models. EEOA had shown Antineoplastic activity in all models on dose dependant manner. Hematological parameters of tumor bearing rats showed significant changes when compared with normal rats. The total WBC count, proteins and PCV were found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased. At the same time of interval, EEOA treatment could change these parameters to near normal on dose dependant manner.

In the control group DEN significantly enhanced the biochemical markers like ALP, SGPT, SGOT, total protein and bilirubin. Pretreatment with EEOA reduced the elevated levels of all above mentioned biochemical indicators.

Histopathological observations reveal that DEN treatment has damaged the liver architecture due to induction of cancer and pretreatment with EEOA prevents/reversed the liver damage in dose dependant manner.

Key words: Antineoplastic Activity, Diethyl Nitrosamine, Hematological Parameters, Oscillatoria Annae, SGPT, SGOT.

INTRODUCTION

Cancer or malignant neoplasm is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis i.e spread to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize[1][2].

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, are common members of the plankton of marine, brackish and freshwaters throughout the world. They also occure on rocks and soil and in symbiosis with plant and fungi. They have a simple structure at subcellular level and lack a nucleus, a characteristic feature defining them, along with bacteria, as prokaryotes[3]. Oscillatoria is a genus of filamentous cyanobacteria which is named for the oscillation in its movement. Filaments in the colonies can slide back and forth against each other until the whole mass is reoriented to its light source. It is commonly found in watering-troughs waters, and is mainly blue-green or brown-green. Oscillatoria is an organism that reproduces by fragmentation. Oscillatoria forms long filaments of cells which can break into fragments called hormogonia. The hormogonia can grow into a new, longer filament. Oscillatoria annae are a morphologically diverse group of oxygenic photosynthetic prokaryotes, which are phylogenetically closed related to each other and to chloroplasts. Oscillatoria annae include unicellular, colonial and filamentous forms some filamentous cyanophytes form differentiated cells called heterocyst, that are speciallized for hydrogen fixation, and resting or spore cells called aconites. Most of the bacteria found in the fresh water, while others are marine occur in damp soil, or even temporarily moistened rocks in deserts[4].

Furthermore, literature survey of Oscillatoria annae revealed that no researcher has yet reported invivo antineoplastic activity of this alga. Therefore it is worth conducting an investigation on the antineoplastic activity of ethanolic extract of O. annae (EEOA).

MATERIALS AND METHODS

1. Materials:

Oscillatoria Annae is an autotropic, filamentous organism. This strain was obtained from national facility for marine cyanobacteria (NFMC), Trichy, Tamilnadu, India.

Preparation of Extract:

About 50 gm of powdered Oscillatoria annae was taken in a round bottom flask, 800 ml of ethanol was added and macerated for 7 days. During maceration the whole content was warmed two times a day at intervals. At the end of the 7 day the extract was filtered through muslin cloth while hot the extract was concentrated to a semisolid mass and dried in desiccators. This extract has been used for various experimental purposes.

Phytochemical Screening:

A preliminary phytochemical screening of extract carried out and following constituents have been detected, terpenoids and steroids.

Animals:

The male Swiss rats weighing 100 – 150 g were used in this study. They were procured from Nandha College of Pharmacy and Research Center, Erode. The animals were housed under 12 hrs day and night conditions for 2 to 3 months. The animals had free access to pellet and tap water ad libitum.

2. Methods:

The adult male Wistar rats (100-150 g) were divided into five groups each containing six animals. All animals of group II - V were injected intraperitoneally with Diethylnitrosamine at a dose of 200 mg/kg on day '0'. Group I was the normal control group received vehicle. Group III and Group IV mice were treated with EEOA started three weeks after injecting the diethylnitrosamine, at a dose of 200 mg/kg/day and 400 mg/kg/day respectively, orally. Group V rats were treated with vincristine at a dose of 0.8 mg/kg/day, orally. On eighth week of studies blood was drawn from each rat by the retro orbital plexus under ether anesthesia in two tubes, one is for hematological parameters such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), protein and packed cell volume (PCV) and blood from second tube was centrifuged and serum was collected which was used for serum glutamate pyruvate transferase (SGPT), serum glutamate oxaloacetate transferase (SGOT), LPO, ALP.

At autopsy, livers were excised and slices of 2–3 mm thick are cut with a surgical blade, fixed in 10% phosphate buffered formalin. They were used for immunohistochemical examination of GST-P positive foci[6][7].

Statistical analysis

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical studies revealed the presence of carbohydrates, proteins, steroids, terpenoids and phenolic compound.

The Antineoplastic activity of EEOA was tested in five models. EEOA had shown Antineoplastic activity in all models on dose dependant manner. Hematological parameters of tumor bearing rats showed significant changes when compared with normal rats. The total WBC count, proteins and PCV were found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased. At the same time of interval, EEOA treatment could change these parameters to near normal on dose dependant manner.

In the control group DEN significantly enhanced the biochemical markers like ALP, SGPT, SGOT, total protein and bilirubin. Pretreatment with EEOA reduced the elevated levels of all above mentioned biochemical indicators.

Histopathological observations reveal that DEN treatment has damaged the liver architecture due to induction of cancer and pretreatment with EEOA prevents/reversed the liver damage in dose dependant manner.

Table No. 1 :- In Vivo Antineoplastic Effect of Ethanolic Extract Of *Oscillatoria Annae*: Hematological Parameters:

Groups	*Hb (g%)*	*RBC (mil/mm^3)*	*WBC (mil/mm^3)*	*PCV (mil/mm^3)*	*Differential count %*		
					Lymphocyte	*Neutrophil*	*Monocyte*
Control Gp.	14.02 ± 0.29	4.59 ± 0.21	7.2 ± 0.26	17.0 ± 0.72	70 ± 1.31	29 ± 1.62	1 ± 0
Toxin Control Gp.	8.51 ± 0.16	2.87 ± 0.10	20.2 ± 0.32	26.0 ± 1.36	31 ± 1.54	24 ± 1.89	2 ± 0
Standard Gp.	12.91 ± 0.67	4.26 ± 0.34	11.6 ± 0.39	19.0 ± 2.30	52 ± 1.76	27 ± 1.29	1 ± 0
EEOA (200mg/kg)	11.73 ± 0.37	3.81 ± 0.29	15.8 ± 0.51	21.0 ± 1.83	49 ± 1.42	26 ± 1.32	1 ± 0
EEOA(400mg/kg)	12.6 ± 0.51	4.09 ± 0.10	13.67 ± 0.38	20.0 ± 1.13	51 ± 1.34	27 ± 1.24	1 ± 0

Values are expressed as Mean ± SEM, of 5 observations, statistical comparison as follows: Significant at *P < 0.05 ,compared to control group.

Fig. 1:- Effect of Ethanolic extract of *Oscillatoria annea* on Hemoglobin:

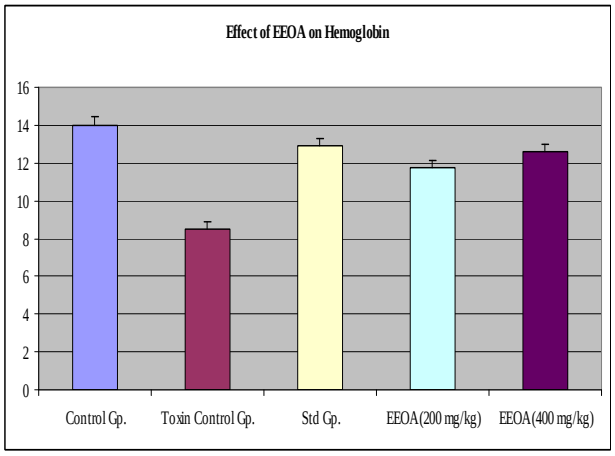


Fig. 2:- Effect of Ethanolic extract of *Oscillatoria annea* on RBC:

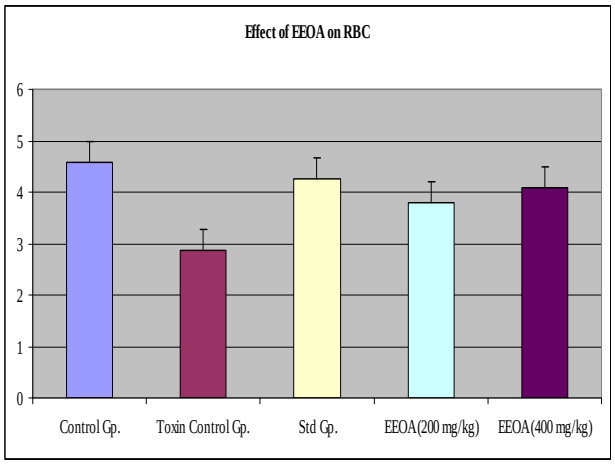


Fig. 3:- Effect of Ethanolic extract of *Oscillatoria annea* on WBC:

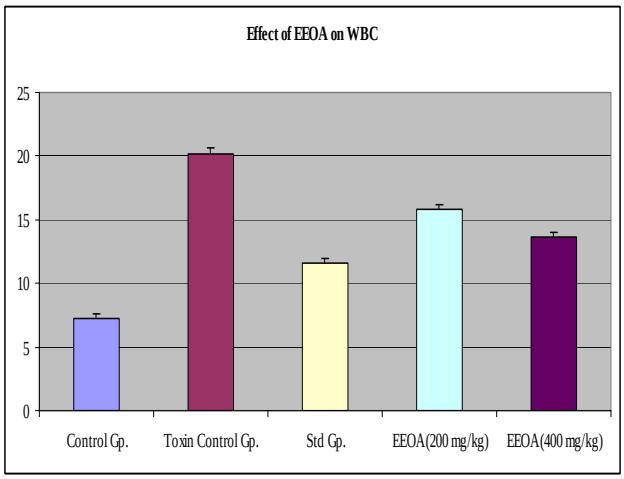


Fig. 4:- Effect of Ethanolic extract of *Oscillatoria annea* on PCV:

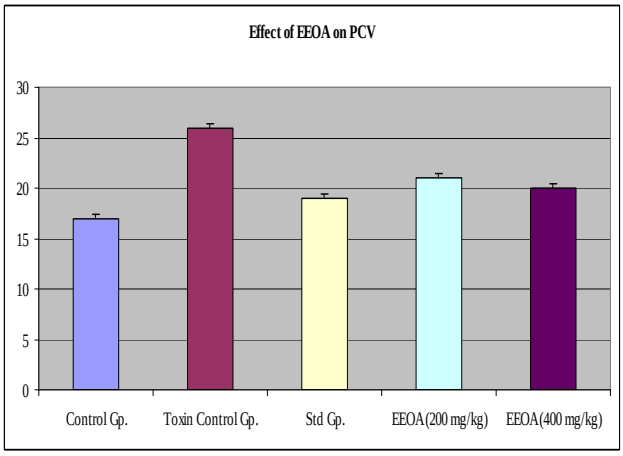


Fig. 5:- Effect of Ethanolic extract of *Oscillatoria annea* on Differntial Count:

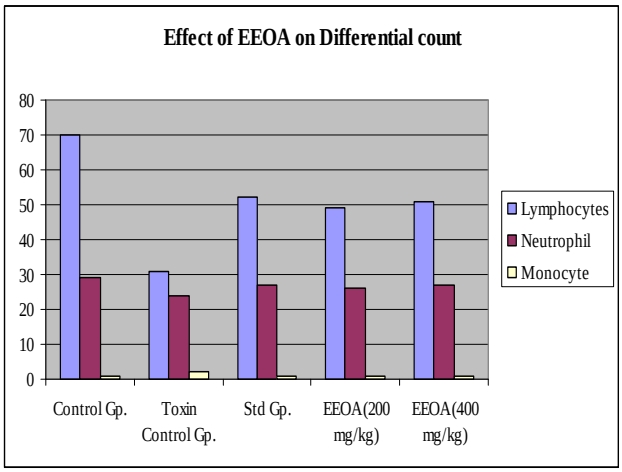


Fig. 6:- Effect of Ethanolic extract of *Oscillatoria annea* on Alkaline Phosphatase:

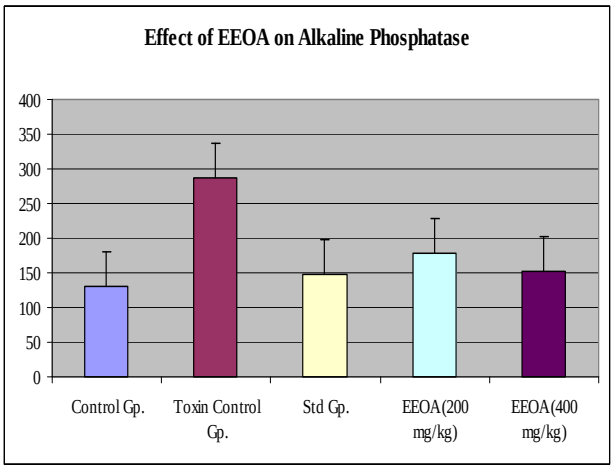


Table 2: In Vivo Antineoplastic Effect of Ethanolic extract of *Oscillatoria annae*: Liver Function Test:

Group	Alkaline Phosphatase	SGOT	SGPT	Total Protein	Bilirubin	
					Direct	Total
Control Gp.	131.42 ± 1.34	0.89 ± 1.11	49.12 ± 1.77	8.56 ± 0.25	0.94 ± 0.56	0.23 ± 0.10
Toxin Control Gp.	286.97 ± 1.39*	31.33 ± 1.14	81.92 ± 1.85	13.8 ± 1.09	15.77 ± 1.74	4.54 ± 0.72
Standard	147.48 ± 1.69	11.76 ± 0.76	62.71 ± 1.19	9.79 ± 0.31	7.71 ± 2.76	2.98 ± 1.14
EEOA(200mg/kg)	178.29 ± 1.45	23.59 ± 2.25	73.96 ± 1.76	10.89 ± 0.23	10.42 ± 2.93	3.75 ± 1.14
EEOA(400mg/kg)	153.39 ± 1.26	13.59 ± 1.25	69.19 ± 0.44	9.98 ± 0.19	8.38 ± 2.92	3.24 ± 1.04

Values are expressed as Mean ± SEM, of 5 observations, statistical comparison as follows: Significant at P< 0.05, *P<0.01, compared to control group.

Fig. 7:- Effect of Ethanolic extract of *Oscillatoria annea* on SGOT:

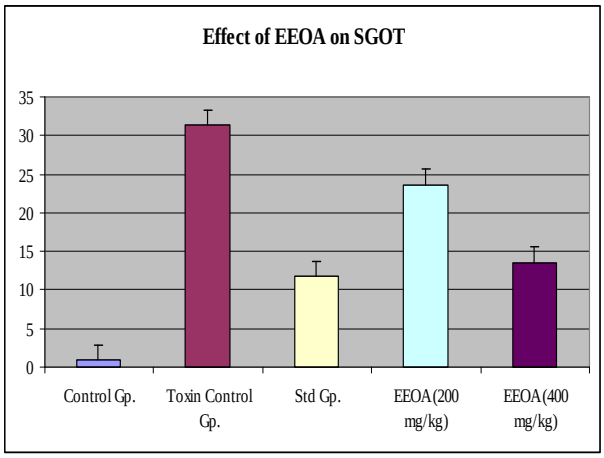


Fig. 8:- Effect of Ethanolic extract of *Oscillatoria annea* on SGPT:

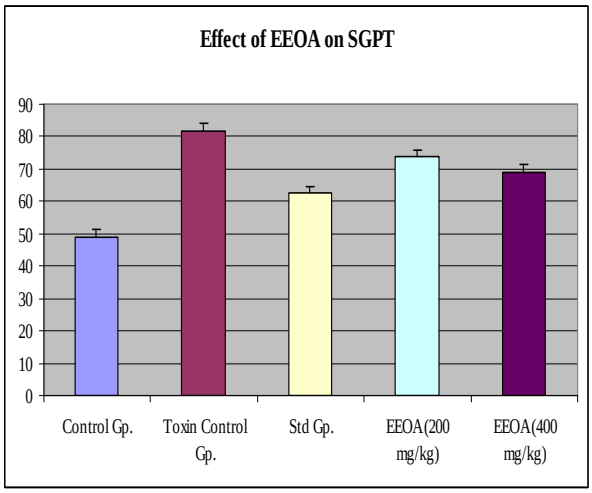


Fig. 9:- Effect of Ethanolic extract of *Oscillatoria annea* on Total Protein:

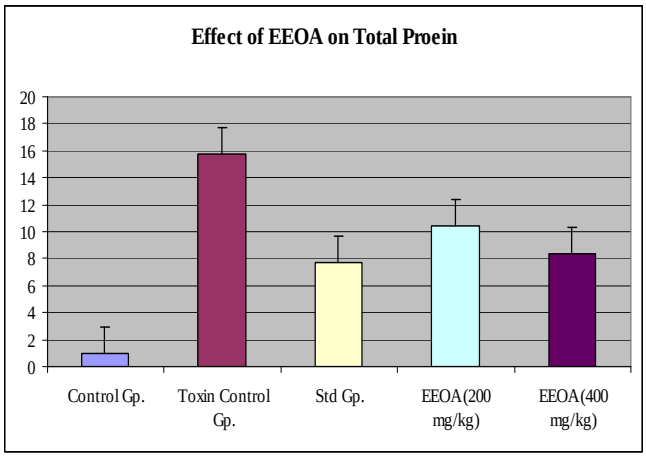
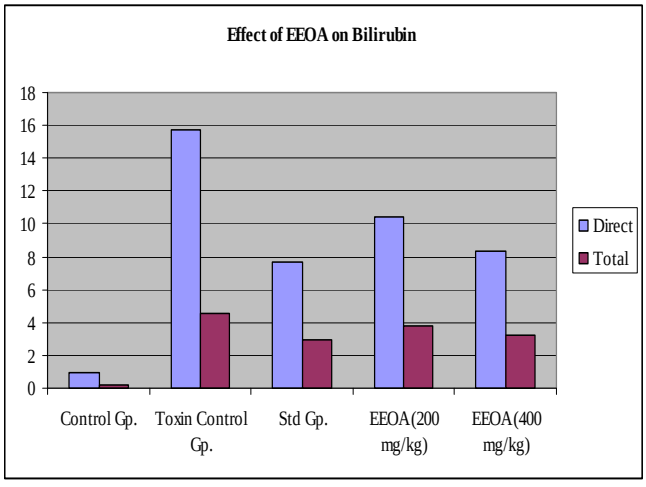
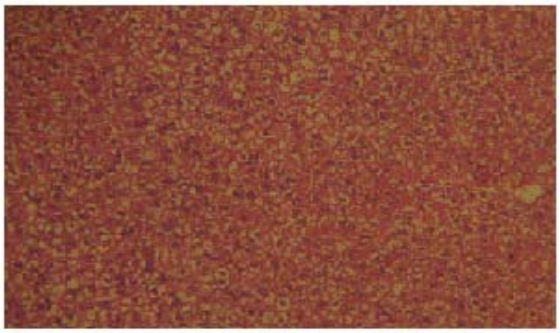


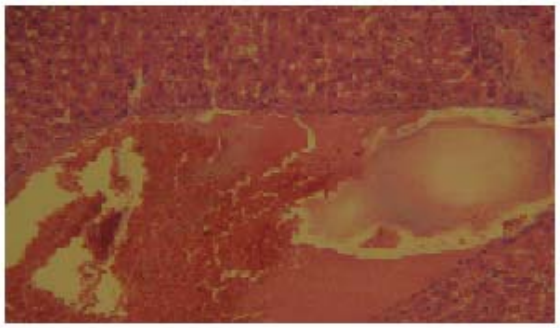
Fig. 10:- Effect of Ethanolic extract of *Oscillatoria annea* on Bilirubin:



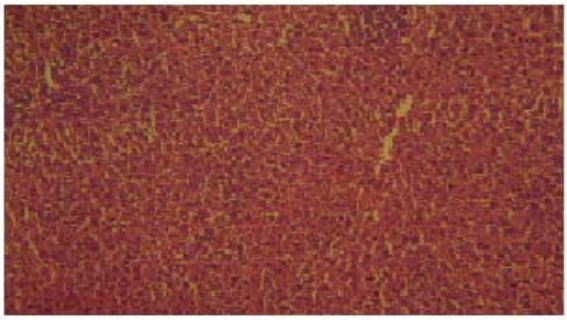
Histopathological Study: Effect of Ethanolic extract of *Oscillatoria annae* on Liver Foci:



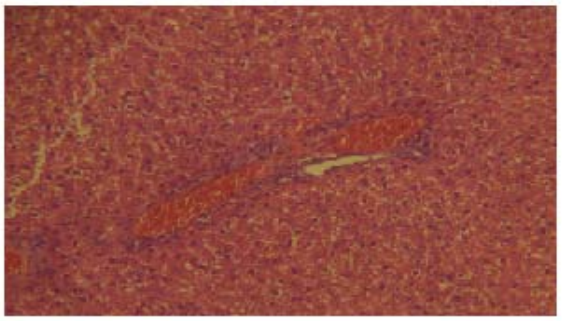
A: Liver architecture of Normal control group



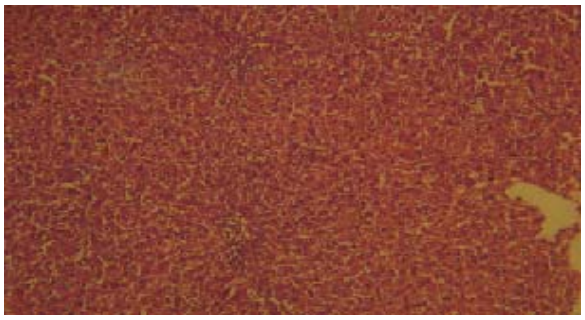
B: Liver architecture of Toxin ctrl. group



C: Liver architecture of Standard group



D: Liver architecture of EEOA (200mg/kg)



E: Liver architecture of EEOA (400mg/kg).

DISCUSSION:

The reliable criteria for judging the value of any anticancer drug are decrease of WBC from blood. The result of the present study show an Antineoplastic effect of EEOA against DEN induced liver cancer in Swiss rats. A significant reduction in the total WBC count, proteins and PCV and increase in the hemoglobin content of RBC. In the control group DEN significantly enhanced the biochemical markers like ALP, SGPT, SGOT, total protein and bilirubin. Pretreatment with EEOA reduced the elevated levels of all above mentioned biochemical indicators.

Histopathological observations reveal that DEN treatment has damaged the liver architecture due to induction of cancer and pretreatment with EEOA prevents/reversed the liver damage in dose dependant manner.

Preliminary phytochemical screening indicated the presence of steroidal compound and terpenoid, flaonoids in EEOA. Flavonoids and terpenoids have been possess antimutagenic and antimalignant effects. The present study points to the potential anticancer activity of *Oscillatoria annae.*

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