ANTI-CANCER POTENTIAL OF POLYSACCHARIDE ISOLATED FROM METHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA STEM BARK

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ABSTRACT

Objective: The exploration of the anticancer potential of polysaccharide isolated from the methanol extract of *Tinospora cordifolia* (*T. cordifolia*) stem bark against breast cancer in DMBA-induced female albino Wistar rat models was examined by various hematological parameters.

Methods: Analysis of Red blood cell (RBC), White blood cell (WBC) and platelet level, Tumor markers Carcino Embryonic Antigen (CEA) and Cancer Antigen 15.3 (CA 15.3) in the serum, was done in the normal, cancer and compound treated rats using specific kits. Histological studies were performed to examine the changes in the tissue morphology and cell patterns in breast tissue.

Results: The decreased levels of RBC, WBC and platelets in 7,12-Dimethylbenz [a] anthracene (DMBA)-induced breast cancer (Group III) animals were revived to the normal conditions in polysaccharide treated breast cancer (Group IV) animals as that of normal (Group I). The level of tumor markers CEA and CA 15.3, was found elevated in serum of DMBA-induced breast cancer groups (Group III) when compared to their levels in the normal groups (Group I) whereas polysaccharide treatment (Group IV) prevented this rise in the levels of tumor markers. The histological studies on the breast tissue samples of all the groups showed the appropriate features where the normal (Group I) animals were characterized with normal cells uniformly arranged without any change in orientation and morphology, DMBA-induced cancer (Group III) animals showed an improper orientation of cells arranged as glandular structures, as nest, or cords of various sizes or as solid sheets foc of necrosis in some areas with margins infiltrating, pushing, circumsised or mixed and the polysaccharide treated (Group IV) animals showed results resembling that of the normal (Group I) animals.

Conclusion: Thus, polysaccharide is proved as an effective chemo preventive agent against breast cancer.

Keywords: Chemoprevention, Blood cells, Cancer antigen, Tissue morphology

INTRODUCTION

Cancer is one of the dreaded diseases taking away millions of life and its prevalence is expected to rise five-fold by 2025 [1]. According to the National Cancer Institute survey reports, the most prominent cancer occurring in males are pharyngeal and oral cancer (62.7%), oesophagus cancer (8.4%), lung cancer (16.8%) and prostate cancer (98.9%) and in females are cervical cancer (67.9%) and breast cancer (89.2%) [2]. There is no single treatment strategy for cancer, and the patients often receive a combination of therapies and palliative care such as surgery, chemotherapy, immunotherapy, hormone therapy and gene therapy, etc. The main drawback with these therapies is that they are accompanied with severe side effects. Hence generally it is necessary to explore effective agents to treat cancer with lesser side-effects. In ancient India, medicinal plants were used to prevent various critical diseases [3]. Plant or plant-based medicines are examined from the past in the system of ayurvedha and medicinal herbs mediated therapeutic approaches are of current interest for their potential biological effect that is free from drastic side-effects [4].

*Tinospora cordifolia* (*T. cordifolia*) belonging to Menispermaceae family has been extensively studied as a potential herb to treat various human illnesses. The stem of *T. cordifolia* was used as an important constituent in several ayurvedic preparations to cure general debility, dyspepsia, fever and urinary diseases, stomach problems, skin burns. The stem is bitter, stomachic, diuretic [5] disorders in bile secretion, constipation, vomiting, jaundice, amenia, etc. *T. cordifolia* rich in secondary metabolites produced certain curative properties such as antioxidant [6], anti-inflammatory [7], antibacterial [8], anti-hyperlipidemic [9], cholesterol-lowering [10], hepatic protective effects [11] and diuretic activities [12], anti-pyretic [13], radioprotective [14] etc. Polysaccharide identified as one of the active principles present in the methanolic extract of *T. cordifolia* stem bark has been previously reported to possess anti-diabetic property on STZ-induced diabetic Wistar rats showing an increase in insulin secretion and glucose utilization in polysaccharide treated animal models [15]. The anti-diabetic potential of a phytocompound is the correlation with its anticancer activity and hence polysaccharide has been explored in the present study for identifying its efficacy in treating cancer.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used were of extra pure and culture Grade, procured from suppliers such as Sigma Chemical Pvt Ltd, USA; Himedia Chemicals, Mumbai. All solvents were obtained from Fischer Scientific Ltd, India.

Isolation of polysaccharide

Polysaccharide was isolated from the methanolic extract of *T. cordifolia* stem bark, by the protocol of Rajalakshmi et al [16].

*In vivo* studies

Animals

Female albino Wistar rats aged between 50 and 55 d was procured from Tamil Nadu Vertynary Sciences, Madhavaram. The animals were maintained under controlled environmental conditions on an alternative 12-h dark/light cycle. Commercial pelleted feed supplied by Sai enterprises Ltd., Chennai and water *ad libitum* were given to animals. This research work on albino Wistar rats was sanctioned and approved by the Institutional Animal Ethical Committee (IAEC No. 07/2013).
Experimental setup
The animals were divided into five groups with six animals each. Group I animals served as normal control. Group II was normal animals supplemented with polysaccharide (20 mg/kg body weight (bwt)). Group III was animals treated with 20 mg of DMBA in 1 ml corn oil to induce breast cancer. Group IV was animals treated with DMBA and simultaneously supplemented with polysaccharide (20 mg/kg body weight). Group V was animals treated with DMBA and simultaneously supplemented with Paclitaxel (1 mg/kg body weight). The overall induction and treatment period was 3 mo* for all groups. After the experimental period, the animals were sacrificed by decapitation, breast and liver tissues were dissected out and tissue homogenates were prepared in 0.1 M Tris-HCl buffer pH 7.4 which were stored at 80 °C, until their use for further analysis.

Hematological parameters
The hematological parameters—Red blood cell (RBC), White blood cell (WBC) and platelet counts were measured using an automated cell counter.

Estimation of tumor markers
The tumor markers Carcino Embryonic Antigen (CEA) and Cancer Antigen 15.3 (CA 15.3) were estimated by the method of Chemiluminescence Immune Assay [17].

Histological studies
Breast tissues from the untreated and the experimental groups were blotted free of mucus, washed in physiological saline and fixed in Bouin-Hollande fixative for 74h. After fixation, the tissues were washed in 70% alcohol for two or three days to remove the excess picric acid and then dehydrated in graded series of alcohol. The tissues were cleared using xylene. The cleared tissues were infiltrated with molten paraffin at 58-60 °C through three changes (20-30 min) and finally embedded in paraffin. Sections of the tissues were obtained using rotary microtome and stained in Ehrlich’s hematoxylin with eosin as the counterstain. The slides were mounted using DPX mountant [18].

Statistical analysis
The data were analyzed using the SPSS Windows Students version software. For all the measurement, one-way ANOVA followed by Duncan’s Multiple Range Test [19] was used to assess the statistical significance of the difference between control and treated groups. A statistically significant difference was considered at the level of p<0.05.

RESULTS
Effect of polysaccharide on RBC, WBC and platelet counts
Analysis of the anticancer potential of polysaccharide on RBC, WBC and platelet counts in DMBA-induced breast cancer rats was done in the present study. When compared to the normal (Group I) animals the levels of RBC, WBC and platelets were found to be in a decreased condition in DMBA-induced breast cancer (Group III) animals. The polysaccharide treatment (Group IV) revived the levels RBC, WBC and platelets as in that of normal animals (fig. 1, 2 and 3). Thus, polysaccharide has a potency to prevent the depletion of blood cells in cancer conditions.

Effect of polysaccharide on CEA
The level of tumor marker CEA, was estimated in the serum of normal, cancer and treated animal groups and the results obtained showed an elevated levels of CEA in the DMBA-induced breast cancer groups (Group III) when compared to their levels in the normal groups (Group I) whereas polysaccharide treatment (Group IV) prevented the elevated of CEA, thus the efficacy of the polysaccharide in preventing tumor development is evident (fig. 4).
Effect of polysaccharide on CA 15.3

The level of breast cancer-specific tumor marker CA 15.3 determined in the normal, cancer-induced and compound treated groups showed that the cancer control groups (Group III) had increased the levels of CA 15.3, whereas an only low level of the antigen was found in the normal control animals (Group I) and this normal level was also maintained in the polysaccharide treated animals (Group IV) (fig. 5). While treating the DMBA-induced rats with the polysaccharide, a normal level of the antigen was observed indicating the effect of the compounds in preventing the tumor development.

Effect of polysaccharide on breast tissue

The breast tissues of all the groups were subjected to histopathological analysis (fig. 6). The Group I breast tissue sections were characterized with normal cells uniformly arranged without any change in orientation and morphology. The tissue sections of DMBA-induced cancer animal (Group III) showed an improper orientation of cells. The tumor cells we rearranged as glandular structures, as nest, or cords of
various sizes or as solid sheets foci of necrosis in some areas with margins infiltrating, pushing, circumcised or mixed. Observations done on the breast tissues taken from the polysaccharide treated animals (Group IV) showed results resembling that of the normal (Group I) animals. Thus, the chemopreventive ability of polysaccharide was substantiated on the DMBA-induced breast cancer rat models.

**REFERENCES**

1. Vanishee Shriaram, Shiraam Mahadevan, Anitharani, Selaviniyagam M, Sathiasekaran BWC. National health programs in the field of endocrinology and metabolism–Miles to go. Indian J Endocrinol Metab 2014;18:7-12.
2. Yong-chuan Wang, Li-juan Wei, Jun-tian Liu, Shi-xia Li, Qing-sheng Wang. Comparison of cancer incidence between china and the USA. Cancer Biol Med 2012;9:128-32.
3. Parekh J, Chanda S. Phytochemicals screening of some plants from the western region of India. Plant Arch 2008;8:657-62.
4. Premalatha B, Raigopal G. Cancer-an ayurvedic perspective. Pharmacol Res 2005;51:19-30.
5. Nayampalli SS, Ainaupure SS, Samant BD, Kudtarkar RG, Desai NK, Gupta KC. A comparative study of diuretic effects of Tinospora cordifolia and hydrochlorothiazide in rats and a preliminary phase I study in human. J Postgrad Med 1998;34:233-6.
6. Tamboli, Saleem B, Sumit P Sontakke, Rahul B Parsode. Study on hypolipidaemic action of alcohol extract of Tinospora cordifolia (Guduchi) on alloxan-induced diabetic rabbits. J Res Indian Med 1969;3:203-9.
7. Raghunathan K, Sharma PV. Effect of T. cordifolia miers (Guduchi) on alloxan induced hyperglycemia. J Res Indian Med 1969:3:203-9.
8. Jeyachandran R, Francis Xavier T, Anand SP. Antibacterial activity of stem extracts of Tinospora cordifolia (Willd) hook. fand thomson. Anc Sci Life 2003:2:340-3.
9. Thahera, Parveen D, Shaik Nyamathulla. Antihyperlipidemic activity of the methanolic extract from the stems of Tinospora cordifolia on sprague dawley rats. Pelagia Res Library Der Pharm Sinica 2011;2:104-9.
10. Stanely Mainzen Prince P, Menon VP. Hypoglycaemic and hypolipidaemic action of alcohol extract of Tinospora cordifolia (Willd) hook. fand thomson. Anc Sci Life 2003:2:340-3.
roots in chemical induced diabetes in rats. Phytother Res 2003;17:410-3.

11. Sharma V, Pandey D. Protective role of Tinospora cordifolia against lead-induced hepatotoxicity. Toxicol Int 2010;17:12-7.

12. Nadkarni KM. Indian material medica. In: Nadkarni KM, editor. Bombay: Popular Book Depot; 1954. p. 1228.

13. Duraisankar M, Ravichandran V. Anti-pyretic potential of polyherbal ayurvedic products. Asian J Pharm Clin Res 2012;5:146-50.

14. Priyanka Sharma, Pradeep K Goyal. Modulation of biochemical and antioxidant enzymes in blood by Tinospora cordifolia against gamma radiation-mediated damage in mice. Asian J Pharm Clin Res 2015;8:106-12.

15. Manikkam Rajalakshmi, Roy Anita. β-cell regenerative efficacy of a polysaccharide isolated from a methanolic extract of Tinospora cordifolia stem on streptozotocin-induced diabetic wistar rats. Chem Biol Interact 2016;243:45-53.

16. Rajalakshmi M, Eliza J, Cecilia Edel Priya, Nirmala A, Daisy P. Anti-diabetic properties of Tinospora cordifolia stem extracts on streptozotocin-induced diabetic rats. Afr J Pharm Pharmacol 2009;3:171-80.

17. Coombes RC. Tumor-markers-their role in clinical cancer management. J Clin Chem Clin Biochem 1981;19:216.

18. Humason GL. Animal tissue techniques. WH Freeman and Company, San Francisco. 4th edition; 1979.

19. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957;13:164-76.

20. Sun Y. Structure and biological activities of the polysaccharides from the leaves, roots and fruits of Panax ginseng C. A. Meyer: an overview. Carbohydr Polym 2011;85:490-9.