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Research Article

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In vitro Cytotoxic Analysis of *Boerhaavia diffusa* Linn.

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Abstract

Cervical cancer is the second most common malignancy and cause of cancer mortality after breast cancer in developing countries. The high risk Human papiloma virus (HPV) types 16 and 18 are responsible for about 70% of all cervical cancer cases. HPV type 16 is sexually transmitted and most commonly causes. The aim of this study is to analysis the prevalence of HPV type16 among women and to evaluate phytochemical investigations and cytotoxic activity of *Boerhaavia diffusa* Linn. A total of 1180 abnormal cervical specimens were tested for HPV type 16. Among of them HPV type 16 was the most prevalent type detected 532 (45%). The non HPV 16 types were 648 (55%). The age wise distribution of HPV type 16 screening showed that 46% of patients were between the age group of 21-35, followed by 36% of the patients with the age group below 20 years. The overall in other group the prevalence ratio is generally lower. The active ingredients of *B. diffusa* were identified by ethanolic extraction method and further subjected to preliminary Phytochemical screening and cytotoxicity activity by MTT assay. Phytochemical testing showed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Terpenes, Anthraquinones and Steriods. Human Papiloma virus type 16 infected SiHa cervical cancer cell line were used to screen the anticancer potential of the extract of *B. diffusa*. The inhibition percentage with regard to cytotoxicity was found to be 47.1% at 125 µg/ml. The results of this research showed that the antioxidant played an important role in protecting the human body against cancer. The plant *Boerhaavia diffusa* Linn showed that significant anticancer activities against SiHa cell line which can be used as an alternative medicine.

Keywords: *Boerhaavia diffusa*, HPV 16, Cervical cancer, Carcinoma

Introduction

Cervical cancer is one of the most commonly occurring cancers of the female reproductive tract[15,17,18]. It is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020. About 90% of the new cases and deaths worldwide in 2020 occurred in low and middle income countries. It was estimated 529,409 new cases and

274,883 deaths in 2008[12,19]. Worldwide in 2012, there are 528,000 cases of cervical cancer were estimated to have occurred, with 266,000 deaths[17]. An estimated 12,900 new cervical cancers and 4,100 cervical cancer deaths will occur in the United States in 2015[16]. In Eastern and Middle Africa, cervical cancer is the most common cancer in women.

In high income countries, programmes are in place which enable girls to be vaccinated against HPV and women to get screened regularly and treated adequately. Nearly all cases of cervical cancer can be attributed to HPV infection. Screening allows pre-cancerous lesions to be identified at early stages when they can easily be treated. Over 100 types of HPVs have been identified till date. HPVs induces mostly benign papillomas. The high-risk HPV strains 16 and 18 cause more than 70% of cervical cancers and transmitted through sexual contact.

Cervical cancer is associated with many risk factors including early sexual debut, having multiple sexual partners or having sex with someone who has multiple sexual partners, HIV positive, a family history of cervical cancer, smoking and poverty etc. Cervical cancer ranks as the first most frequent cancer among women between 15 and 44 years of age in Tanzania. It is one of the most common type of cancer in women and the one with the highest mortality rate[7] in Tanzania. It has been reported that the high rate of infection with HPV type 16 and 18 are the frequent presence of viral DNA in cervical cancer among the population of Tanzanian women[6]. A recent estimate showed that approximately 7500 new cases are diagnosed every year and that about 4000 dies from cervical cancer yearly.

Boerhaavia diffusa Linn. commonly known as 'Punarnava' is an abundant creeping weed found all over India[1]. It is commonly known as Mukkurattai in Tamil language, Hog Weed and Pig Weed in English. The plant has drawn lot of attention due to its uses in Indian Traditional Medicine. The various parts of the plant are used in the treatment of cancer, jaundice, dyspepsia, inflammation, enlargement of spleen, abdominal pain and as an anti-stress agent[2,3] The whole plant extract is hepatoprotective in nature[2].It is also used for the treatment of diabetes [10] and to treat seminal weakness and high blood pressure[3]. The plant *B. diffusa* containing phenolic compounds, in particular alkaloids and amino acids have been reported to exhibit strong antioxidant properties. It also contains quinolizidine alkaloids and potassium salts[13]. The present investigation has been carried out to evaluate the cytotoxic effects of *B. diffusa* whole plant extracts. The whole plants of *Boerhaavia diffusa* are reported to have good medicinal values in traditional system of medicines.

Materials and Methods

A total of 1180 abnormal Pap smear specimens were tested for HPV type 16. The cases were women aged 18 to 65 years. Cervical cancer specimens obtained from women attending Cancer hospital in Tanzania, East Africa. They were recruited from the out and in-patient departments. The cases were women aged 18 years or more, with newly diagnosed histological confirmed cancer of the cervix.

Data will be analyzed by using SPSS.Cervical specimens were taken for cytological analysis. Cervical cancer was confirmed on the basis of histological results of cervical specimens and hybrid Capture 2 technology HPV DNA test. Human Papiloma virus type 16 infected SiHa cervical cancer cell line was obtained from National Centre for Cell Sciences (NCCS) at Pune in India.

The whole plant *B. diffusa* was collected, identified and authenticated by Dr. V. Balasubramanian, Senior Agriculture Officer at Salem in Tamil Nadu. The plant material was cut into small pieces and washed with sterile water, dried in shade, finely powdered and stored in air tight bottles. The active ingredients of *B. diffusa* were identified by ethanolic extraction method and further subjected to preliminary Phytochemical screening and Cytotoxicity activity by MTT assay. Phytochemical screening

of plants was carried out to detect bioactive compounds using qualitative tests. Phytochemical test and MTT assay test done at Life Teck Research centre in Vadapalani at Chennai in India.

Results and Discussion

A total of 1180 abnormal cervical specimens were tested for HPV type 16 (Table: 1). Among of them HPV type 16 was the most prevalent type detected 532 (45%). The non HPV 16 types were 648 (55%). The age wise distribution of HPV type 16 screening showed that 46% of patients were between the age group of 21-35, followed by 36% of the patients with the age group below 20 years. 11% of them belong to the age group of 36-40, 5% in the age group between 41- 49 and 2% between the age group of 50-60 (Table: 2). There is no cases in above 60 age group.

Table 1. Prevalence of HPV type 16 in women cases

S. No	Contents	Total numbers	Percentage (%)
1.	Total case study	1180	
2.	HPV type 16 Positive	532	45%
3.	Other than HPV type 16	648	55%

Table 2. Age wise distribution of HPV type 16 in Tanzanian women

S. No	Age group	HPV 16 positive sample Size = 532	Percentage (%)
1.	Below 20	191	36%
2.	21 - 35	241	46%
3.	36 - 40	59	11%
4.	41 - 49	28	5%
5.	50 - 60	13	2%
6.	61 and above	0	0%

Among the total abnormal cervical specimens, 45% had HPV type 16 positive. Hence, the incidences of HPV type 16 were higher among Tanzanian women than the other types. Similarly, higher prevalence of HPV 16 were observed in Europe, with a few studies in Spain and Italy reporting type-specific prevalence of over 25% for HPV 16 [1,4,14,20]. This may be true in the present study also.

In the present findings clinically apparent genital HPV type 16 infections are correlated with age and suggesting that these viruses are primarily transmitted in adulthood through sexual intercourse[5]. It has been expected that at least 50% of sexually active men and women acquire genital HPV infection at some stage in their lives. Nearly 80% of women will have acquired genital HPV by age 40 years, which makes HPV infections the norm rather than the exception suggested by Myers *et al* [9]. In Tanzania, screening is still at best very scarce and in the majority places not available at all.

The Phytochemical testing result showed that, the plant *B. diffusa* has contains major photochemicals such as Tannins, Alkaloids, Saponins, Flavonoids, Terpenes, Anthraquinones and Steriods (Table:3)are well known natural antioxidant. Antioxidants thus play an important role in protecting the human body against damage by reactive oxygen species. Currently available drugs and synthetic drugs do have potential adverse reactions. There are many natural drugs which are yet to be explored systematically.

Table 3. Preliminary phytochemical analysis of Ethanol extract of *Boerhaavia diffusa* Linn

S. No	Test	Ethanol extract of *B. diffusa*
1.	**Tannins** Test -1 Test -2	 Positive Positive
2.	**Alkaloids**	Positive
3.	**Saponins**	Positive
4.	**Flavonoids**	Positive
5.	**Terpenes**	Positive
6.	**Anthroquinones**	Positive
7.	**Steriods**	Positive

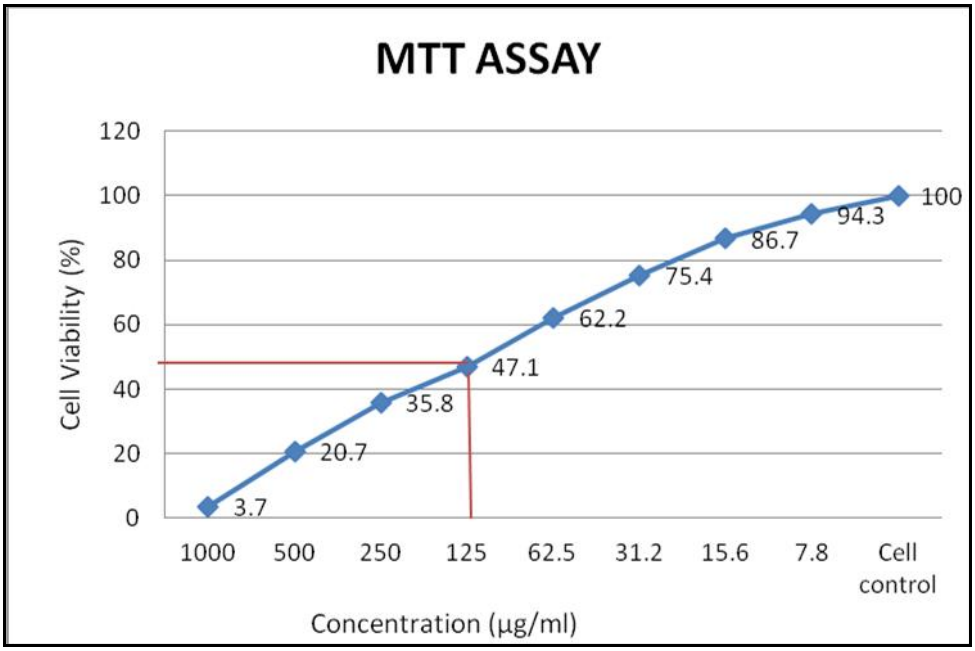
The inhibition percentage with regard to cytotoxicity was found to be 47.1% at 125 µg/ml (Table: 4).Rakhi Srivastava results demonstrated that *B. diffusa* fraction inhibits the proliferation of human cervical cancer cell line, HeLa and the cell cycle via S-phase inhibition plays some roles in *B. diffusa* induced antiproliferative activities in the HeLa cell line[11]. In the present study also showed that *B. diffusa* fraction inhibits SiHa cervical cancer cell line.

Table 4. Anticancer effect of *B.diffusa* Linn on SiHa cell line

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1.	1000	Neat	0.02	3.7
2.	500	1:1	0.11	20.7
3.	250	1:2	0.19	35.8
4.	125	1:4	0.25	**47.1**
5.	62.5	1:8	0.33	62.2
6.	31.2	1:16	0.40	75.4
7.	15.6	1:32	0.46	86.7
8.	7.8	1:64	0.50	94.3
9.	Cell control	-	0.53	100

Hence the plant *B. diffusa* has significant anticancer activities against SiHa cell line which can be used as an alternative medicine. The results of the study revealed that the plant extracts have strong antioxidant activity and remarkable cytotoxic activity (Fig: 1).The cytotoxic activity may be due to the presence of alkaloids in the plant.

Figure 1. Anticancer effect of *Boerhaavia diffusa* Linn on SiHA cell line (IC_{50} Value)



Further investigations are needed to provide some additional insight into the in-vivo antioxidant activity and cytotoxic activity of the plant. The above results suggest that *Boerhaavia diffusa* Linn were found to reveal antioxidant potential which supports the use of this plant in cervical cancer treatment.

Conclusion

This present study showed prevalence of HPV type 16 in women, is similar to those reported in other regions of the world. Similarly, it appears that HPV type 16 is the most common type associated with cervical cancer. The above study shows those young groups are more vulnerable to HPV 16 virus infection. This will also require the treatment of male partner if infected. Cervical cancer is one of the most successfully curable forms of cancer when diagnosed in early stage. Chemotherapy, radiotherapy and the other conventional cancer treatments used nowadays, are too expensive and cause many side effects. Currently Gardasil and Cervarix vaccinations are available. The vaccines are useful only for women before exposure to HPV infection. So, it is important to develop novel potent anti-cancer agents from natural products for the treatment of this virus disease. *Boerhaavia diffusa* Linn has inhibition percentage with regard to cytotoxicity was found to be 47.1 % at 125 µg/ml. The cytotoxic activity can be due to the presence of alkaloids in the plant, which needs further analysis. Further in-vivo studies will help us to investigation this plant in order to identify, isolate its active anticancer principles and its mechanism of action.

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