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Analysis of Radiotherapy and chemotherapy induced toxicity on rodents (Swiss albino) and pharmacological importance of *Pistia stratiotes*

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Abstract

Herbal medicines has been reported to play an important role in the discovery of new drugs for the treatment of human diseases, including cancer [1]. Many herbal remedies have shown to play anticancer role through multiple mechanisms. Our present work is an attempt to screen some essential components from the water weed Pistia stratiotes and to study its potentiality against the side effects induced by radiotherapy and chemotherapy

Key words: Radiotherapy, chemotherapy, Pistia stratiotes, Herbal medicine, Phytochemical screening.

Introduction

Cancer is the leading cause of death worldwide. Different treatment modalities are on their way to treat cancer. The most common treatment modalities include: Chemotherapy and Radiotherapy. Many of the studies has shown severe damage caused by exposure to radiation and chemotherapy.

Being the most common treatment, radiotherapy and chemotherapy itself causes many alterations in the cellular and the genetic level. Many herbal crude drugs including Ocimum sanctum, Basil etc (P U Devi et. al) has shown a good response to reduce the cytotoxicity caused by radiation. The herbal crude extract of Pistia stratiotes is used in this work to study its potentiality to reduce toxicity caused due to radiation and chemotherapy drugs. The crude extract derived from the water weed; Pistia stratiotes has shown to contain Alkaloids and steroids which may play an important role in reducing the toxicity induced by radiotherapy and chemotherapy. In this work we have shown that the crude extract of Pistia stratiotes are rich in anti-oxidants which are the potential candidate to reduce the toxicity induced by both radiation and chemotherapy in Swiss albino mice model.

Methods

Collection of plant materials

Pistia stratiotes, the total plant with its leaves and roots were collected from the upper and the lower lake of Bhopal. Weight of the plant sample (Pistia stratiotes) collected was taken immediately just after the sample collection.

Extraction

Powder of leaves of Pistia stratiotes (2-3 weeks after shade drying) was weighed & loaded in a separating funnel with 50% methanol. The concentrated crude extract was formed as a result of evaporation of the solvent (50 % methanol) in a water bath at 45□C,
thereby forming a brown colored marc, with fine crystals. This collected crude extract crystals was then used as 50% methanolic extract (50 % ME) for the experiment.

Phytochemical screening

The methanolic extract of the crude drug (Pistia stratiotes) was subjected to systematic phytochemical screening to assess the presence of different phytoconstituents such as alkaloids, glycosides, proteins, amino acids, sterols, carbohydrates compounds, acidic compounds, resins etc.

Thin layer chromatography

Thin layer chromatography (TLC) was performed using petroleum ether, chloroform, methanol, ethyl acetate and water extracts. All the extracts were used after dissolving them in their respective solvents to prepare sample solutions.

Column chromatography

The crude drug 50 % methanolic extract (Pistia stratiotes) was used for column chromatography. Silica gel (100-200 mesh) was used as a stationary phase and successive solvent systems were
used ranging from non-polar to polar solvents. The rate of elution was fixed at 10 drops per minute. Different fractions of the different solvents used, were collected and used for λ max calculation using UV-spectrophotometer (data not shown).

Experimental Animals

The Swiss albino mice of the mean weight of 31gm and 6-7 weeks old were obtained from the animal house, Jawaharlal Nehru Cancer Hospital & Research center, Bhopal. The use of animal was as per CPCSEA norms. Approval for experimental work was as per ethical committee of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal (M.P.).

Preparation and Administration of Test Drug (50% ME of Pistia stratiotes) solution

The amount of the test drug (50% ME) to be administered was calculated depending on the body weight of the animal and the design of experiment. The crude drug was administered orally to each animal by using a cannula.

Experimental Design

Animals were divided into six groups of four animals each, for both Radiotherapy as well as chemotherapy groups. The groups were treated as follows:

Induction of Radiation on experimental animals

Unanesthetized mice were restrained in ventilated Perspex box and the groups including: B, C, E and F were given 9 Gy as discussed in the protocol. The whole body dose was given at the rate of 150 cGy/min by Co-60 Tele-therapy unit (Theratron 780 C, Co unit manufactured by M/S Theratronics Ltd, Canada), at a distance of 80 cm from the source, at Jawaharlal Nehru cancer hospital and Research center, as previously described by M. R. Adhvaryu et al. (2008). The commercial radio-protective drug (Antioxidant: Antoxid) dose was given to the animals belonging to group B, along with the γ – radiation (9 Gy). The stock solution was prepared in water for injection and the dose was given as

per the

Groups	Treatment
A	Normal control (No crude drug or radiation)
B	Standard Radio-protective Drug (Antioxidant; Antoxid + γ-Radiation: 9Gy)
C	Radiation control (only γ-Radiation: 9 Gy)
D	Test crude drug control (only 50 % ME, conc. 500 mg/kg B.wt)
E	Test crude drug (100 mg/kg B.wt) + γ-Radiation: 9 Gy)
F	Test crude drug (500 mg/kg B.wt) + γ-Radiation: 9 Gy)

Chemotherapeutic group

Groups	Treatment
P	Normal control (No crude drug / chemo drug)
Q	Standard Chemo-preventive Drug (MVI + Cyclophosphamide)
R	Chemotherapy drug control (only cyclophosphamide)
S	Test crude drug control (only 50 % ME, conc. 500 mg/kg B.wt)
T	Test crude drug (100 mg/kg B.wt + Cyclophosphamide)
U	Test crude drug (500 mg/kg B.wt + Cyclophosphamide)

body weight (100 mg/kg body weight).

Induction of Chemotherapeutic drugs on experimental animals

A freshly prepared solution of Cyclophosphamide (50 mg/kg body weight as previously discussed, Ralf Paus, Bori Handjiski *et. al,* 1994) in DDW was injected intra-peritoneal to the mice belonging to groups (Group Q, R, T, U). The stock solution of Multi-vitamin Infusion (MVI) was prepared in water for injection and the dose was given to group Q as per the body weight (100 mg/kg body weight).

Histopathological Studies

Major organs (including Intestine, Liver, kidney and spleen) of both the group of animals were flushed with normal saline & fat tissues were removed and fixed in 10% formalin solution for histo-pathological evaluation to assess chronic toxicity of the crude herbal extract on these organs and also the effect of radiotherapy and chemotherapy on these biological specimens. The histo-pathological section was done by Pal Diagnostic Centre, Guru Khalsa Hospital, Bhopal (M.P).

Chromosomal, Apoptosis and Micronucleus Studies

Weight of the mice was recorded. 0.1 ml/gm body weight colchicine (0.025%) was injected into peritoneal cavity of the mice. After two hrs of injection, the mice were killed by cervical dislocation. Bone marrow from the femur bone was used for the chromosomal, Apoptosis and Micronucleus studies as described M. R. Adhvaryu*, S. P. Srivastav et. al, 2008. It was proposed independently by Schmid [68] and Heddle [69] that an alternative and simpler approach to assess chromosome damage in vivo was to measure micronuclei (MNi), also known as Howell–Jolly bodies, in dividing cell populations such as the bone-marrow. Number of cells having abberations per 50 normal cells were counted and Data was analyzed by ANOVA.

Results and Discussion

Phytochemistry

Phytochemical analysis of the 50 % crude Methanolic extract of the leaves of Pistia stratiotes revealed high sufficient amount of deoxysugars were found during the phytochemical screening (Table No. 1).

Table 1: Phytochemical screening of crude drug extract (50 % ME *Pistia stratiotes*)

Components	Tests	*Pistia leaves*
Alkaloids	Mayer's test	+
Glycosides	Keller Killani test	+
Sterols	Salkowski reaction	+
Carbohydrates	Molish's test	+
Phenols	Fehling's test	+
Flavanoids	Ferric chloride test	-
Saponins	Shinoda test	-
Tanins	Foam test	-

Chromatography

The crude drug extract (50% methanolic extract) of Pistia stratiotes was seen to show best resolution in Chloroform: Methanol (9:1) (Data not shown). Different fractions of each of the solvents used in column chromatography were collected and λ-max was recorded using UV double beam spectrophoto-meter (Data not shown).

Histopathological studies

Different histopathological studies of organs revealed the damaged Crypt & Villi to be prominent in both the groups, whereas there was much less toxicity found in the test drug group of both conc. 100 & 500 mg/kg body weight, as shown in the Fig.1.

In vivo study

Six groups each for both Radiotherapy and chemotherapy were used for the In vivo study, so as to evaluate the ability of crude extract of Pistia stratiotes against toxicity induced by chemo-therapy and radiotherapy.

Anthropometry (body & spleen weight profile)

Table 2 and 4 shows no any significant difference in the body weight of the two groups, whereas Table 3 shows a very significant decrease in the spleen weight within the radiation treated groups.

Table 2: Body Weight Difference in mice before and after γ -Radiation exposure (9 Gy)

Groups	Day 1 Wt.	Day 15 Wt.
A	L = 34.30	L = 35.60
	R = 22.40	R = 25.90
	LR = 30.60	LR = 34.90
	NM = 25.80	NM = 29.00
B	L = 25.50	L = 21.40
	R = 25.15	R = 22.00
	LR = 22.30	LR = 22.85
	NM = 26.80	NM = 28.85
C	L = 24.85	L = 23.90
	R = 28.10	R = 23.55
	LR = 22.10	LR = 25.25
	NM = 25.60	NM = 26.55
D	L = 25.35	L = 24.70
	R = 26.30	R = 27.70
	LR = 29.70	LR = 27.80
	NM = 27.70	NM = 27.10
E	L = 28.15	L = 29.40
	R = 24.35	R = 27.00
	LR = 31.40	LR = 33.85
	NM = 31.70	NM = 31.85
F	L = 27.50	L = 29.95
	R = 25.10	R = 30.70
	LR = 28.00	LR = 29.90
	NM = 27.20	NM = 29.60

* L : Left ear mark, R : Right ear mark, LR : Left & Right ear mark, NM : No mark.

Radiation Group

Group A : Normal control (No any treatment of any crude drug
or radiation), Group B : Standard Radioprotective Drug (Antioxidants; Antoxid + γ-Radiation; 9 Gy), Group C : Radiation control (only γ-Radiation : 9 Gy), Group D : Test drug control (only 50 % ME of Pistia stratiotes, conc. 500 mg/kg B.wt), Group E : Test Drug (conc. 100mg/kg B.wt) + γ- Radiation (9 Gy), Group F : Test Drug (conc. 500 mg/kg B.wt) + γ-Radiation (9 Gy)

Table 3: Spleen weight difference in mice after γ-Radiation exposure (9 gy)

Group / Mice	A (mg)	B (mg)	C (mg)	D (mg)	E (mg)	F (mg)
L	122.0	90.08	19.1	100.0	60.0	75.3
R	100.0	99.90	14.9	110.0	72.2	78.0
LR	111.0	92.50	15.0	104.	71.6	72.7
NM	100.0	100.2	16.3	99.00	68.10	80.30

Table 4: Body Weight Difference in mice before and after Chemotherapy

Group	Day 1 Wt.	Day 15 Wt.
P	L = 34.30 R = 22.40 LR = 30.60 NM = 25.80	L = 35.60 R = 25.90 LR = 34.90 NM = 29.00
Q	L = 29.20 R = 24.30 LR = 31.40 NM = 30.35	L = 33.30 R = 24.30 LR = 32.00 NM = 30.00
R	L = 30.30 R = 28.60 LR = 22.85 NM = 28.20	L = 33.30 R = 30.00 LR = 26.20 NM = 32.30
S	L = 25.35 R = 26.30 LR = 29.70 NM = 27.70	L = 24.70 R = 27.70 LR = 27.80 NM = 27.10
T	L = 30.80 R = 31.25 LR = 26.00 NM = 26.70	L = 32.10 R = 32.50 LR = 26.30 NM = 27.10
U	L = 31.90 R = 28.80 LR = 35.90 NM = 30.80	L = 33.70 R = 29.70 LR = 35.70 NM = 31.80

*** L : Left ear mark, R : Right ear mark, LR : Left & Right ear mark, NM : No mark.**

Chemotherapy Group

P : Normal control (No any treatment of any crude drug or cyclophosphamide), Q : Standard Chemopreventive drug (MVI)+ cyclo- phosphamide, R : Chemotherapy control (only cyclophosphamide), S : Test Drug control (50 % ME Pistia stratiotes), T : 100 mg Test Drug + cyclophosphamide, U : 500 mg Test Drug + cyclophosphamide.

Histopathological studies; Tissue protection (Crypt & villi count)

Both the Radiation as well as the chemotherapy groups show much difference in the crypt and villi counts in the crude drug treated as well as controls as shown in Table: 7, 8 and Graph: 1,2,3 and 4, showing the protective nature of the crude drug (50 % ME Pistia stratiotes) at both the concentrations: 100 and 500 mg/kg body weight.

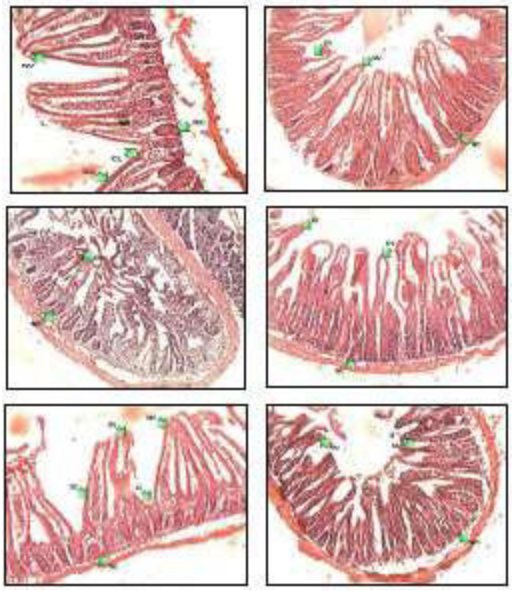
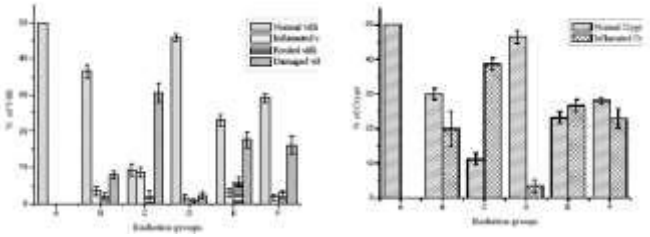


Fig 1: Histopathological sections of Intestine belonging to Radiation group
(A) **Normal control (No any treatment of crude drug or γ -Radiation) showing intact crypt & villi,** (B) **Standard Radioprotective Drug control (γ –Radiation: 9 Gy + Antoxid) showing radioprotective nature of commercially available drug,** (C) **Radiation control (only γ –Radiation: 9 Gy) showing damaged crypt & villi counts compared to normal,** (D) **Crude Drug control (only 50% ME of *Pistia*) showing normal crypt & villi counts,** (E) **100 mg Crude Drug + γ –Radiation: 9 Gy showing protective nature,** (F) **500 mg Crude Drug + γ –Radiation: 9 Gy showing better protective nature than 100 mg, compared to radiation control. * NC: Normal crypt, CL: Crypt of Lieberkuhn, GC: Goblet cells, NV: Normal villi, DV: Damaged villi, IV: Inflamated villi, IC: Inflamated crypt**



Graph 1, 2: Comparison between the Normal & Inflamated crypt & villi counts in Radiation group

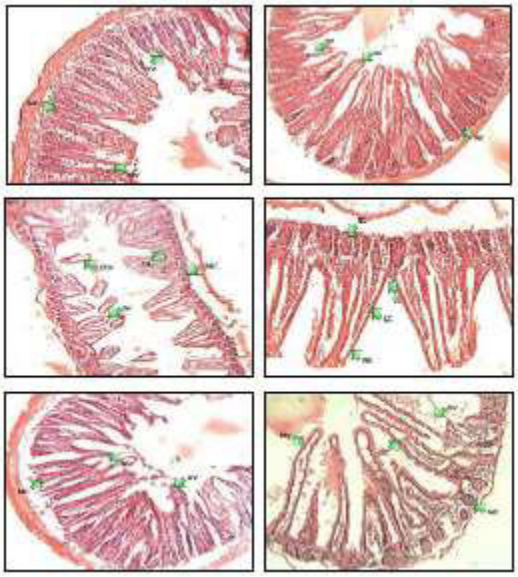
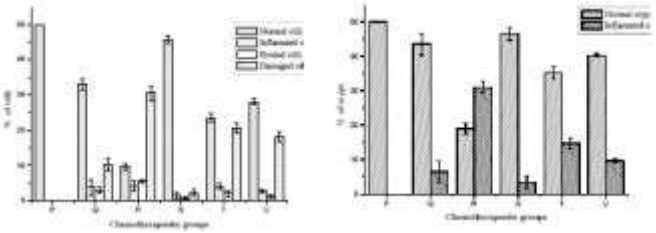


Fig 2: Histopathological sections of Intestine belonging to Chemotherapy group (P) Normal control (No any treatment of crude drug or cyclophosphamide) showing intact crypt & villi, (Q) Standard Chemopreventive Drug control (cyclophosphamide + MVI) showingchemopreventive nature, (R) Chemotherapy control (only cyclophosphamide) showing damaged crypt & villi counts compared to normal, (S) Crude Drugcontrol (only 50% ME of Pistia) showing normal crypt & villi counts, (T) 100 mg Crude Drug +cyclophosphamide, showing protective nature, (U) 500 mg Crude Drug + cyclophosphamide, showing better protective nature than 100 mg, compared to chemotherapy control.

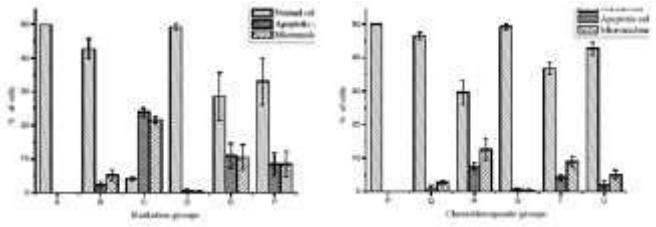


Graph 3, 4: Comparison between the Normal & Inflamated crypt & villi counts in chemotherapy group

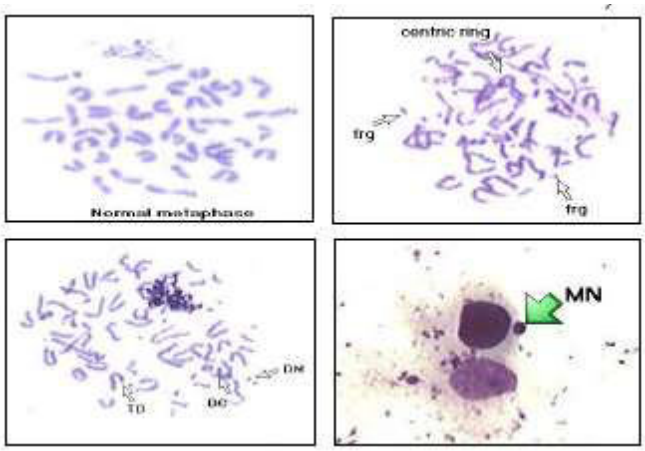
Genotoxicity study (Chromosomal

Apoptosis and Micronucleus)

The micronucleus and the apoptotic cell counts in group E and F does not vary significantly, but as compared to the group B (standard Radioprotective drug: Antoxid + γ-Radiation; 9 Gy), group F with the crude drug concentration 500 mg/kg b.wtshows nearly equal protective nature (Table: 9, Fig: 5). In the chemotherapy group, group U with the crude drug concentration 500 mg/kg b.wt shows nearly equivalent protective nature when compared with the group Q (cyclo-phosphamide + MVI) (Table: 10, Fig: 6).

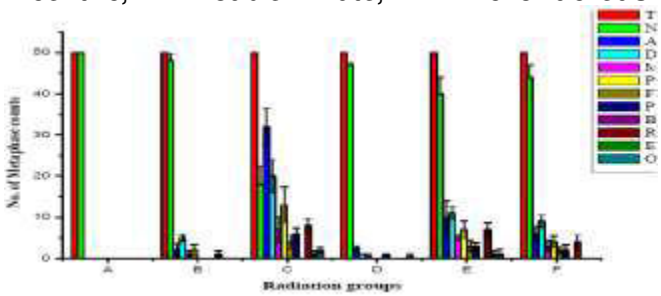


Graph 5, 6: Showing number of Normal versus micronucleus & apoptotic cells in Radiation and Chemotherapy groups types of aberrations have been seen in the Radiation group treated with γ-Radiation (9Gy). The toxicity induced by the radiation is most prominent in the radiation control group (C). The crude drug extract (50 % ME Pistia stratiotes)



given in two different concentrations (ie. 100 mg and 500 mg/kg b.wt), in the groups E and F respectively, shows a good protective ability. The group F with 500 mg/kg B.wt shows a significant decrease in the toxicity levels as compared to the radiation control group C (Table 11, Graph

Fig 3: Chromosomal aberrations due to γ –Radiation (9 Gy)
in Radiation group
(a) Normal Metaphase, (b) & (c) Aberrant Metaphase,
(d) Micronucleus. * frg: Fragment, TD: Terminal Deletion, DC: Dicentric, DM: Double Minute, MN: Micronucleus



Graph 7: Comparative study of different aberrations in all groups caused due to irradiation Radiation Group: A: Normal control, B: Standard Radio-protective (γ -Radiation 9 Gy + antoxid), C: Radiation control (only γ- Radiation: 9 Gy), D: Crude Drug control (only 50 % ME of Pistia), E: 100 mg Crude Drug + 9 Gy, F: 500 mg Crude Drug + 9 Gy * TM: Total Metaphase, FBD: Fragment Break Deletion, NM: Normal Metaphase, PP: Polyploidy, AM: Aberrant Metaphase, BB: Bubbling, DC: Dicentric, ER: Endo-reduplication, MIN: Minute, OT: Others, PCD: Premature Centromeric Division, ICD: Inter-Calary Deletion

References

[1]. Evans HJ. Molecular mechanism in theinduction of chromosome aberrations Holand Biomedical Press. 1977; 57-74.

[2]. Ganesh C Jagetia et al. Radioprotectivepotential of plants and herbs against the effect of ionising radiation. Journal of clinical biochemistry, Nutrition. 2007;40:74-81.

[3]. Schuchter L M et al. The current status of W R 2721 (Amifostine : a chemotherapy and radiotherapy protector". Biologic Therapy Cancer updates. 1993 3; 1-10,.

[4]. Jagitia G C et al. Radiation modifyingeffect of Ocimum santum on mousesurvival. Annual of National academy of sciences. (India), 1986.

[5]. Goel H. C et al. Radioprotection by herbal preparation of Hippobhae rhamnoides, RH-3againt whole body lethal irradiation in mice. Phytomedicine. 2002 9;15-25.

[6]. Salin C A et al. Protection of mousejeujenum against the lethal irradiation by Podophyllum hexandrum. Phytomedicine. 2001;8: 413-422,.

[7]. Mittal A et al. Influence of Podophyllum hexandrum on endogenous anti-oxidant defence system in mice : Possible role in radio protection. Journal of ethnopharmacol. 2002;76:253-262.

[8]. Goel H. C et al. Radioprotective potentialof herbal extract of Tinospora cordefolia. Journal of Radiation Research. 2004;45:61-68.

[9]. Dische S et al. A radomized multicentertrail of CHART vs conventional radiotherapy in head and neck cancer". Radiotherapy Oncology. 1979;44:123-136,

[10]. NHGRI Chromosome Abnormalities, 2006. Gupta, SS. Prospects and Prespective of natural plants products in medicine, Indian Journal of Pharmacology :26:1-12, 1994.

[11]. Farns Worth NR. Medicinal plants in therapy Bulletin of the World Health Organization 1985 63 no.6:965-981.

[12]. Andrew Vickers, Catherine Zollman and Roberta Lee, Herbal medicine. BMJ 1999 319:1050-1053.

[13]. Arora R et al. Radioprotection by plant products: Present status and future prospects. Phytotherapy research. 2005;19:1-22.

[14]. Borris RP. Natural products research prespectives from a major pharmaceutical company, J Ethanopharmacol. Moreman D.E, "An analysis of food plants drug plants of native north America", J. Ethanopharmacol, 1996;52.

[15]. Thomas M Zennie and Jerry W.McClure, 2004. Pietro Monaco and Lucio Previtera, 1991

[16]. Anamika Tewari et al., "Amelioration of muncipal sludge by Pistia stratiotes L:Role of antioxidant enzymes indetoxification of metals." 2008.

[17]. M D Mukhtar et al. 2004.

[18]. Ling Y et al. 1999. Medicinal plants of east and southeast Asia. By L.M Perry. MIT Press, Cambridge`. Hand book of Energy Crops. By J.Duke, 1980.

[19]. Tropical feeds. Feed informationsummaries and nutritive value. By B.Gohl. FAO Animal Production and Health Series 12. FAO, Rome. Handbook of Energy Crops. By J.Duke, 1981.

[20]. Making aquatic weeds useful. National Academy of Sciences (or N.A.S.), Washington, DC. 1976.

[21]. Amirhosein Ahemed et al., "Preventive effects of hesperidin as a flavanoid, against genotoxicity induced by cyclophosphamide. 2008.

[22]. Yogeshwari Shukla and MadhulikaSingh, "Cancer preventive property of ginger : A brief review". Food and chemical Toxicology. 2007;45; 683-690.

[23]. Deendayal Patel et al., "Epigenin and cancer chemoprevention : Progress, potential and promise". International Journal of Oncology. 2006;30:233-245,.

[24]. Niture et al., "Chemopreventive strategies, targeting the MGMT repairprotein : Augmented expression inhuman lymphocytes and tumor cells byethanolic and acqueous extracts ofseveral indian medicinal plants." International Journal of Onology. 29;1269-1278, 2006.

[25]. A A Izzo et al., "Eating garlic andonion : A matter of Life or death". British Journal of Cancer. 2004;91; 194,.

[26]. Gauri Shankar S A, Tani Das, "Anticancereffects of Curcumin : Cycle of lifeand death." Biomed. (Cell Division). 2008 3-14.

[27]. Annelyse Duvoix et al., "Chemopreventive and therapeutic effectsof Curcumin". Cancer Letters. 2-3; 181-190, 2004.

[28]. Gajulla D. et al., "Determination of totalPhenolics, Flavanoids and Antioxidant andchemopreventive potential of Basil". International Journal of Cancer Research. 2009;5(4):130-143,.

[29]. Shilpa S and Ganesh N et al., "Tumorinhibition and cytotoxicity assay byaqueous extract of onion and garlic : Invitro analysis". International Journal ofPhytomedicine. 2010;2;80-84.

[30]. Chi Chen et al., "Dietary chemopreventivecompounds and ARE / EpRE signalling". Free radical Biology and Medicine. 36;1505-1516, 2004.

[31]. Imad Shureiqr et al., "Chemoprevention :A general prespective". OncologyHematology. 2000;33:157-167,.