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PRECLINICAL EVALUATION OF *INVIVO* ANTICANCER ACTIVITY OF *PUNICA GRANTUM* L AND *ZIZIPHUS MAURITIANA* USING EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE.

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ABSTRACT

The present investigation was designed to preclinical evaluation of *invivo* anticancer activity of *Punica grantum* L and *Ziziphus mauritiana* using Ehrlich Ascites Carcinoma in Swiss albino mice. Experimental tumor was induced by inoculation of $1x10^6$ Ehrlich ascites carcinoma cells from the tumor bearing mice aseptically. Group 1 mice (n=12) served as normal control, group 2 mice (n=12) were Ehrlich ascites carcinoma cells control. Group 3 mice (n=12) received standard drug 5- Flourouracil 20 mg/kg b.w, i.p., group 4, 5 and 6 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of *Punica grantum* L of 200 mg/kg b.w, respectively, for nine days, group 7, 8 and 9(n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of *Ziziphus mauritiana* of 200 mg/kg b.w, respectively, for nine days, whereas group 10, 11 and 12 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of combination of both plants of 200 mg/kg b.w, respectively, for nine days. Change in body weight, survival time, ascites fluid volume and packed cell volume were noted. At the end of study, 6 animals from each group were sacrificed, blood samples were collected and WBC, RBC, Hb content were estimated. Ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* showed significant reduction in body weight, tumor volume, packed cell volume and percentage increase in life span. Significant increase in RBC, Hb content and reduction in WBC count were observed. The results suggest that the ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* exhibited significant anticancer activity ($r<0.001$).

KEYWORDS: *Punica grantum* L, *Zizipus mauritiana* , Ehrlich ascites carcinoma.

1. INTRODUCTION:

Cancer is one of the most common devastating disease affecting millions of people per year. Cancer has been estimated as the second leading cause of death in humans. The plant kingdom has provided a variety of medicines for cancer treatment, currently over 60% of the drugs are derived in one or other way from natural source including plant, marine organism and micro-organism. There are worldwide efforts to discover anticancer agents from plants (Rajandeep et al,2011). Plants have proved to be an important natural source of anti-cancer therapy for several years (Nirmala et al, 2011). The first agents to advance into clinical use were the so-called vinca alkaloids, vinblastine (VLB) and vincristine (VCR) as active agents, isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae). These compounds reported potential activity against lymphocytic leukemia in mice. The mechanism of action of Vinca alkaloids is that they inhibit the cell proliferation by affecting the microtubular dynamics during mitosis, and this causes a characteristic block during mitosis leading to apoptosis (Rajandeep et al,2011). Podophyllotoxin is obtained from the roots of Podophyllum species, namely, *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich. Epipodophyllotoxin is an isomer of podophyllotoxin. The two clinically important semi-synthetic analogs generated from Epipodophyllotoxin are Etoposide and Teniposide which were found very potential in treating lymphomas, bronchial and testicular cancers (Nirmala et al, 2011). Paclitaxel (Taxol) is obtained from the bark of the Pacific Yew, *Taxus brevifolia* Nutt.(Taxaceae). Another species, *Taxus baccata*, an Indian Ayurvedic medicine have also been in use for cancer therapy. Research is still undergoing in the area of anti-cancer therapy (Nirmala et al, 2011). Curcumin (diferuloylmethane), a polyphenolic compound is isolated from the Indian plant spices, *Curcuma longa* (commonly known as turmeric), now finds its application as potential anti-cancer compound. Curcumin is involved in modulating the cell cycle pathway and induces apoptosis of various cancer cells (Nirmala et al, 2011). A plant alkaloid, Ellipticine (5, 11-dimethyl-6H-pyrido [4,3-b] carbazole) and its derivatives were isolated from Apocynaceae plant species (eg. *Ochrosia borbonica,Excavatia coccinea, Ochrosia elliptica).* They exhibit significant anti-tumor properties. The primary function of this drug is that it intercalates with DNA and also causes inhibition of Topoisomerase II activity. It is also reported that this drug, inhibits cell growth and causes apoptosis of human hepatocellular carcinoma HepG2 cells (Nirmala et al, 2011). Major research programs are being undertaken in a number of laboratories in various parts of the world, screening plant extracts for anticancer activity (Naik et al, 2006). Therefore, the present study taken up for screening the anticancer potential of *Punica grantum* L and *Ziziphus maritiana*. The recent studies focused on the potential role of the leaves extraction of *Ziziphus mauritiana* as prevention or regression agent affecting the growth of certain tumors (kunwar et al, 2009). The extracts from fruits (Ndhala et al,2006), leaves (Dahiru et al, 2005; Dahiru and Obidoa, 2007) of *Ziziphus mauritiana* have been reported to exhibit antioxidant activity, whereas bark (Pisha et al, 1995; Ramadoss et al, 2000) and pulp (Vahedi et al, 2008) are reported to possess cytotoxicity against different cancer cell lines. *Ziziphus mauritiana* belongs to the family *Rhamnaceae*, It contains flavonoids, oleic and linoleic acid, triterpene oligoglycosides (Yoshikawa et al, 1997) *Ziziphus mauritiana* is used for many of their ailments such as burns, wounds, conjunctivitis, cold, cough, mouth ulcer, fever, hypertension, diuretic, anthelmintic, antiemetic, etc (Chopda and Mahajan, 2009; Jyotsana et al, 2010; Ashok et al, 2010; Jain et al, 2010). Several studies have indicated immunomodulatory activity (Wadekar and Patil, 2008) ([16]),hepatoprotective activity (Dahiru et al, 2005; Dahiru and Obidoa, 2007), antioxidant and free

radical scavenging activity (Kalidose and Krishnamoorty, 2011; Abalaka et al, 2011), antiulcer activity (Panchal et al, 2010), antidiarrhoeal activity (Dahiru et al, 2006), antimicrobial activity (Abalak et al, 2010; Mahesh and Satish, 2008), antiplasmodial and antimycobacterial activity (Panomwan et al, 2011), antihyperlipidaemic activity (Dahiru and Obidoa, 2009). Recent studies have shown that pomegranate is a potent anticancer agent that causes the induction of apoptosis and cell cycle arrest in cancer cells, inhibition of multiple signaling pathways in cancer cells, inhibition of tumor genesis in animal models of various carcinomas (Sartor and Pal, 2013; Ryan et al, 2013; Yakes et al, 2011; Smith et al, 2013). *Punica grantum* L belongs to family *Punicaceae* commonly called Pomegranate, recently described as nature's power fruit (Jurenka, 2008). It contains antocyanins, glucose, ascorbic acid, punicic acid, sterols, polyphenol and flavonols (Aviram et al, 2000; Sharaf and Nigm, 1964). Traditionally *Punica grantums* is used as anti-inflammatory (Ahmed et al, 2005), antidiabetic (Huang et al,2005), and neuroprotective (Loren et al, 2005). It has been reported for treatment against influenza virus (Haidari et al, 2009), diarrhea and dysentery (Ismail et al, 2012), anti-atherosclerotic (Cam et al, 2009) and antioxidant activity (Chidambara et al, 2002). Keeping above in view, the present study was taken up to predict potential antitumor effect of aqueous, ethanol and chloroform seed extracts of *Ziziphus mauritiana* and *Punica grantum* L in combination to evaluate the synergic effect of both plants in EAC tumor bearing mice as there is no previous anticancer activity reported for the combination of the two plants. This research will enable us to suggest, increase the frequency of the more potent extract of these plants in diet for prophylactic/curative purpose**.**

2. MATERIALS AND METHODS

2.1. Plant material and preparation of extracts:

The authenticated aqueous, ethanol and chloroform extracts of *Punica grantum* L and *Ziziphus mauritiana* were obtained from "Green Chem", Bangalore-560071.The seeds of *Punica grantum* L and *Ziziphus mauritiana* were obtained from local markets. The seeds were washed with water for the removal of adhering material and sun dried. Seeds were powdered with a mechanical grinder, passing through sieve # 40 and stored in airtight container. The seed powder (1kg) was extracted in a soxhlet with hexane (4000ml) for 6h for the removal of fatty matters. The hexane extract was discarded and residues were successively extracted with distilled water, ethanol and chloroform (3200ml each) for 8h each. The extracts were filtered and concentrated under vaccum (Buchi, Switzerland) to get concentrated extracts (60g), which was dried in vaccum oven and stored in a desiccator.

2.2. Animals:

Healthy male adult Swiss albino mice weighing 25±5 g were obtained from the Drug Control Laboratory (DCL), Bangalore, India, housed in well ventilated cage under standard laboratorial conditions, i.e. room temperature of 25 ± 3 ^{0}C, relative humidity 45-55 % and natural day and night cycle. The animals were allowed free access to standard laboratory cube pellets and drinking water *ad libitum.* The study protocol was approved by Institutional Animal Ethics Committee.(Registration No: 152/1999, renewed in 2012).

2.3. Tumor Cells:

EAC cells were obtained by Amala Cancer Research Center, Thrissur, Kerala, India and were maintained by weekly intraperitonial (i.p) inoculation of 10^6 cells/mouse in the laboratory. Ehrlich Ascites Carcinoma (EAC) cells maintained in the peritoneal cavity of Swiss albino mice were collected from an animal having 7 days old ascitic tumor by aspirating the ascitic fluid in sterile

isotonic saline. The viable EAC cells were counted (trypan blue indicator) under microscope. A fixed number of viable cells 10^6 cells were inoculated into the peritoneal cavity of each recipient mouse.

2.4.Grouping and treatment:

Experimental tumor was induced by inoculation of $1x10^6$ Ehrlich ascites carcinoma (EAC) cells from the tumor bearing mice aseptically. Healthy, adult Swiss albino mice were divided into 12 groups of 12 animals each and following treatment was given: Group 1 mice (n=12) served as normal control, group 2 mice (n=12) was EAC control. Group 3 mice (n=12) received standard drug 5- Flourouracil 20 mg/kg b.w, i.p., group 4, 5 and 6 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of *Punica grantum* L of 200 mg/kg b.w, respectively, for nine days, group 7, 8 and 9(n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of *Ziziphus mauritiana* of 200 mg/kg b.w, respectively, for nine days, whereas group 10, 11 and 12 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of combination of both plants (ZP) of 200 mg/kg(100 mg from each plant) b.w, respectively, for nine days.

2.5. Tumor growth response:

The anticancer effect of plant extracts were assessed by change in tumor volume, packed cell volume, median survival time (MST) and percentage increase in life span (%ILS). The tumor volume was measured by taking the ascetic fluid in a graduated centrifuge tube following its collection from the peritoneal cavity; the total volume of fluid was centrifuged at 2000 rpm for 20 minutes for determining the packed cell volume. MST and %ILS were determined using following formulae (Durairaj et al, 2009).

$$\text{MST} = \frac{\text{day of first death} + \text{day of last death}}{2}$$

$$\%\,\text{LS} = \frac{(\text{MST of treated group} - \text{MST of control group})}{\text{MTS of the control group}} \times 100$$

2.6. Hematological parameters:

At the end of experimental period, on day 10, after an overnight fasting, blood was collected from freely flowing tail vein and used for the estimation of the hemoglobin (Hb) content, red blood cells (RBC) count and white blood cell (WBC) count by standard procedures.

2.7. Statistical analysis:

The data were expressed as mean ± S.E.M (n=6). The statistical analysis was performed by means analysis of variance (ANOVA) followed by Dunnett's post hoc test where the difference was considered significant if $p < 0.05$.

3. RESULTS

3.1. Survival Time:

Table 1 shows the effect of different extracts of *Punica grantum* L and *Ziziphus mauritiana* on mean survival time and % ILS against EAC. In the EAC control group, the mean survival time was 14 days and it increased significantly to 23 and 21 days with ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* L ZP (E) and ethanol extract *Punica grantum* L P(E) of treated groups respectively. The mean survival time in 5-Flurouracil treated mice found to be 26 days (r <

TREATMENT GROUP	MST (Days)	ILS%
EAC CONTROL	14.20 ± 0.58	-
5-FLUOROURACIL	26.20 ± 0.73^{r}	84.51
P-E	21.60 ± 0.51^{rz}	52.11
P-Aq	19.80 ± 0.37^{rz}	39.44
P-Ch	19.00 ± 0.71^{rz}	33.80
Z-E	20.20 ± 0.58^{rz}	42.25
Z-Aq	18.60 ± 0.93^{rz}	30.99
Z-Ch	17.60 ± 0.40^{qz}	23.94
ZP-E	23.20 ± 0.86^{rx}	63.38
ZP-Aq	20.20 ± 0.86^{rz}	42.25
ZP-Ch	19.40 ± 0.74^{rz}	36.62

0.001). The %ILS was found to be 63.38 %, 52.11%, 84.51% in EAC induced animals treated with ZP (E), P (E) and 5-Flurouracil respectively when compared with vehicle treated cancerous animals.

Table1: Effect of different extracts of *Punica grantum* L and *Zizipus mauritiana* on mean survival time and percentage increase in life span in EAC tumor bearing mice.

n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: q< 0.01, r< 0.001, as compared with EAC control; x< 0.05, z< 0.001, as compared with 5-Fluorouracil treated group.

3.2. Effect of drugs on body weight and tumor growth response:

Figure 1 show the percentage decrease in the body weight after treatment with different extracts of *Punica grantum* L and *Ziziphus mauritiana* on day 10. The percentage decrease was found to be 41.46%, 50.47% and 64.77% in EAC induced animals treated with P (E), ZP(E) and 5-Flurouracil respectively when compared with vehicle treated cancerous animals. ZP (E) significantly ($r<0.001$) decreased the tumor volume and packed cell volume as compared to that of the EAC control group. [Figure 2,3].

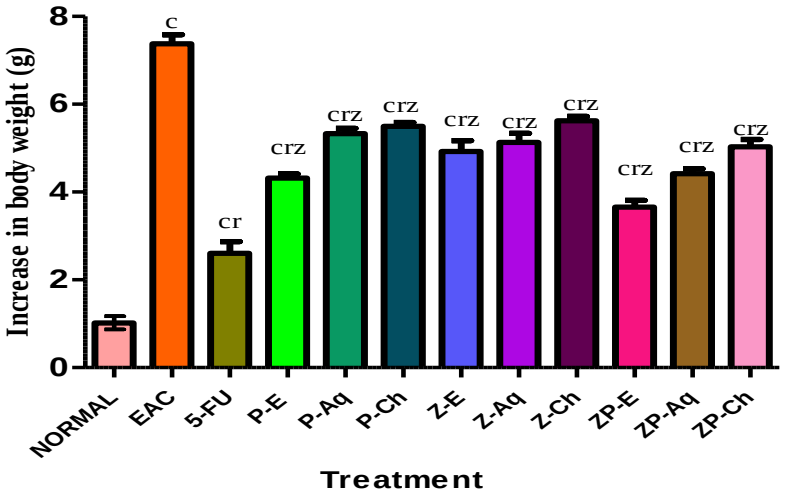

Fig 1: Effect of different extracts of *Punica grantum* L and *Zizipus mauritiana* on body weight in EAC tumor bearing mice. n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: c< 0.001, as compared with normal group; r< 0.001, as compared with EAC control; z< 0.001, as compared with 5-Fluorouracil treated group.

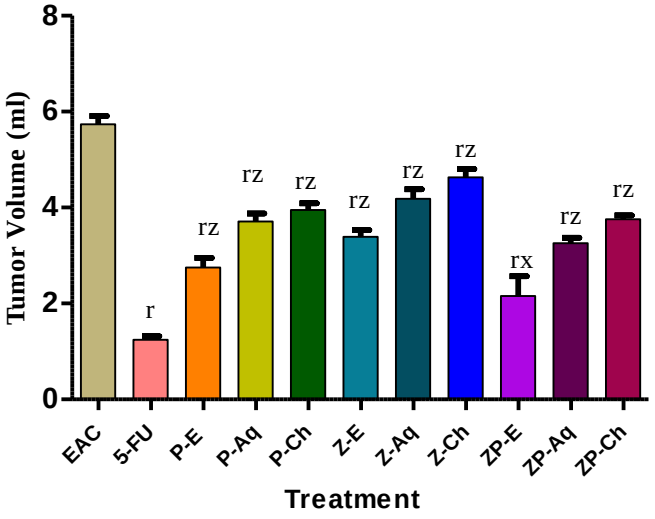

Fig 2: Effect of different extracts of Punica grantum L and Zizipus mauritiana on tumor volume in EAC tumor bearing mice. n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: r< 0.001, as compared with EAC control; z< 0.001, x< 0.05, as compared with 5-Fluorouracil treated group.

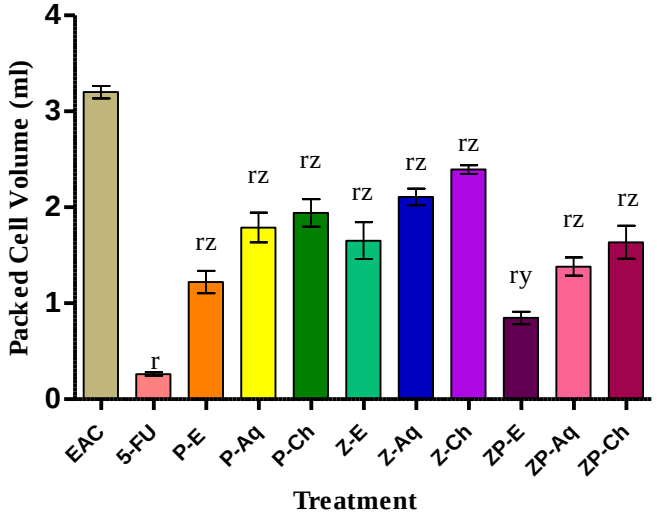

Fig 3: Effect of different extracts of Punica grantum L and Zizipus mauritiana on packed cell volume in EAC tumor bearing mice. n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: r< 0.001, as compared with EAC control; z< 0.001,y< 0.01 as compared with 5-fluorouracil treated group.

3.3. Hematological parameters:

Table 2 reveals the effect of different extracts of *Punica grantum* L and *Ziziphus mauritiana* on hematological parameters against EAC induced animals estimated on 10th day of treatment. The total WBC count found significantly increased in the EAC control group when compared with the normal group (c < 0.001). Ethanol and aqueous extracts of combination of *Ziziphus mauritiana* and *Punica grantum* L (ZP) as well as ethanol extract of *Punica grantum* L P(E), showed activity at par with 5-

Fluorouracil as standard and these differences were statistically non-significant for ethanol extract of ZP and y<0.01 for both aqueous of ZP and P(E). Ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* L (ZP) when administered to EAC bearing mice significantly decreased the WBC count as compared with EAC control. RBC count and Hb content in the EAC groups were significantly (c < 0.001) decreased as compared to normal group. Treatment with ZP(E) 200 mg/kg showed significant (r < 0.001) increase in the RBC count and Hb content when compared to the EAC control group, and restored these values towards normal [Table 2].

Table 2: Effect of different extracts of *Punica grantum* L and *Zizipus mauritiana* on hematological parameters in EAC tumor bearing mice.

TREATMENT GROUP	RBC COUNT (x 10^6/mL)	WBC COUNT (x 10^3/mL)	Hb (g%)
NORMAL	5.68 ± 0.11	6.96 ± 0.18	15.99 ± 0.39
EAC	2.17 ± 0.1 ^{c}	18.58 ± 0.55 ^{c}	7.07 ± 0.42 ^{c}
5-FU	4.65 ± 0.25 ^{r}	6.50 ± 0.34^{r}	12.29 ± 0.56 ^{br}
P-E	3.95 ± 0.34 ^{bq}	9.94 ± 0.70 ^{ary}	9.74 ± 0.76 ^{cp}
P-Aq	3.61 ± 0.32 ^{cp}	11.30 ± 0.52 ^{crz}	8.66 ± 0.57 ^{cy}
P-Ch	3.07 ± 0.33 ^{cy}	11.85 ± 0.67 ^{crz}	8.26 ± 0.43 ^{cy}
Z-E	3.29 ± 0.25 ^{cx}	10.95 ± 0.38 ^{crz}	9.42 ± 1.00 ^{cx}
Z-Aq	3.07 ± 0.30 ^{cy}	12.22 ± 0.82 ^{crz}	8.93 ± 0.30 ^{cy}
Z-Ch	2.79 ± 0.37 ^{cy}	13.58 ± 0.80 ^{crz}	8.09 ± 0.68 ^{cz}
ZP-E	4.26 ± 0.31^{ar}	8.06 ± 0.78 ^{r}	10.89± 1.00 ^{cq}
ZP-Aq	3.89 ± 0.32^{cq}	10.36 ± 0.69 ^{bry}	9.70 ± 0.81 ^{c}
ZP-Ch	3.66 ± 0.40^{cq}	11.70 ± 0.72 ^{crz}	9.27 ± 0.28 ^{cx}

n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. p values: a < 0.05, b< 0.01, c< 0.001, compared to the normal group; p<0.05, q< 0.01, r<0.001, as compared with EAC control; x< 0.05, y< 0.01, z< 0.001, as compared with 5-Fluorouracil treated group.

4. DISCUSSION:

Search for a selective and less toxic molecule for cancer treatment is an ongoing process. Plants have played an important role as a source of effective anticancer agents and about 60% of the currently available anticancer drugs are derived from plant sources (Rajandeep et al, 2011). The global trend is also towards natural bioactive substances due to their low toxicity and cost. The exploration of medicinal plants for their therapeutic efficacy still holds the hope for the treatment and prevention of cancer. The present study was designed to explore the possible *in vivo* anticancer activity of plant extracts of *Ziziphus mauritiana* and *Punica grantum* L (ZP). The ‘‘appropriate’’ transplantable mouse tumors models still have their place in the drug development programs and are used to investigate the antineoplastic effects of plant extracts. Hence in the present study the *in vivo* antitumor efficacy of plant extract were assessed in EAC cell lines in mice respectively. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma and is able to grow in almost all mice strains (Rajandeep et al, 2011). It has reported that Ehrlich ascetic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascetic fluid formation (Rajandeep et al, 2011). The ascetic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Panchal et al, 2010). In this ascites tumor model, a substantial increase in body weight of the animals was observed in EAC-bearing control

mice owing to the rapid and progressive accumulation of ascites tumor cells. The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of animal (Clarkson and Burchneal, 1965). The present study showed that ethanol extract of *Ziziphus mauritiana* and *Punica grantum* L (ZP) 200 mg/kg administered to EAC mice significantly increased the life span than that of EAC control . Furthermore the reduced volume of tumor and increased survival time of mice suggest the delaying impact of ethanol extract of ZP on cell division (Shi et al, 2008). In the present study both ethanol extract of *Ziziphus mauritiana* and *Punica grantum* L (ZP) and ethanol extract of *Punica grantum* L showed the significant decrease in body weight, tumor volume ($r< 0.001$), and packed cell volume ($r<0.001$) when compared with the EAC control group. Since the prolongation of life span is a reliable criterion for judging the anticancer efficacy of any compound (Rajandeep et al, 2011). An enhancement of life span of group treated by ethanol extract of ZP by 63.38 % more over that of EAC control was considered as effective antitumor response .The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb content, this occurs either due to iron deficiency or due to hemolytic or myelopathic conditions (Liu, 2004). Treatment with ethanol extract of *Punica grantum* L P(E) at 200 mg/kg dose significantly increased the RBC level ($q< 0.01$) and Hb content ($p< 0.05$) when compared to the EAC control. Ethanol extracts of combination of *Ziziphus mauritiana* and *Punica grantum* L (ZP) at 200 mg/kg dose showed significant effect than the ethanol extract of *Punica grantum* L P(E) for RBC level ($r< 0.001$) and Hb content ($q< 0.01$). The WBC count has been decreased significantly ($p<0.001$) when compared with the EAC bearing mice and restored more towards the normal level in the group which is treated with ethanol extract of ZP at 200 mg/kg. Ethanol extract of ZP at 200 mg/kg dose showed non-significantly decrease in WBC level when compared to 5-FU at 20 mg/kgthus, increase in life span, and decrease in body weight, tumor volume and packed cell volume and the restoration of hematological parameter as a result of treatment and in the group treated by ethanol extract of *Ziziphus mauritiana* and *Punica grantum* L (ZP) at 200mg/kg may be attributed to antioxidant as well as anticancer activity. The previous phytochemical analysis of *Punica grantum* L with *Zizipus mauritiana* has also revealed the presence of phenolic, alkaloid and flavonoids, which could also be expected to be responsible for apoptosis as well as anticancer activity (Shah and Mello, 2004).

5. CONCLUSION: Based on these data, ethanol extract of combination of *Punica grantum* L with *Zizipus mauritiana* exhibited significant anticancer activity ($r<0.001$).

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7-REFERENCES:

Abalak ME, Daniyan SY and Mann A. (2010). Evaluation of antimicrobial activities of two *Ziziphus* species on some microbial pathogens. Afr J Pharm Pharmacol. 4(4):135-39.

Abalaka ME, Mann A and Adeyemo SO. (2011). Studies on in-vitro antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L compared with Ascorbic acids. J Med Genet Genomics. 3(2):28-34.

Ahmed S, Wang N, Hafeez BB, Cheruvu VK and Haqqi TM. (2005). *Punica granatum* L Extract inhibitors IL-1 beta-induced expression of matrix metallo proteinases by inhibiting the activation of MAP kinases and NF-kappa B in human chondrocytes in vitro. J Nutr. 135:2096-2102.

Ashok KJ, Mohan GV and Rajdeo S. (2010). Folklore claims on some Medicinal Plants used Bheel Tribe of Guna District, Madhya Pradesh. Ind J Tradit Knowl. 9(1):105-07.

Aviram ML, Dernfeld M, Rosenblat N, Volkova M and Kaplan R. (2000). Pomegranate juice consumption reduce oxidative stress, atherogenic modifications of LDL and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr. 71:1062-76.
Cam M, Hisil Y and Durmaz G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food chem. 112:721-26.

Chidambara MK, Javaprakasha GK and Singh RP. (2002). Study on antioxidant activity of pomegranate (*punica granatum*) peel extract using in vivo models. J Agric Food chem. 50:4791-95.

Chopda MZ and Mahajan RT. (2009). Wound Healing Plants of Jalgaon District of Maharashtra. Ethnobotanical leaflets. 13:1-32.

Clarkson D and Burchneal JH. (1965). Preliminary screening of antineoplastic drugs. Prog Clin Cancer. 1:625-29.

Dahiru D and Obidoa O. (2009). Effect of aqueous extract of *Ziziphus mauritiana* L leaf on cholesterol and triglyceride levels in serum and liver of rats administered alcohol. Pak J Nutr. 8(12):1884-88.

Dahiru D and Obidoa O. (2007). Pretreatment of albino rats with aqueous leaf extract of Zizyphus mauritiana protects against alcohol induced liver damage. Trop J Pharm Res. 6:705–10.

Dahiru D, Sini JM and John AL. (2006). Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodents. Afr J Biotechnol. 5(10):941-45.

Dahiru D, William ET and Nadro MS. (2005). Protective effect of *Ziziphus mauritiana* leaf extract on carbon tetrachlorideinduced liver injury. Afr J Biotechnol. 4(10):1177-79.

Durairaj AK, Vaiyapuri TS, Mazumder UP and Gupta M. (2009). Antineoplastic and antioxidant activities of *Oxystelma esculentum* on swiss albino mice bearing ehrlich ascites carcinoma. Pharm Biol. 47:195–202.

Haidari M, Ali M, Casscel SW and Madjid M. (2009). Pomegranate (*punica granatum*) purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir. Phytomedicine. 16:1127-36.

Huang TH, Peng G, Kota BP, Yamahara J and Roufogalis BD. (2005). Antidiabetic action of *punica granatum* flower extract: activation of PPAR-gamma and identification of an active component. Toxicol Appl Pharmacol. 207:160-9.

Ismail T, Sestili P and Akhtar S. (2012). Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti–infective effects. J Ethnopharmacol. 143:397-405.

Jain, S.P., Singh, S.C., Sarika, S., Singh, J., Mishra, N.P. and Anil, P. (2010). Hitherto unreported Ethanomedicinal use of plants of Betul District of Madhya Pradesh. Ind J Tradit Knowl. 9(3):522-25.

Jurenka J. (2008). Therapeutic Applications of Pomegranate (*Punica grantum* L): A Review. Alternative Medicine Review. 13:128-44.

Jyotsana S, Painuli RM and Gaur RD. (2010). Plants used by Rural Communities of District of shahjahanpur, Uttar Pradesh. Ind J Tradit Knowl. 9(4):798-803.

Kalidose A and Krishnamoorty P. (2011). Antioxidant efficacy of endocarp with kernel of *Ziziphus mauritianab lam* in p-dimethylaminoazobenzene induced hepatocarcinoma in *Rattus norvigicus*. Ind J Nat Prod Resour. 2(3):307-14.

kunwar RM, Uprety Y, Burlakoti C, Chowdhary CL and Bussmann RW. (2009). Indigenous use and ethnopharmacology of medicinal plants in far-west Nepal. Ethanobotany Res Appl. 7(1547):5-28.

Liu RH. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr. 1(34):3749-85.

Loren DJ, Seeram NP, Schulman RN and Haltzman DM. (2005). Maternal dietary supplementation with pomegranate juice is neuroprotective in an animal model of neonatal hypoxic-ischemic brain injury. Paediatrics. 57:858-64.

Mahesh B and Satish S. (2008). Antimicrobial activity of some important medicinal plants against plant and human pathogen. World J Agricult SCi. 4:839-43.

Naik GH, Priyadarsini KI and Mohan H. (2006). Free radical scavenging reactions and phytochemical analysis of triphala, an ayurvedic formulation. Curr Sci. 90:8-11.

Ndhala AR, Mupure CH, Chitindingue K, Benhura MA and Muchuweti M. (2006). Antioxidant potentials and degree of polymerization of six wild fruits. Sci Res Essay. 1:87–92.

Nirmala MJ, Samundeeswari A and Sankar D. (2011). Natural plant resources in anticancer therapy. Research in plant biology. 1(3):1-14.

Panchal S, Panchal K, Vyas N, Modi K, Patel V and Bharadia P. (2010). Antiulcer activity of methonolic extract of *Ziziphus mauritiana* stem bark. Int J Pharmacog and Phytochem Res. 2(3):6-11.

Panomwan P, Kanlaya L, Samran P, Palangpon K, Apichart S and Somsak R. (2011).Antiplasmodial and Antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana.*Ind J Phytochem. 72:909-15.

Pisha E, Chai H and Lee IS. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis.Nature Med. 1(10):1046–51.

Rajandeep k, Karank K and Harpreet K. (2011). Plant as a source of anticancer agents. J Nat Prod Plant Resour. 1(1): 119-124.

Ramadoss R, Jaggi M and Siddiqui MJ. (2000). Use of betulinic acid and its derivatives for inhibiting cancer growth and a method of monitoring this. Dabur Res Found. 8:320-35.

Ryan, C.J., Smith, M.R., Bono, J.S., Molina, A., Logothetis, C.J. and Souza, P. (2013). Abiraterone in metastatic prostate cancer without previous chemotherapy.N Engl J Med. 368:138–48.

Sartor O and Pal SK (2013). Abiraterone and its place in the treatment of metastatic CRPC. Oncol. 10: 6–8.

Shah PP and Mello PM. (2004). A review of medicinal uses and pharmacological effects of *Mentha piperita*. Nat Prod Rad. 3(4):214-21.

Sharaf A and Nigm SA. (1964). The oestrogenic activity of Pomegranate seed oil. J Endocrinol Invest. 29:91-2.

Shi Y, Sahu RP and Srivastava SK. (2008). Triphala inhibits both *in vitro* and *in vivo* xenograft growth of pancreatic tumor cells by inducing apoptosis. BMC Cancer. 8:294.

Smith, D.C., Smith, M.R., Sweeney, C., Elfiky , A.A., Logothetis, C., Corn, P.G., Vogelzang, N.J., Small, E.J., Harzstark, A.L., Gordon, M.S., Vaishampayan, U.N., Haas, N.B., Spira, A.I., Lara, P.N., Srinivas, S., Sella, A., Schoffski, P., Scheffold, C., Weitzman, A.L. and Hussain, M. (2013). Cabozantinib in patients with advanced prostate cancer: Results of a phase II randomized discontinuation trial. J Clin Oncol. 31:412–19.

Vahedi F, Najafi MF and Bozari K. (2008). Evaluation of inhibitory effect and apoptosis induction of *Ziziphus jujube* on tumor cell lines, an *invitro* preliminary study. Cytotechnology. 56(2): 105-11.

Wadekar R and Patil KS. (2008). Effect of *Ziziphus mauritiana* leaf extracts on phagocytosis by human neutrophilis. J pharm Res. 1(1):78-83.

Yakes, F.M., Chen, J., Tan, J., Yamaguchi, K., Shi, Y., Yu, P., Qian, F., Chu, F., Bentzien, F., Cancilla, B., Orf, J., You, A., Laird, A.D., Engst, S., Lee, L., Lesch, J., Chou, Y.C. and Joly, A.H. (2011). Cabozantinib (xl184): a novel met and vegfr2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther. 10:2298–2308.

Yoshikawa, M., Murakami, T., Ikebata, A., Wakao, S., Murakami, N. and Matsuda, H. (1997). Bioactive saponins and glycosides X On the constituents of *Ziziphi spinosi* semen, seeds of *Zizyphus jajuba*. Mill var spinosa H4(1): Structure and Histamine release inhibitory effect of jujuboside A and C and acetyl jujuboside B. Chem Pharm Bull.45(7):1186-92.