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RESEARCH ARTICLE

Protective Effect of *Persia americana* Mill. Fruit Extract Against 9 Gy Gamma Radiation Insult In Swiss Albino Mice.

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Abstract

Introduction: Gamma rays are well known for their high penetrating capacity and deleterious side effects. In this connection we had investigated *Persia americana* (avocado) as herbal alternative.

Methodology: Adult male Swiss albino mice were treated orally for 30 days with *Persia americana* fruit extract before 9 Gray whole body gamma radiation exposure to investigate the protection potential of *Persia americana*. Animals were sacrificed 24 hours post irradiation for assessment of chromosomal aberration, hepatic abnormality, blood and liver reduced glutathione level and erythrocytes abnormalities.

Results: Pretreatment with *Persia americana* fruit extract significantly contributes to cellular protection, as fewer abnormalities were found in pretreated group. The number of chromosomal abnormalities and cellular abnormalities and hepatic abnormalities were also found significantly low in *Persia americana* fruit extract pretreated groups.

Conclusion: In conclusion, pretreatment with *Persia americana* fruit extract has been reported to compensate the adverse effect of gamma radiation incident by improving the antioxidant reservoir of body.

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Introduction:-

Gamma radiation is widely known for different uses due to its highest penetration ability (Devi and Ganasoundari, 1999). Gamma radiation induces cellular degradation due to DNA damage and disruption of cellular structures. (Devi and Rao, 2000) Radiation damage mimics the oxidative stress associated with oxygen toxicity. Gamma radiation is capable to produce immediate, as well as delayed effects on living organisms. However adverse effects of gamma rays exposure is well defined, still gamma rays are used for many purposes in society. Gamma therapy is widely used for cancer treatments where the penetration ability works as a knife to cut down tumor cells. Therefore, investigation of new molecules to compensate the adverse effects of gamma radiation is of great significance.

As gamma rays are highly penetrating to whole body of any subject, search of novel edibles to compensate the adverse effect of gamma rays exposure is very important. However many synthetic compounds have been investigated within last decade to compensate radiation induced cellular damage, but often they are associated with many known side effects. Hence it is very considerably beneficial to investigate novel, natural and herbal products against adverse effect of gamma radiation. (Elzebrook and Wind, 2008).

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Persea americana Mill. generally known as Avocado or butter pear is a member of lauraceae family. The fruits have fatty, strongly flavored, smooth, almost creamy texture. They are high in monounsaturated fat. Avocado as a cardio-supportive, chemo-protective and hypoglycemic agent is well documented. It contains significant amounts of monounsaturated fat, protein, 9 vitamins, and 7 minerals, 16 antioxidants, 15 cholesterol lowering agents, 12 anti-carcinogenic substances, 8 cardioprotective chemicals, 7 anti-inflammatory compounds, 6 anti-atherosclerotic constituents, 6 anti-tumor elements, Alanine, alpha and beta-carotene, vitamin C and E, beta-sitosterol, and pensenone A & B are among avocado's most potent. This rich nutrient status makes this fruit a strong candidate for investigation against many clinical conditions (Kim,2000;Wang et al,2015;Drecher,2012;Yasir and Kharya,2010).

Material and Methods:-

Experimental Design:-

Hydro-ethanolic extract of whole fruit pulp was prepared by Simple cold extraction method. 2 % gum acacia is used as a suitable vehicle for delivery of dried extract. Toxicological studies have been performed for the above fruit extract 500 gm/kg body weight of *Persia americana* extract (PAE) were selected against 9 Gy radiation exposure. Two combinations were prepared with combination of irradiation and doses. After thirty days of PAE oral gavages (twice a day), animals were irradiated and autopsied 24 hours post irradiation.

Un-anaesthetized animal were whole-body irradiated by Cobalt teletherapy unit (Co-60 as radiation source) at the Radiotherapy Department of Jawaharlal Nehru Cancer hospital & Research Centre Bhopal (M.P.) India. Each mice was placed in a close fitting Perspex box (3x3x11) and exposed to single time whole body exposure to 9 Gy radiation for 9 min (dose rate = 1 Gy /min) with a surface distance of 150 cm. Non-irradiated (control) and Irradiated (radiation only) only mice were treated by suitable vehicle with definite volume.

Adult Male Swiss albino mice (*Mus musculus*) 4-6 weeks old, Weighing 26±8 g from inbred colony maintained on the standard mice feed (procured from Hindustan Lever Ltd., India) and water ad libitum. Five animals of each group were housed in Polypropylene cage containing paddy husk (procured locally) as bedding throughout the experiment. The mice were maintained under controlled conditions of temperature and light (12 h: light; 12 h: dark). Animal care and handling were performed according to guidelines issued by the World Health Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). Institutional animal Ethical Committee has approved the present study.

Experimental groups:-

All animals were divided into eight groups. Each group contains five mice. Different experimental groups and their treatments are described above.

Group 1- Control: Normal Control.

Group 2-PAE Only: Mice treated with 500 gm/kg body weight drug only.

Group 3- Radiation Only: Mice treated with 9 Gy radiations only.

Group 4: Radiation +PAE: Mice pretreated with 500 gm/kg body weight drug along with 9 Gy radiations

Estimation of the concentration of reduced glutathione in the liver and blood of mice:-

Reduced glutathione (GSH) content of blood was measured by spectrophotometer using Ellmans reagent. Fresh blood sample were collected by orbital puncture just before GSH estimation. Absorbance was recorded at 412 nm using UV 1601 by Shimadzu spectrophotometer (Beulter *et al*, 1963). Levels of hepatic glutathione were measured by the method described by (Moron *et al*, 1979).

Preparation of hepatocytes slide:-

After dissection liver was perfused with normal saline properly to get a good cell suspension. Centrifuge it for 30 min. at 1400 rpm. Pellet was treated with freshly prepared carnoy's fixative. Above step of treatment with carnoy's fixative was repeated twice to get a debris free pellet. Cytological smear slides were prepared by air-drop method and stained slides with giemsa stain.

Histopathology of Liver

Liver was removed and fixed in formalin buffer solution. Sequential steps of dehydration, cleaning, impregnation, embedding, staining and mounting were performed sequentially before histological observations. Hematoxyline & Eosin staining was performed to visualize gross anatomical abnormalities (Muller and Jacks, 1975).

Blood Smear Preparation:-

Blood films were made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. After fixation, the slide is stained with Field's stain to distinguish the cells from each. Blood smears were examined microscopically (Nayak, 2008).

Chromosomal Aberration Assay:-

The animals were injected with colchicine to arrest the cells in metaphase and sacrificed them by cervical dislocation. The femur marrow was excised and aspirated by flushing with normal saline and processed for chromosome analysis. Metaphase plates were prepared by the air-drying method. Slides were stained with giemsa (Sigma) and aberrations were monitored under a light microscope (Devi and Prasanna, 1990).

Statistical Analysis:-

The Student's test was used for statistical comparison between the groups and significance level (P Value) was determined for reduced blood glutathione, reduced liver glutathione, hepatocytes smear and chromosomal aberration. Mean \pm SEM values have been estimated using Apple mac book excel software. 50 metaphases and 1000 Hepatocyte cells were analyzed in each group. Liver histopathology was analyzed to observe the changes in tissue arrangement after different treatments.

Results:-

Animals subjected to 9 Gy radiations were exhibited signs and symptoms of radiation sickness. Food and water consumption were reduced. However, no mortality was evident in any of the group. No specific toxic effects in the terms of sickness were observed in animals treated with PAE alone. There is no significant change reported in urination and defecation pattern. However, variation in body weight was observed in respect of dose and treatment. Radiation treated mice have shown severe abnormalities while pretreated groups have shown significantly lower adverse side effects. (P Value 0.05) After radiation exposure blood smear revealed many kind of abnormal blood cells among normal R.B.Cs distributions. Change in RBCs shape was very prominent. Presence of poikilocytes, elyptocytes, burr cells were also observed frequently. However, pretreated groups have comparative reduction in abnormalities. (Figure-1).

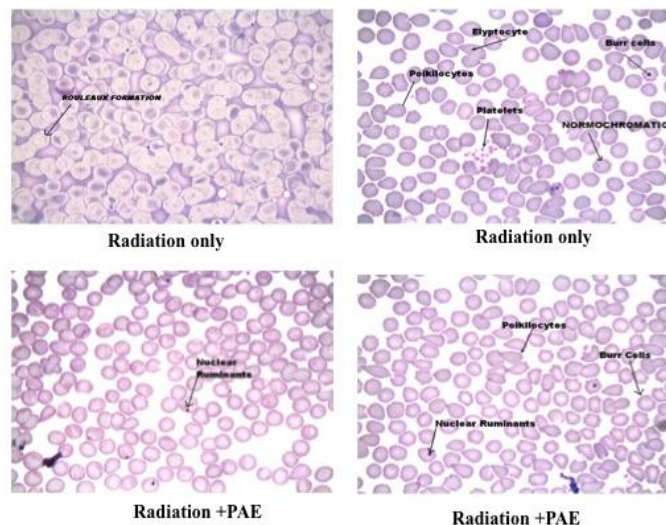


Figure -1 Effect of PAE pre-treatment on red blood corpuscles

Hepatic smear revealed, presence of normochromatic, bi-nucleated hepatocytes among subject control group and PAE only treated group. Bi-nucleated cells are more common than mono nucleated hepatocytes. (Figure-2,9).

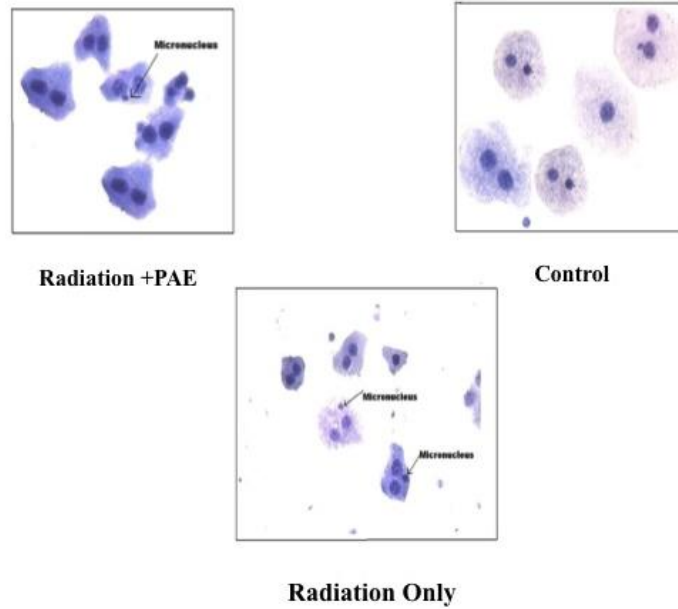


Figure-2 Comparative analysis of hepatocytes smear among PAE in different groups

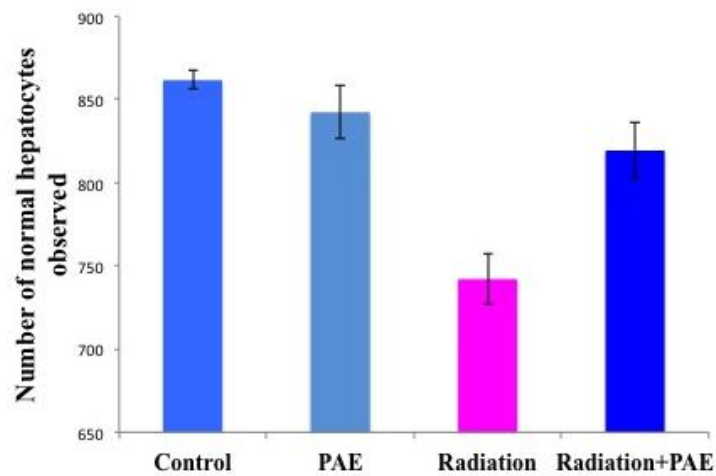


Figure-9 Comparison of normal hepatocytes reported in different groups (P value 0.05)

9Gy Radiation induced severe damage in liver tissue was clearly visible in histological observation in animals of radiation only treated group. Disruption in normochromatic tissue architecture was observed in radiation alone groups. Transverse Sections (T.S.) of liver of animals treated with PAE only revealed normal distribution of hepatic tissues. Hepatic tissue architecture is similar as vehicle only treated control group. (Figure-3).

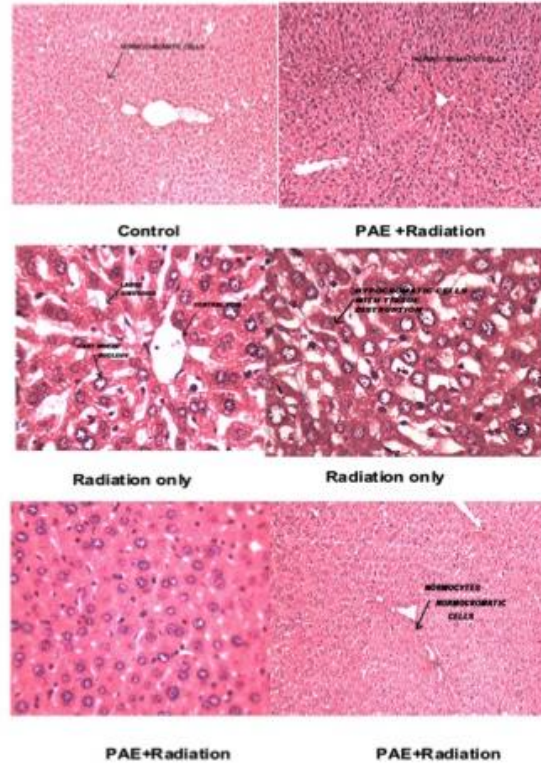


Figure-3 Transverse section of liver histo-architecture of different treatment groups

The number of chromosomal abnormalities and cellular abnormalities were noticeably less among the animals of PAE pretreated group in compare to radiation only treated animals. Radiation only treated mice have shown severe abnormalities while pretreated groups have shown significantly lower adverse effects. (Figure-4,5,8).

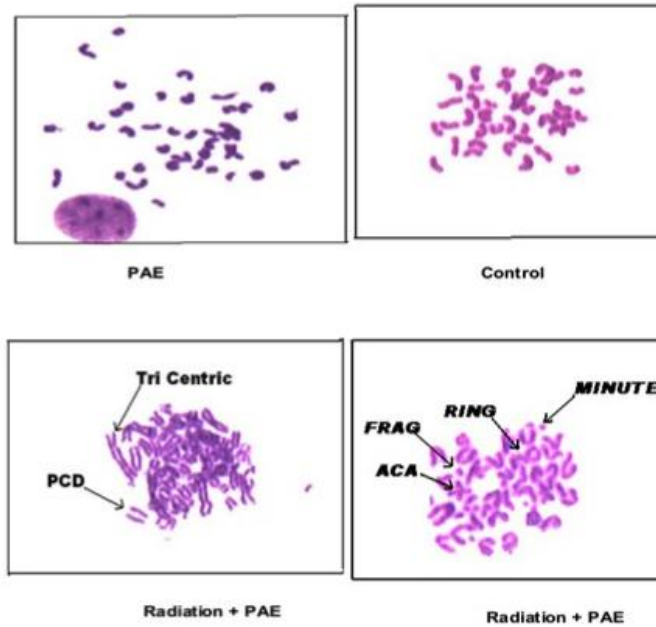


Figure-4 Metaphases of different groups

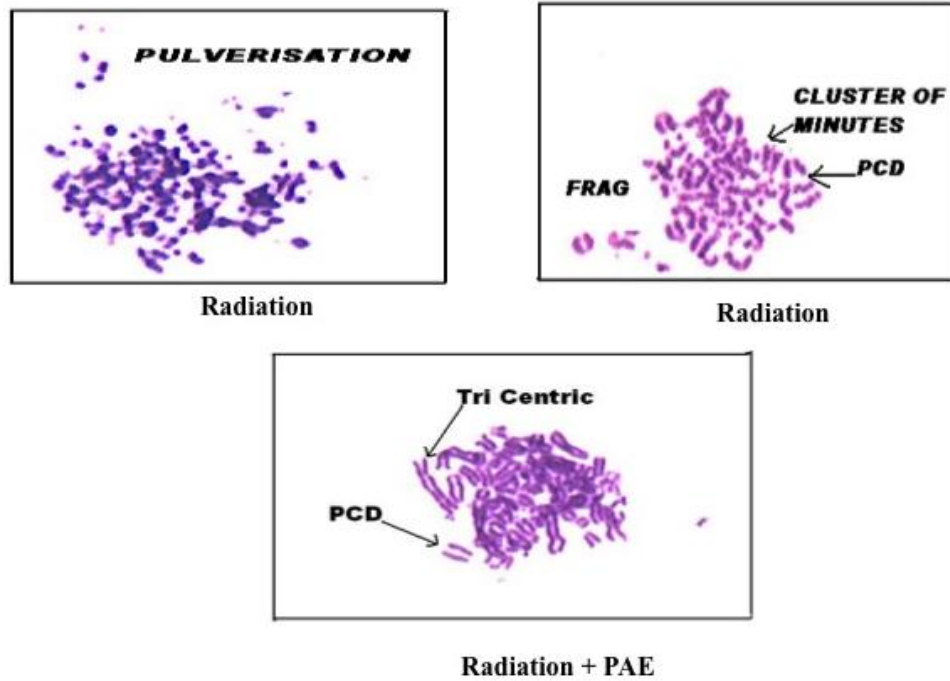


Figure-5 Metaphases of different groups

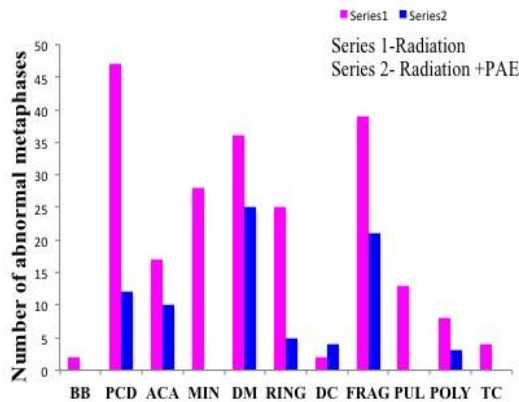


Figure-8 Comparison of different kind of chromosomal abnormalities reported in metaphases of Radiation alone and PAE pretreated group

BB-Bubbling,PCD-precentromeric deletion,ACA-acrocentric association,MIN-minutes ,DM-double minutes,RING-ring,DC-dicentric chromosomes ,FRAG-fragmentation,Pul-Pulverisation,POLY-polyploidy,TC-tricentric chromosomes

A significant elevation was reported in blood and liver GSH in *Persia americana* extract pretreated groups in compare to normal controls. 9 Gy gamma irradiations caused significant reduction in GSH level of blood and liver in compare to normal control group and PAE pretreated groups. Significant difference (P value 0.049) in hepatic GSH level was reported, along with a significant difference in blood GSH level. (P value 0.043) (Figure-6,7)

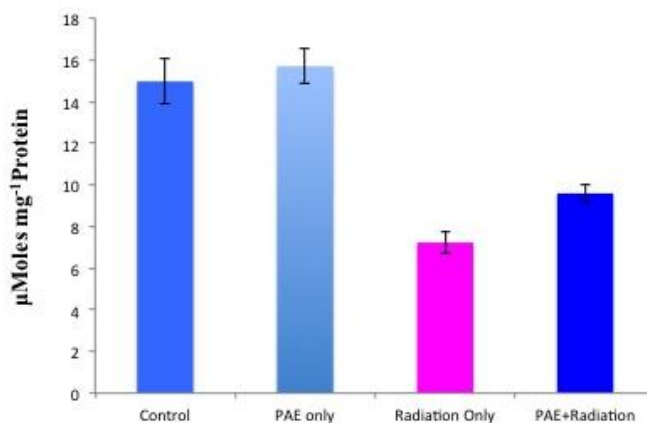


Figure-6 Effect *Persia americana* extract (PAE) pretreatment on of hepatic GSH level of different groups (P value 0.049)

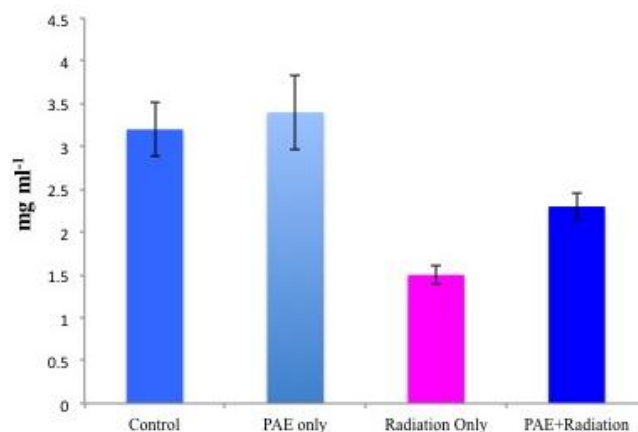


Figure-7 Effect *Persia americana* extract (PAE) pretreatment on blood GSH level of different groups (P Value 0.043)

Discussion:-

In current experiment blood and hepatic GSH both were found to be involved in radioprotection mechanism.(Figure-6,7) Hepatic and blood GSH level were reduced by gamma radiation exposure, because GSH was being oxidized by donating its proton to versatile protectors and execute its radio protective function through free radical scavenging restoration of the damaged molecule by hydrogen donation reduction of peroxides and maintenance of protein thiols in the reduced state. (Biaglow,1987).

The exact mechanism of action of chromosome protection by avocado is still not well defined. However, scavenging of radiation-induced free radicals may be one of the important mechanisms of radioprotection by *Persia americana*. Therefore, the reduction in chromosomal damage observed in the present study may be due to free radical scavenging activity of *Persia americana* fruit extract as well as protection against gamma radiation induced DNA damage. Chromosomal aberrations are highly quantifiable manifestations of radiation-induced damage to DNA that may be observed in the first post-irradiation mitosis, and studies conducted in plants employed scoring of chromosome aberration as a method to quantify levels of radioprotection by various sulfhydryl compounds (SH) (Lomaestro and Malone, 1995). Further investigations are advisable to explore the exact mechanism behind the phenomena at molecular level.

Irradiation generates free radicals, which generates oxidative stress within the cell. GSH contributes to maintain cell's inner environment reduced by donating proton. GSH oxidized itself and convert its structure as GSSG (oxidized glutathione). Higher oxidized glutathione level interprets that cell has coped up with an oxidative stress. In present study it was observed that any one of them either blood glutathione and liver glutathione involves in cellular protection mechanism at a time (Hamilton and Batist,2004;Plaza *et al* ,2009).

Gamma radiation exposure is well known to generate cellular damage by many mechanisms including chromosomal aberration, DNA damage, and disruption of cellular structures by reactive oxygen species (ROS) formation (Hallenback, 1994). Gamma radiation is capable of inducing genomic instability in mammalian cells and instability is thought to be the driving force responsible for induction of radiation sickness. Genomic instability is characterized by a large collection of diverse endpoints that include large-scale chromosomal rearrangements and aberrations. The capacity of radiation to induce genomic instability depends to a large extent on radiation quality or linear energy transfer (LET) and dose (Smith *et al*, 2003).

After radiation exposure, formation of reactive oxygen species within body is an important cause for cellular damage. Antioxidants like glutathione plays an important role in removal of oxidative stress generated as the result of radiation insult. *Persia americana* fruit is very rich in nutrients, vitamins, amino acids and antioxidants which possibly protects against radiation. (Ganasaundari et al, 1997).

Persia americana (Avocado) extract is capable to increase GSH levels when radiation encounters. Once the cells have an antioxidant pool, it tries to compensate the adverse changes induced after ionizing radiation exposure. If the tissue system already in lack of antioxidants and other compensating factors within body the possibility of damage increases (Kim, 2000). Avocado is a natural source of many active components with already known function. Avocados contain β -Sitosterol and Persenone A, which are known to prevent prostatic carcinoma and breast cancer. Avocado also contains few antioxidants like Tartaric acid and Cryptoxanthin, (xanthophyll). Cryptoxanthin is a potential chemo preventive agent. It prevents DNA damage and contributes in DNA repair stimulation (Lu *et al*, 2005). In addition avocados are a rich source of bioactive phytochemicals such as vitamin E, some carotenoids, vitamin C, phenols, and sterols (mainly β -sitosterol). These bioactive carotenoids are likely to be absorbed into the bloodstream, where in combination with other diet-derived phytochemicals they may contribute to the significant cellular protection (Fulgioniet *al* ,2001;Ding *et al*,2007).

Avocados also have the phytochemical glutathione, more than the other common fruits, even three times more than oranges, which have the second highest level. As an antioxidant, glutathione helps neutralize free radicals that can cause cell damage in the body leading to cancer and heart disease. Glutathione Prevents Cancer via Numerous effects (Unluet *al*,2005). Adding avocado fruit in diet significantly enhances carotenoid absorption from salad and salsa, which is attributed primarily to the lipids present in avocado.

Radiation induced oxidative stress was removed by the master cellular antioxidant glutathione. GSH offer protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation (Mikaelsen,1952). Glutathione also helps in other biochemical functions such as energy utilization, immune system activity, detoxification and disease prevention.

Conclusions:-

In summary, present study concludes that *Persia americana* fruit extract is compensating the adverse effect of gamma radiation incident against 9 Gy gamma radiation by contributing in maintenance of cellular homeostasis. Intake of *Persia americana* fruit is advisable to patients of radiotherapy and those who want to boost immunity against gamma radiation exposure. We are proposing that *Persia americana* fruit is a candidate for adjuvant therapy to alleviate the side effects of gamma radiation to normal cells of cancer patients.

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