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ADHATODA VASICA: EFFECT OF ADMINISTERING ETHANOLIC EXTRACT OF *ADHATODA VASICA* ON BLOOD COUNT AND SOD ENZYME ACTIVITY AGAINST EXPOSURE TO γ – RADIATIONS

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ABSTRACT: In the present studies, antibacterial studies and medicinal effects of the ethanolic extract of *Adhatoda vasica* on the physiological variations including blood count variations w.r.t. RBC, WBC, Hb and enzyme SOD activity of the subjects under study, Swiss Albino mice, on exposure to gamma radiations, have been evaluated. Phyto-chemical analysis of *A. vasica* showed the presence of alkaloids, saponins, tannins and reducing sugars. It was observed that RBC, WBC and Hb count of the control group mice increased with the administration of extract where as these parameters decrease on exposure to gamma radiations. However, in case of group of mice administered with the dose of the extract and exposed to gamma radiations, the decrease is much less as that observed in the mice exposed to only radiations. In case of enzymatic SOD activity, it was observed that the animals with extract dose showed lower value of SOD specific activity while the animals with extract dose and γ radiations also showed lower SOD specific activity as compared to the animals exposed to gamma radiations without the extract dose. These results substantiate the medicinal effects on the blood count and enzymatic activity in the subject animals. Antibacterial studies showed positive results against *B. Subtillis, and Shigella*.

INTRODUCTION: These days due to total dependence on allopathic medicinal system, our immune system has become so adaptive that allopathic medicines have no more positive effects on the body; rather, various side effects on the body are emerging day by day. On the other hand, herbal products are safer than synthetic drugs. Therefore, in the present scenario, the general opinion about use of medicines is tilting towards herbal medicines, which is indicated in the general rise in the trade of herbal drugs.

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Leaves and bark of *Taxus baccata* are the prime source of Taxol [1], an anti-cancer drug. Ethanolic extract of *T. baccata* has shown activity against gram negative bacteria and fungi [2]. *Baccopa monnieri* is well known for its sedative tranquilizing, broncho-dilatory, anti-cancer and many more activities [3]. Outer bark and stem of *Cedrus doedra* are astringent and possess carminorative and anti-spasmodic biochemical actions.

It is reported to possess digestive functions, remove toxins from bowel, alleviate coughing and cure skin disorders [4]. *Aloe* products have long been used in health foods and for medicinal and cosmetic purposes. The gel derived from *Aloe* possesses therapeutic properties such as anti-histamine laxatives [5] and also helps in peptic ulcers relief [6]. Ethanolic extract of Ipomoea pes-tigridis, well known for treatment of headaches, swelling, snake bites, has been tested against A. mice and it was found that the extract has significant analgesic activity [7].

Adhatoda vasica is a well-known plant drug in Ayurvedic and Unani medicine, well documented for therapeutic potentials and is described as anti-bleeding and anti-asthmatic. It has been used for the treatment of various diseases and disorders particularly for the respiratory tract ailments. It is used as an expectorant, bronchodilator and to liquify sputum [8]. Most frequently mentioned medicinal uses are the treatment of respiratory diseases and disorders including cough, bronchitis and phthisis. Leaves and roots are those parts of plants that are most frequently used in the form of decoction or powder.

Pharmacologically, the most studied chemical in *A.vasica* is a bitter quinazoline alkaloid, vasicine, which is present in leaves, roots and flowers. Vasicine shows bronchodilatory activity both in vitro and in vivo studies. It is found to have uterotonic activity in different animals. It was shown that the effect was influenced by degree of priming of uterus by esterogens [9]. Ethanolic extract from leaves showed hypoglycemic as well as radio modulatory influences when checked on rats [10]. An essential oil from leaves showed smooth muscle relaxant activity in isolated guinea pig chain [11].

Ionizing radiations (external or internal) produce a wide variety of biological effects. The early effects of radiations are anorexia, nausea, diarrhea, hypertension, tachycardia, leucopenia, cataract, sterility, shortening of life span, neoplasms developmental anomalies and mutations. Extracts of different plants have been shown to prevent the adverse effects of these radiations. Kumar *et al* [12] reported that extract of *Podophyllum hexandrum*, a drug known for allergy and inflammation and inhibiting apoptosis, provides about 80% radioprotection against gamma radiations in mice thereby reducing the mortality rate. Troxerutin, a class of medications known as flavonoids, is used in the treatment of varicose veins and other disorders which cause blood to pool. The use of Troxerutin as a radioprotective agent has been made by Ping *et al* [13]. They observed that the drug increased the 30-day survival rates of irradiated mice dramatically thereby indicating that troxerutin is an effective radioprotective agent.

Slow release of penicillamine and potassium iodide (KI), selected as radioprotectors for both chronic radiation exposure and acute radiation exposure, from biodegradable polymer poly (Lactide-co-Glycolide) (PLGA) used as the carrier, was carried out to study the role of PLGA-Nanoparticles formulation in improving the radioprotective activity of the drug in gamma-irradiated mice [14].

High efficiency protection exhibited by Rosemary extract against morbidity and mortality in mice exposed to gamma radiations was studied employing chemiluminescence method by Maris *et al* [15].

Because of their highly proliferative nature, hemopoietic organs and blood cells have been considered very important parameters for radiation damage. Since the experiments of Heinke, who studied that damage to hemopoietic tissue after whole body irradiation, the hemopoietic tissue has received special attention in radiations studies.[16] Changes in blood cells count are still considered most important biological evidences for excessive acute exposure to both internal and external radiations. This is understandable because of high sensitivity of blood and blood forming tissue to ionizing radiation.

It has been seen that after whole body irradiation, whether it is carried out with single or repeated dose, manifestations to the blood forming tissue may appear in peripheral blood even in the absence of histopathological changes in blood forming or lymphatic tissue [17]. Rugh and Pardo [18] found a mild depression in red blood cell count at four to eight weeks post irradiation and complete recovery by twelve weeks, in young mice after a whole body exposure to 0.03kGy. The average hemoglobin percent appeared to be unaffected by 0.03 kGy in young mature mice.

In view of the medicinal uses of *Adhatoa vasica* and adverse effects of gamma radiations, in the present manuscript, we present a detailed study on the medicinal effects of the extract of *Adhatoa vasica* on RBC, WBC, Hb and enzyme SOD activity of the subjects, Swiss Albino mice, exposed to gamma radiations. Antibacterial activities of hexane, methanol and ethyl acetate extracts and the alkaloid, vasicine, extracted from the extract, have also been evaluated.

EXPERIMENTAL:

Materials and Method: *Adhatoda vasica* plant, collected from the Herbal Garden, Joginder Nagar, Mandi, Himachal Pradesh, was identified by Prof. M. K. Seth, Department of Bio-Sciences, H.P. University, Shimla. The leaves from plant were washed thoroughly with water and dried under shade for one month. Dried leaves were ground to a coarse green powder (1.650 Kg).

Extraction: Dried leaves powder was extracted with 80% ethanolic solution (1L) at room temperature for 24 h. The ethanolic extract was percolated and the powder was fed with fresh 80% ethanolic solution and was left for 24h at room temperature. The ethanolic extract was again percolated and the process was repeated five times. The combined ethanolic extract was concentrated under reduced pressure and a gummy residue was obtained. The gummy extract was acidified with 2% aqueous organic acid (acetic acid) and continuously stirred for 24h at room temperature. The acidic solution obtained was fractionated with $CHCl_3$ (50mL × 4). Aqueous acidic layer thus obtained after extraction with chloroform was basified with ammonia (pH=9). The basic layer was again extracted with $CHCl_3$ and the organic layer obtained was concentrated under reduced pressure to give amorphous residue. The amorphous residue was tested for components on TLC and was found to contain number of components.

Column chromatography: Different components were separated by column chromatography. The column was packed with Silica gel by wet method using ethyl acetate/hexane as solvent. The amorphous residue obtained above was added at the top of the column. The column was run by gradient elution method using mixture of solvents with varying polarity (Hexane: EtOAc) in different proportions starting with 90:10 v/v and continued till 45:55 v/v mixture. The different elutes were tested on TLC which indicated the presence of number of constituents.

Phytochemical Analysis: As the amorphous residue obtained was found to show the presence of many components when run on the TLC, it was considered to carry out the phyto-chemical investigations to determine the chemical nature of the constituents such as alkaloids, terpenoids, saponins, reducing sugars, tannins, carbonyls, flavonoids, phlobatanins and steroids by simple chemical reactions reported in literature [19].

1. **Test for Alkaloids:** Plant extract (0.2 g) was warmed with 2% sulfuric acid (3mL) for 2 min and was filtered in a test tube. Few drops of Dragon dwarf's reagent were added. The presence of orange red precipitates indicates the presence of the alkaloids.

2. **Test for Terpenoids:** To 0.5g of the extract, 2mL of chloroform was added. To it concentrated sulfuric acid was added carefully along the walls of test tube to form a layer. The presence of reddish brown colored interface shows the positive results for the presence of terpenoids in the extract.

3. **Test for reducing Sugars:** In a test tube, 2mL of the crude plant extract and 5mL of distilled water was added, shaken well and filtered. The filtrate was boiled with 3-4 drops of Fehling solutions, A and B for 2 min. Formation of orange red precipitates indicates the presence of reducing sugars.

4. **Test for Saponins:** To about 0.2g of the plant extract in a test tube, 5mL of distilled water was added, shaken well and filtered. The filtrate was boiled. Occurrence of frothing indicates the presence of saponins.

5. **Test for Tannins:** Small quantity of the plant extract was added to water (3mL) in a test tube and was placed on a boiling water bath for 5 min. The mixture was filtered and ferric chloride was added to the filtrate. Appearance of dark green solution indicates the presence of tannins.

6. **Test for Carbonyls:** To 2mL of plant extract in a test tube, few drops of 2, 4-dinitrophenylhydrazine solution was added and shaken immediately to observe the presence of yellow colored precipitates for the presence of an aldehyde.

7. **Test for Flavonoids:** 0.2 g of the plant extract was weighed in a test tube and was dissolved in a cold dilute solution of NaOH. To it was added dilute hydrogen chloride. Appearance of the yellow colored solution that turns colorless indicates the presence of flavonoids.

8. **Test for Phlobatanins:** 0.5g of the plant extract was dissolved in distilled water in a test tube and filtered. The filtrate was boiled with 2% hydrogen chloride solution and observed for red precipitates that show the presence of phlobatanins.

9. **Test for Steroids:** To 0.5g of ethanolic extract of the plant extract 2mL of acetic anhydride and 2mLof sulfuric acid was added to observe the color change from violet to blue green indicating the presence of steroids.

Extraction of Vasicine: Direct isolation of major alkaloid, vasicine, present in the leaves of *Adhatoda vasica,* was carried out by the method reported in literature [20].

Radiomodulatory studies: In order to study the therapeutic effect of the extract of *A. vasica,* the physiological variations including blood count variations w.r.t. RBC, WBC and Hb and enzyme SOD were under taken.

Experimental Subjects: Physiological variations were evaluated on Swiss Albino mice, 6-8 weeks old with 20-24g body weight, procured from Central Drug Research Institution, Kasauli, H.P., India. The animals were maintained under controlled conditions of room temperature and light in an animal house and were regularly provided with food and water.

The mice were divided into four groups viz. a control group, a group of mice administered with extract dose, a group exposed to gamma radiations and the group fed with extract dose and also exposed to gamma radiations.

***Adhatoda vasica* leaf extract:** Washed and shade dried leaves were crushed to a coarse powder (200g). To it was added 80% ethanol (200 mL) and kept at room temperature for 24h. The ethanol was percolated and the residue was treated with fresh ethanol. Process was repeated three times. The extract was dried and stored under 4˚C and used as drug as and when required.

Irradiation: Cobalt-60, source of gamma radiations, housed in Gamma chamber 900, supplied by Bhaba Atomic Research Center, Trombay, Mumbai, India, was used for irradiation. Animals were restrained in well ventilated boxes and exposed to whole body radiations for a total dose of 4Gy and 6 Gy.

Animal Ethics clearance: All experimental procedures were conducted after the approval of Institutional Ethics Animal Committee (IAEC/ BIO/12-2009) Himachal Pradesh University, Shimla, Himachal Pradesh.

Experimental Setup: Radiomodulatory studies were based on the examination of variations in the Swiss Albino Mice. Initially total 32 mice were taken and divided into four groups.

- **Group 1** was control group consisting of 8 animals which were supplied neither with drug nor with radiations.
- **Group 2** consisting of 8 animals was given extract dose every day for 10 days.
- **Group 3** consisting of 8 animals that were not administered with the extract drug dose, but exposed to gamma radiations only on the 10^{th} day.
- **Group 4** consisting of 8 animals was administered extract drug dose for 10 days and was exposed to the radiations the next day i.e. the 11^{th} day.

Extract Dose: The amount of the extract dose given to group 3 and group 4 was 600 mg/Kg of body weight. Group 2 and 4 were exposed to a radiation dose of 6 Gy.

Blood Count studies: Blood samples from the heart of sacrificed mice were collected after puncturing the heart in separate vials. Heparin/EDTA was added as an anticoagulant to prevent clotting of blood.

Peripheral count (hemoglobin, red blood cells, white blood cells) were measured. Following parameters of the blood samples were investigated.

Total red blood cells count: Hayem's solution, used for red blood cells count, was prepared as under: sodium chloride (1 g), sodium sulphate (5 g) mercuric chloride (0.5 g) and distilled water (200 ml).

Blood was drawn into diluting pipette (carrying graduated marks 101 on stem above bulb) by gentle solution. Any blood sticking outside the pipette was wiped off with cotton and tip was dipped in diluting fluid and the bulb of pipette was filled by gentle suction up to 101 mark on stem above bulb.

Tip of the pipette was closed with finger and the pipette was rotated gently on its horizontal axis to ensure uniform mixing. Counts were taken on haemocytometer (Neubar's chamber). RBC was expressed as No/mm^3.

Total White blood cells count: For white blood cells count, Toison's reagent was prepared as follows: sodium chloride (100 mg), sodium sulphate (800mg), glycerol (3ml), methyl violet (pinch) and distilled water (18 ml).

Blood was drawn into pipette with graduation mark 11 on the stem above the bulb up to 0.5 mark and the procedure followed was same as that of the RBC counting. Counts were measured on Neubar's haemocytometers. WBC counts were expressed as No/mm^3.

Hemoglobin Estimating: Hemoglobin value was estimated on Sahli's haemoglobiometer. Blood was drawn into Haemometers pipette up to graduation mark (20 µl) and was transferred to the haemometer tube containing N/10 HCl up to 1 mark. The blood was stirred with speculates glass rod provided. Distilled water was added drop by drop with continuous stirring so that color of diluted blood matches with the color of reference comparometer tubes fixed each side.

Acid blood solution was allowed to stand for 3 min and reading was noted. Hemoglobin content was noted and expressed as g/100 µl.

Enzymatic studies:

Superoxide Dismutase (SOD): Superoxide dismutase activity was determined by the method by Misr and Fridovich [21]. This method involves essentially as auto-oxidation of Epinephrine as per following equation:-

$$\text{Epinephrine} \xrightarrow{\text{SOD}} \text{Adrenochrome (Pink colored complex)}$$

Known amount of tissue was homogenized in 0.15M tris buffer pH=7.4. Tissue homogenate was centrifuged at 9168 g for 15 min and supernatant was collected for SOD activity assay. The assay mixture (1 ml) contained 185 µl CBC (carbonate bicarbonate buffer pH=10.3), 800 µl of distilled water and 10 µl of supernatant. Reaction was started by addition of 5 µl of 0.3M epinephrine stock solution. The blank solution contained 195 µl of CBC, 800 µl of distilled water and 5 µl of epinephrine. Change in absorbance of reduced epinephrine per minute was immediately measured at 490nm.

Percentage autooxidation=

$$\frac{\text{Change in Absorbance per minute of Blank}}{\text{Change in Absorbance per minute of Sample}} \times 100$$

Inhibition of auto-oxidation is given by 100-A where A is the percent auto-oxidation

One unit of SOD activity= 50% inhibition of auto-oxidation of epinephrine

SOD specific activity was finally calculated in units/ mg/protein/min (U/mg/protein/min.)

Antibacterial Study: Antibacterial activity of extract of the plant material collected during soxhlet extraction with solvents of different polarity i.e. hexane, methanol, ethylacetate and vasicine directly from the leaves, was tested against both gram positive *(Staphylococcus aureus, Bascillus subtilus)* and gram negative bacteria *(Pseudomona aeruginosa, Escherichia coli, Schigella).*

Phytochemical Analysis: Ethanolic extract of the plant was tested for identification of different classes of phytochemical constituents present in the

leaves. Colored reactions were carried out as reported by Venkatesan and coworkers [19] during phyto-chemical analysis of *Aegle marmolis.* The results are presented in the **Table 1**. It is observed from the Table that the extract gave positive test for alkaloids, saponins, flavonoids, tannins and sugars. Terpenoids, steroids, carbonyl and phlobatanins were found to be absent in the extract.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF *A. VASICA*

Constituents	Ethanolic Extract
Alkaloids	+
Terpenoids	-
Reducing Sugars	+
Saponins	+
Tanins	+
Carbonyls	-
Flavonoids	+
Phlobatanins	-
Steroids	-

Radiomodulatory studies (Blood Count parameters):

Effect of γ-rays on physiological behavior: Exposure to γ radiation has been found to have adverse effect on the physiological behavior of animals. Numerous studies have been undertaken to see the effect of radiation on blood count and SOD activity. To study the medicinal effect of extract on the blood count and SOD activity of the mice after irradiation, mice were divided into different groups.

Group I consisted of control, another group of 8 mice were given whole body irradiation (6 Gy), another group was given the extract 0.6g/kg of body weight of mice and the fourth group was given the extract dose and also irradiated with 6Gy radiation dose. The extract dose was administered for 10 days and on the next day mice were irradiated with 6Gy total dose.

After 2h of the radiation, total 8 mice were sacrificed (2 from control, 2 from control and radiation, 2 from extract and 2 from extract and radiation). All the mice were tested for blood count variations; with respect to the RBC, WBC, Hb and SOD activity.

Remaining animals were sacrificed after two days of irradiation and blood count studies were carried out. It was found that mice that were given only radiation and no extract dose did not survive while the mice with extract dose survived after irradiation and were tested for the blood count variations. The results are presented in **Table 2**.

TABLE 2: BLOOD COUNT FUNCTIONS, RBC, WBC AND HB OF CONTROL, IRRADIATED, WITH EXTRACT AND WITH EXTRACT & IRRADIATED SUBJECTS AFTER 2 AND 48H IRRADIATION

Subject	Blood Count functions of mice after					
	RBC × 10^6 mm^3		WBC × 10^3 mm^3		Hb × gm 10^{-2} ml^{-1}	
	2h	48h	2h	48h	2h	48h
Control	2.16 ± 0.037	1.97 ± 0.085	26.08 ± 24.98	20.37 ± 2.24	10.10 ± 0.10	9.28 ± 0.1
Irradiated	1.39 ± 0.023	N. S.	24.98±1.30	N. S.	9.90 ± 0.10	N. S.
With Extract	3.15 ± 0.040	2.42 ± 0.039	28.30 ± 1.20	30.22 ± 2.36	11.60 ± 0.20	11.50 ± 0.2
With Extract & Irradiated	2.05 ± 0.031	1.97 ± 0.042	24.70 ± 1.97	28.52 ± 3.03	11.20 ± 0.05	11.20 ± 0.6

N.S. = not survived

It is observed from the Table that RBC, WBC and Hb count of mice increases in the subjects administered with the extract dose while it decreases in the subjects exposed to radiations for 2h (mice exposed for 48h did not survive) in comparison to the control indicating the positive effects of the extract dose. This was further substantiated, when the subjects administered with the extract dose were irradiated for 2h and 48h.

It is observed from the Table 2 that RBC and WBC counts are although lower but near to the control values in case of 2h irradiation while in case of 48h irradiation, RBC counts remains the same and WBC count increases from 20.37 x $10^3 mm^3$ to 28.52 x 10^6 No/mm^3.

Hemoglobin value increases both in 2h and 48h irradiation and is close to the one observed for the subjects with extract dose.

The increased values of the blood count functions of the subject administered with the extract dose indicates that the ethanolic extract of *A. vasica* induces medicinal effect in improving blood count function of the mice. It is further concluded that the adverse effects of radiations on the blood count functions are overthrown by supplementing with the extract dose of *A. vasica*. It was further observed that the mice which were irradiated showed physiological deformation with swollen internal body organs while the mice with extract and irradiated showed that the internal damage was much less substantiating the positive medicinal effect of extract, inhibiting adverse effect of radiations. Since the mice did not survive after two days of irradiation, the exposure dose was reduced to 4Gy and the amount of extract dose given to the mice was increased to 0.9g/ Kg of body weight of the mice. The number of the days for which the extract was given was 7 days, 14 days, 21 days, 28 days and 35 days and the mice were exposed to the radiations the next day. After 24 h of irradiation the animals were sacrificed and the blood count studies were carried out. The results are presented in **Table 3** and **Figures 1-3.**

TABLE 3: BLOOD COUNT FUNCTIONS, RBC, WBC AND HB OF CONTROL, IRRADIATED, WITH EXTRACT AND WITH EXTRACT & IRRADIATED SUBJECTS AFTER 24H IRRADIATION

Subjects	Blood Count function of mice irradiated after 24 h of				
	7 days	14 days	21 days	28 days	35 days
			RBC × 10^6 No/mm^3		
Control	1.24 ± 0.27	2.11 ± 0.048	2.07 ± 0.043	2.16 ± 0.037	3.0 ± 0.084
Irradiated	1.12 ± 0.38	1.49 ± 0.059	1.15 ± 0.09	1.35 ± 0.71	2.20 ± 0.06
With Extract	1.85 ± 0.32	2.49 ± 0.049	2.11 ± 0.048	3.04 ± 0.061	5.24 ± 0.24
With Extract & Irradiated	1.45 ± 0.31	2.10 ± 0.041	1.49 ± 0.037	1.86 ± 0.03	3.97 ± 0.05
			WBC × 10^3 No/mm^3		
Control	26.49 ± 9.65	19.00 ± 1.68	19.40 ± 1.25	26.95 ± 1.50	29.60 ± 3.09
Irradiated	24.89 ± 2.63	15.43 ± 1.50	19.00 ± 1.68	24.70 ± 2.00	24.98 ± 0.04
With Extract	27.20 ± 9.47	27.97 ± 2.85	29.90 ± 1.43	30.93 ± 1.34	40.00 ± 4.6
With Extract & Irradiated	26.69 ± 3.68	22.62 ± 3.72	26.00 ± 3.87	28.30 ± 2.88	38.50 ± 0.05
			Hb × gm 10^{-2} ml^{-1}		
Control	9.28 ± 0.02	9.26 ± 0.075	11.2 ± 0.08	11.4 ± 0.02	11.82 ± 0.11
Irradiated	7.13 ± 0.006	8.48 ± 0.06	9.5 ± 0.04	9.9 ± 0.01	9.0 ± 0.40
With Extract	10.80 ± 0.08	9.5 ± 0.04	12.4 ± 0.02	14.6 ± 0.06	14.70 ± 0.13
With Extract & Irradiated	10.20 ± 0.075	10.90 ± 0.04	10.80 ± 0.06	9.9 ± 0.01	12.80± 0.040

Total dose = 4 kGy

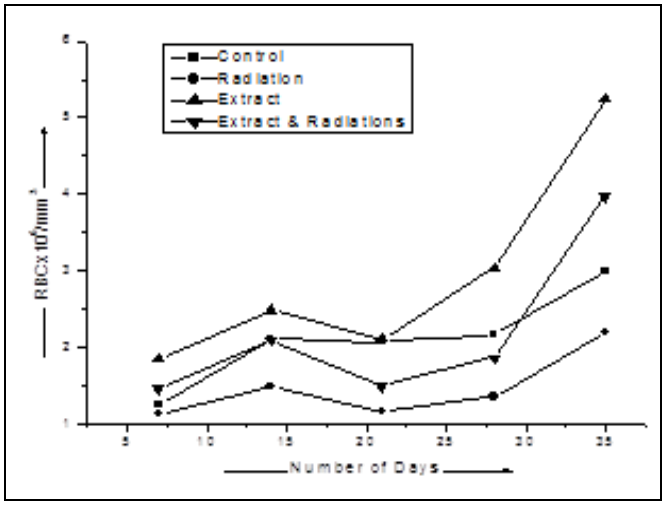

FIG. 1: EFFECT OF NUMBER OF DAYS ON VARIATION OF RBC COUNT OF MICE

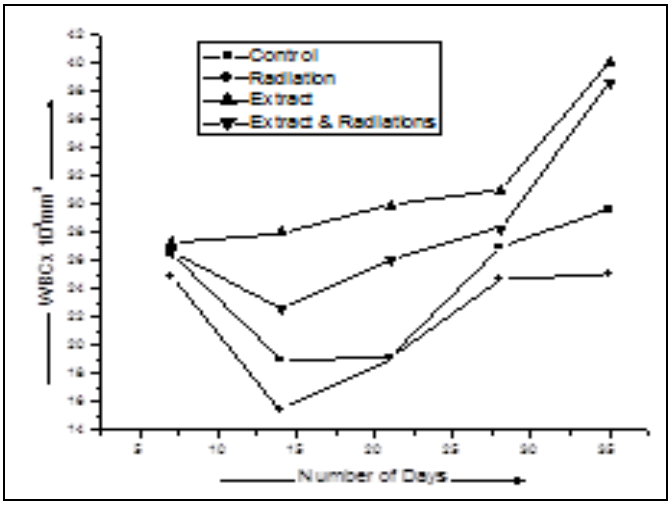

FIG. 2: EFFECT OF NUMBER OF DAYS ON VARIATION OF WBC COUNT OF MICE

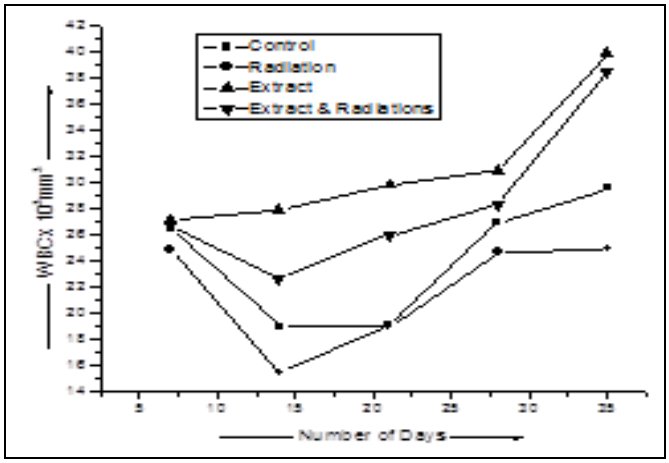

FIG. 3: EFFECT OF NUMBER OF DAYS ON VARIATION OF HB COUNT OF MICE

It is observed from the Figures and the Table that the RBC, WBC and Hb count of all the subjects under each group increases with increasing number of days. The control group from 7days to 35days shows the maximum value of each of the parameter i.e. RBC (3.0 $\pm$ 0.084 $\times 10^6$ No/mm^3); WBC (29.60 $\pm$ 3.09 $\times 10^3$ No/mm^3) and Hb (11.82 $\pm$ 0.11g/100ml) in 35 days. These values increase in the subjects administered with the extract dose and after 35 days the respective values are 5.24 $\pm$ 0.24$\times 10^6$ No/mm^3, 40.00 $\pm$ 4.6 $\times 10^3$ No/mm^3 and 14.70 $\pm$ 0.13g/100ml.

The higher values of the group administered with extract dose for 35 days in comparison to the control indicates the medicinal effect of the extract. Irradiation has been shown to affect the blood count functions and gives lower values of RBC (2.20 $\pm$ 0.06 x 10^6 No/mm^3), WBC (24.98 $\pm$ 0.04 x 10^3 No/mm^3) and Hb (9.0 $\pm$ 0.40g/100ml) after 35 days, but the subjects administered with the extract dose and then irradiated showed higher values than the control and little lower than the subjects with the extract dose (RBC = 3.97 $\pm$ 0.05 x 10^6 No/mm^3; WBC = 38.50 $\pm$ 0/05 x 10^3 No/mm^3; Hb = 12.80 $\pm$ 0.04g/100ml), thus indicating that the extract has inhibiting tendency towards the adverse effects of irradiation on the blood count functions.

Superoxide Dismutase (SOD) activity: Superoxide dismutase (SOD) is a family of metallo- enzymes which protect the cells against the toxic effects of superoxide anions (O_2^-) produced during oxidative stress. Under the oxidative stress, the activity of SOD increases to counter act the stress.

The effect of extract dose on SOD activity on the animals (mice) exposed to gamma radiations has been studied and the results are presented in **Table 4**.

TABLE 4: SOD SPECIFIC ACTIVITY OF MICE (control, irradiated, with extract and with extract + radiation groups)

Subjects	Specific activity of mice irradiated after 24 h of			
	7 days	14 days	21 days	28 days
	SOD Activity (units /mg protein / min)			
Control	7.10 ± 1.40	8.00 ± 0.50	8.40 ± 0.80	7.45 ± 1.10
Irradiated	16.02 ± 2.10	15.10 ± 1.25	17.10 ± 2.50	18.02 ± 1.50
With Extract	6.95 ± 1.50	7.50 ± 0.60	8.50 ± 1.10	7.10 ± 0.14
With Extract & Irradiated	11.97 ± 0.50	13.01 ± 2.60	12.50 ± 1.10	14.15 ± 1.48

It is observed from the Table that the control group of mice shows increasing value of SOD activity (8.40 $\pm$ 0.80 units/mg protein/min) up to 21 days that decreases in the next 7 days i.e. the 28th day to 7.45 $\pm$ 1.10 units/mg protein/min. The subjects that were fed with the extract dose showed lower values in comparison to the control, the minimum (7.10 $\pm$ 0.40 units/mg protein/min) being observed on the 28th day.

The group of animals that were exposed to radiation showed high level of stress with SOD specific activity values up to 18.02 $\pm$ 1.50 units/mg protein/min.

In case of the animals fed with extract dose and then irradiated, have values lower than those given only radiations but higher than the control and the extract fed animals. The lower values of SOD specific activity in the subjects fed with extract dose to control and lower values of the activity of the animals with extract dose and irradiation to irradiated animals indicate that the extract has the ability to balance the oxidative stress. Similar observations were made by Srinivasarao *et al* [22] during their studies on anti-oxidant and anti-inflammatory activity of vasicine against lungs damaging in rats.

Antibacterial Studies: Nutrient agar (N/A) media containing a mixture of 3.25g of nutrient broth and 5 g of agar in 250 ml of distilled water was prepared by autoclaving at 121^{o}C for 20 min. N/A plates were prepared by pouring the media on petri plates and were incubated at room temperature for overnight to check contamination. Stock solution of each extract of the plant material i.e. hexane extract, methanol extract, ethyl acetate extract and vasicine isolated from the plant material was prepared by dissolving 5 mg of each of the extract and vasicine in 10 ml of DMSO. Antibacterial activity was tested against both gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Schigella*).

Agar plates were punctured to create a well of 40 µl capacity to which respective extracts were applied separately and the effect of the extract on the growth of bacteria was observed. The results are presented in **Table 5**.

TABLE 5: BACTERIOLOGICAL STUDIES OF EXTRACT OF LEAVES OF *ADHATODA VASICA*

Bacteria	Extracts			
	Hexane	Methanol	Ethyl acetate	Vasicine
Pseudomonas	-	-	-	-
Staphylococcus aeureus	-	-	-	-
Bacillus subtillis	+	+	-	+
Eschirichia coli	-	+	-	-
Schigella	+	-	+	+

It is observed from the Table that all the extracts and vasicine did not show any effect on the growth of *Staphylococcus* while except for the ethyl acetate extract; all others showed positive response towards the growth of *Bacillus strain*. Among them, vasicine showed lower concentration (5mg/ml) for inhibiting the bacterial growth in comparison to hexane extract (7.2mg/ml) and methanol extract (12.48mg/ml) indicating that the single compound, an alkaloid, vasicine is more effective than the extract containing different constituents.

The antibacterial activity of different extracts and vasicine was also tested against gram negative bacteria and it was observed that both hexane and ethyl acetate extracts and vasicine were active against *Schigella* bacterial culture only and did not show any activity against *E. coli.*

Here too, vasicine with lower concentration (5mg/ml) was found to be effective in comparison to hexane extract (7.2mg/ml) and ethyl acetate extract (12.00mg/ml).

RESULTS AND DISCUSSION: The harmful effects of gamma radiations on the living being are well established and in view of this, comparative study on correlation of gamma radiations and the medicinal effect of ethanolic extract of *A. vasica* on hematological constituents of blood of Albino mice has been investigated.

It has been observed that an exposure to a total body dose of 3Gy can cause a detectable depression in total red blood cells but exposure to a moderate dose range between 5 to10 Gy leads to a decrease in concentration of all the cellular elements in blood. In the present study, the mice were irradiated with a total dose of 6Gy and an extract dose of 600mg/Kg body weight was administered to the subjects.

The values of RBC, WBC and Hb functions of the animals, fed with the extract dose, were found to increase while the exposure on radiations, these values decreased in comparison to the control. However, in case of the animals fed with the extract dose and then exposed to radiations, the values of these functions are either near or higher than the control and little lower than those of the extract fed animals.

In a similar study carried out by Kumar et al. [23], animals were exposed to 8.0Gy radiations and extract dose of 800mg/Kg body was administered. It has been observed that the mice were found to be lethargic with symptoms of nausea and diarrhoea resulting in reduced food and water intake. The values of RBC, WBC and Hb of the mice with the extract dose and exposure to the radiations show a decrease and then increase but the values were less than the control even after 30 days. However, exposing the mice to a lower dose (6Gy) and lower extract dose, in the present study, the radiation

appalling effects were avoided and the values of hematological constituents, RBC, WBC and Hb were found to be higher in comparison to the control (except for RBC and Hb after 21 and 28 days, which showed lower value than control).

The decrease in the hematological constituents of peripheral blood of the animals, exposed to radiations, has been attributed to the direct damage by radiation dose. Samarth *et al* [24] explained these variations due to direct destruction of mature circulating cells, loss of production of cells and also loss of cells by hemorrhage or leakage through capillary walls.

Radiation is one of the physical agents that induce oxidative stress which is defined as an increase in reactive oxygen species (ROS) and/or a decrease in the antioxidant defence mechanisms [25]. Free radicals such as peroxide ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$), peroxyl radical ($RO_2^{\cdot}$) as well as non-radical species such as hydrogen peroxide (H_2O_2) may be present as ROS. These radicals are produced as part of the body's normal metabolic process or by extraneous stimuli such as heavy metals and other toxicants. Biological damage induced by ionizing radiations is known to be due to the free radicals generated during radiolysis of water [26]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system [27] which removes superoxide anion by converting it to hydrogen peroxide, which is then removed by glutathione peroxidase and catalase, thus diminishing the toxic effect caused by this radical.

The effect the extract dose of *A. vasica* on SOD activity on the Albino mice, exposed to gamma radiations, has been studied and it is observed that the animals fed with extract dose and then irradiated have values lower than those, given only radiations but higher than the control and the extract fed animals indicating that the extract has the ability to balance the oxidative stress.

Radio-protective effects of Spirulina in liver of Swiss albino mice exposed to γ- radiations has been attributed to the presence of singlet oxygen quencher, beta-carotene, and vitamin E, which is an effective lipid soluble antioxidant and free radical scavenger which protect cell membrane from peroxidative damage [28].

Sharma *et al* [29] also observed that administering extract of *Withania somnifera* to Swiss Albino Mice, exposed to lead nitrate, showed significant decrease in brain SOD, catalase, reduced glutathione (GSH), and total protein. Antioxidant and hepatoprotective effects of the red ginseng essential oil (REO) in H_2O_2-treated HepG2 Cells and CCl_4-treated mice were studied and it was observed that REO diminished the H_2O_2-mediated oxidative stress and also restored both the activity and expression of antioxidant enzymes such as SOD, catalase and glutathione peroxidase by directly scavenging reactive oxygen species [30].

Manavalan *et al* [31] observed that there was a marked decreased in the percentage inhibition of SOD, catalase and the level of GSH in carbon tetrachloride (CCl_4) treated rats when compared with normal control group due to the hepatocellular damage by CCl_4. However, when the rats were administered with the aqueous extract of *Teramnus labialis* (Linn.) in CCl_4, it significantly increased the percentage inhibition of GSH, SOD and catalase.

The antibacterial activity of methanolic extract of *A. vasica* against all tested microorganisms was greater than that of the aqueous extract. *Adhatoda's* antibacterial properties have been clinically evaluated by Brantner and Chakraborty [32]. Different solvent extracts and vasicine, an alkaloid from *A. vasica,* showed antibacterial properties against *Bacillus strain* and *Schigella.* So results are in consonance with previous results. These findings suggest a possible application of *Adhatoda* in the improvement of drinking water quality [33].

CONCLUSION: The medicinal effects of ethanol extract of *A. vasica* on deteriorating effects of gamma radiations on the blood count functions, taking Albino mice as the subject of study, have been well accomplished. The feeding of the animals with the extract dose increases the values of RBC, WBC and Hb functions, the exposure to the radiations decreases these values in comparison to the control but the animals fed with the extract dose when exposed to radiations, the values are either near or higher than the control and little lower than those of the extract fed animals. Different solvent extracts and vasicine also showed antibacterial properties against *Bacillus strain* and *Schigella.*

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