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Protective potential of *Cassia fistula* on heamatological parameters of swiss albino mice against radiation

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Abstract
Fruit, stem bark and leaves of *Cassia fistula* Linn. Retain a biologically active compound such as flavonoids, glycosides, tannin, anthraquinone, saponin, steroids and alkaloids and many other compounds. The stem bark, fruit and leaves extract shows various activities like antipyretic, ant inflammatory, antidiabetic, analgesic, laxative, hypolipidemic, hepatoprotective, anti-ulcer, antitumor, antimicrobial, antifungal and antioxidant. Traditionally used in many problems in form of lotion, syrup, quath, powder etc. The main aim and objective of this study is to find out the phytochemical constituents responsible for antioxidant activity of *Cassia fistula* Linn. Barks extract and evaluate the total antioxidant activity by reducing power assay. Activity of *Cassia fistula* bark methanol extract (CFBE) on hematological parameter of Swiss albino mice induced by radiations is also assess.

Keywords: *Cassia fistula* Linn. Stem bark, reducing power assay, hematological parameter of swiss albino mice

Introduction
Radiations exist all over the place; in the environment, in soil, plants, water, rocks. Radiations are very important implement in our new world. Our life and the whole world are full of miscellaneous types of radiations. These radiations influenced the formative process of flora fauna, biological system and human kind also. So we are uncovered to radiations during diagnostic and industrial purpose, during the use of mobile, televisions and other electronic equipments, during travel by air, nuclear power plants and their accidents and background radiations also. When radiations fall down on the surface of earth and other object, they give some or entire amount of energy to them. Much larger doses of radiations are used to kill bacteria (harmful) in food technologies, their sterilization and also used in the medical equipment and tools sterilization. Tools and technologies, which are based on radiations and their isotopes, play a huge role in various sectors such as in health, agriculture, industries, mining etc.
Radiotherapy is one of them, a valuable approach for cancer patients. It is used in the treatment of especially those cancers, which cannot be excised by surgical treatment. Radiations are classified on the basis of their wavelength and energy.
But it is more useful when classify on the basis of biological effect and penetrating power. Penetrating radiations can cross or penetrate our skin such as X-rays, gamma rays. Non Penetrating radiations do not cross our skin, as an alpha rays and beta rays. More penetrating radiations are much damaging and destructive for living organisms. Prospective biological effects depend on the dose of radiations and the sensitivity of the object. Dose of radiation may be acute and chronic. Acute dose- is a large dose delivered during a short time. Effects of these doses can be observed within hours/days/week and in a month. Chronic dose- is a small amount of rays given to long period. After radiation exposure object show effects they may be stochastic and non-stochastic. Stochastic effects- when effects increase with the dose of radiations are known as stochastic effect. Non-stochastic effects- are dose threshold, acute or late response effects.
X rays and gamma rays are electromagnetic ionizing radiation with high energy and frequency. They can affect the cell by mutation (change or effect the genetic material) and sometime kill the cells or initiate the cancer; it's all depending upon the dose of radiation. Leukemia may be embarking by the exposure to ionizing radiation. Medical radiations that used in cancer treatment, is one of the general sources of rays overdose, So the patient treat with chemotherapy, may be have chances of chromosomal abnormalities such as Down Syndrome offspring. Workers who did their job in oil refining, rubber manufacturing or any kind of benzene source also have a great chance of leukemia. Some risk factors are most strongly associated with specific types of leukemia.
IARC (International Agency for Research on Cancer) classified the "X rays and gamma radiation" as known human carcinogen, whereas NTP (National Toxicological Programme) classified the ionizing radiation as "known to be a human carcinogen". After complete body exposure, manifestation of injury to mammalian tissues is well reflected in peripheral blood. [4]

Review of Literature
First revelation of any chemical against X-rays induced damage was reported in 1942 by Dale. Experimental evidences suggested that UVB and UVC radiations are most hazardous component to causing skin cancer [1]. However, UVC radiation has been completely filtered by stratospheric ozone layer and therefore, it has not much biological relevance to skin cancer [1]. Majority of solar UV radiation reaching the surface of the earth is UVA (90-99 %) and only 1-10 % is composed of UVB radiation. Earlier studies with animal models showed the complete skin carcinogenic

potential of both UVA and UVB radiations [2]. However, higher exposure doses are needed for UVA caused photo carcinogenesis when compared to UVB in terms of both dose and duration and also has a longer tumor latency period, which is attributed to its weak tumor initiating potential [3]. Ultraviolet radiation B produce cancer by straight DNA break/ damage while malignant melanoma cause by UV A rays via indirect DNA smash which is caused by free radicals and ROS. UVB radiation is a well-recognized generator of ROS and reactive nitrogen species (RNS) which play a key role in mediating its biological effects. Radiation exposure is gradually increasing due to depletion of ozone layer. 232,000 people were documented with melanoma with 55,000 deaths in 2012.

Table 1

Name of investigator	Activity
Purohit R.K. (1990)	Radio response of liver in heteropneustes fossils Bloch
Verma, 1991	Effect of 10 Gy gamma radiations on house sparrow kidney
Yadav (1994	histological changes against gamma rays on kidney of Swiss albino mice
Purohit *et al*. (2001)	1 or 2 Gy gamma rays induced biochemical changes in the skin of Swiss albino mice and Compared it to with Liv.52
Purohit *et al*. (2002	Radiation (1-2 Gy) induced hepatic lesions of Swiss albino mice Compared the result with std. drug Liv.52
Hari Kumar *et al*. (2004)	Anticancer activity of 2.5kg/b. wt dose of *E. officinalis* fruit pulp against single dose of 7Gy radiation after complete body exposure
Sharma and Purohit (2014)	3.0 and 5.0Gy induced hematological changes in the Swiss albino mice Against Liv.52

Collection and Authentication of Plants

Cassia fistula Linn. (Golden shower) has been selected for the study of anticancer antioxidant activity on Swiss albino mice. The Aerial parts of *Cassia fistula* Linn. (Golden shower) stem bark was collected from SPC Govt. College, District: Ajmer, (Rajasthan)

Drying and Size Reduction of Plant Material

The plant material (Bark) was washed and dried in shade then cut into small pieces and dried at room temperature in shade for 30 days then powdered the material with the help of mechanical grinder and stored in air tight container.

Collection of Experimental Animals

For the study, adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. The temperature of the room was maintained between 22-27ºC. 6 week old and 20 – 25gm weight healthy male mice were assigned into 3 groups and 6 animals in each.

- First Group- In a first group normal control mice (positive-control)
- Second Group- mice treated with radiation only (3Gy).
- Third Group- This group mice were treated with 100mg/kg b.wt. Of methanolic extract of Cassia *fistula Linn*. Orally once a daily with the exposure of radiations.
- Forth Group- Mice of this group were given 200mg/kg body weight dose of MESB of *Cassia fistul*a orally once a day with radiation exposure till the experiment was completed.
- Fifth Group- Mice were treated 250 mg/kg by weight methanol extract of *Cassia fistula* with radiations expose till the 30th day of experiment.

Collection and Detemination of Samples and Haematological Parameter

Body weight of the animals was observed on 10th, 20th, 30th days. Blood was collected from tail vein in separate EDTA tubes on the first, 10th, 20th, 30th day. R.B.C. and W.B.C. evaluated with the help of Neubaur's chamber. PCV and hemoglobin was measured by microhematocrit method and Sahli's method respectively. Other hematological indices were calculated with the help of formula. To find out the phytochemical constituents in the stem bark of *Cassia fistula* Linn. Preliminary test were performed.

Table 1: Preliminary Phytochemical Screening

Phytoconstituents	Observation	Result
Carbohydrate	Molish test, Fehling test and Iodine test	Present
Proteins	Biuret test	Present
Anthraquinone	Borntranger's test, Modified Anthraquinone test	Present
Glycosides	Foam test and Heamolytic test	Present
Alkaloids	Mayer's reagent, Wargner's test and Dragendroff reagent	Present
Flavonoids	Shinoda test	Present
Saponin	Foam test	Present
Tannins	Gelatin test and Chlorogenic test	Present
Anthocyanin	Sodium Hydroxide Reaction and Ferric Chloride Reaction	Absent
Sterols and terpenoids	Liebermann Bruchard Test and Salkowski Test	Absent

In-Vitro Pharmacological Investigation

The present study was aimed to investigate the *in vitro* free radical scavenging activity of methanol extract of bark *of Cassia fistula* Linn. In-vitro free radical scavenging activity of the extracts was assessed against different free radical systems as following. Assay repeated 3 times and the result were taken as mean value of triplicates.

Reducing Power Assay [12]

Reducing power of Methanol extract of Cassia fistula stem bark determined by Oyaizu (1986) method. This is a

suitable and fast method for determination of antioxidant activity in the sample solution. Reducing power assay is based on the reduction potential of substances; react with potassium ferricyanide to form potassium ferrocyanide ($Fe^{3+} \rightarrow Fe^{2+}$). After reaction with $FeCl_3$ a complex ferric-ferrous is formed, show maximum absorption at 700nm.

METHOD-

1 ml of different concentrations of the plant extract solutions were added to 2.5 ml of 1 % potassium ferricyanide in different investigation tubes as well as the resultant mixture incubated at 50° C for 20 minutes. Then 2.5 ml of 10 % trichloroacetic acid was added to each tube. The tubes were centrifuged for 10 minutes at 3000 rpm. The supernatant from each tube (2.5 ml) was taken in a separate investigation tube as well as 2.5 ml of distilled water 0.5 ml (0.1 %) ferric chloride solution were added to each investigation tube. The absorbance of these assay mixtures was measured at 700 nm. Increased absorbance of reaction mixture indicated the increased reducing power. In parallel to this the reducing power of B.H.T was also determined for comparison.

Estimation of W.B.C and R.B.C with the Help of Hemocytometer

Neubauer's chamber is specially designed for the counting of red blood corpuscles and leukocytes. It's very difficult and almost impossible to calculate these manually under the microscope. So a peculiar chamber was designed, which is known as Haemocytometer or Neubauer chamber. It is a thick glass plate with 30×70×4mm size and 0.1mm thick space in it, therefore each square contain $1×1×0.1=0.1mm^3$. There are depressions on both edges or in between the area, on which the squares are marked as 'H' shape. The ruled area is divided into 9 large squares at the border or corner of the slide while mid squares have 25 medium sized chambers with double lines. These medium sized squares are further divided into 16 small squares that result in every smallest square having $1/400mm^3$ area.

Procedure

Sample was filled up to the mark 0.5 of the RBC pipette. Then filled the pipette again up to the mark 101 with normal saline or Hyme's solution for dilution of the blood sample. The blood has been diluted 200 times. Remove the extra blood from the capillary channel of the RBC pipette. After that vigorously shaken the pipette 2-3 minutes. Put a drop of sample on the slide and covered it with provided cover slip. Observation was done under a microscope. And RBC/mm^3 was calculated with the below formula-

Calculation

RBC/ µL or mm3 = Average number of RBC Conted × dilution × Depth

No. of chamber counted

Procedure for WBC Count

Sample was filled up to the mark 0.5 of the WBC pipette. Then filled the pipette again up to the mark 11 with normal saline or Hyme's solution for dilution of the blood sample. The blood has been diluted 20 times. It was also cautious that there were no air bubbles in the pipette. After that WBC pipette with a sample was shaken properly for 2-3 minutes. Put the blood sample drop on the slide and cover it with provided cover slip. Observation was done under a microscope. And calculation was done with the below formula-

Calculation

WBC/ µL or mm3 = Number of WBC Counted × dilution × Depth

No. of chamber counted

Method for Heamoglobin Estimation

Principal

Haemoglobin is a conjugated protein whose main function is to carry oxygen, because they have red pigment haemoglobin. Every haemoglobin molecule contains globin a protein part and heams non protein part. Single molecule of Hb can carry 4 oxygen molecules because of 4 heme groups. 23 percent of Carbon dioxide is also carried by Hb. Carbon dioxide binds with the amino part of globin to form carbaminohemoglobin. Hb was measured by Sahli's method. Base of this method is, Hb is converted into acid hematin in the presence of HCl. It gives brown yellowish colour. Colour of the sample measured with standard colour reference. 02 ml of sample was taken in haemoglobin pipette. First drop of blood is not taken and is blown into a graduated tube containing dil. HCl up to mark 20 ml. After this it was kept aside for 5-10 minutes. During this haemoglobin was converted into haematin due to oxygenation. Then compared the test sample colour with the standard colour kit. If darker in colour then add some water or dil. HCl. Then recorded it as gm/100 ml.

Result and Discussion

Reducing Power Assay- The reducing power of bark extract of *Cassia fistula* Linn. Extract was compared with B.H.T. The reducing power of bark extract was found to increase with the increasing concentration of the extract, which is compared to the standard drug B.H.T.

Table 2: Reducing Power Assay of Stem Bark Methanolic Extract of *Cassia fistula Linn.* With Reference to BHT

S. No.	Concentration (µg/ml)	Absorbance of BHT	Absorbance of (SBME) Stem Bark Methanolic Extract of *Cassia fistula* Linn.
1	50	0.171	0.127
2	100	0.348	0.298
3	150	0.628	0.556
4	200	0.918	0.654
5	250	0.987	0.865

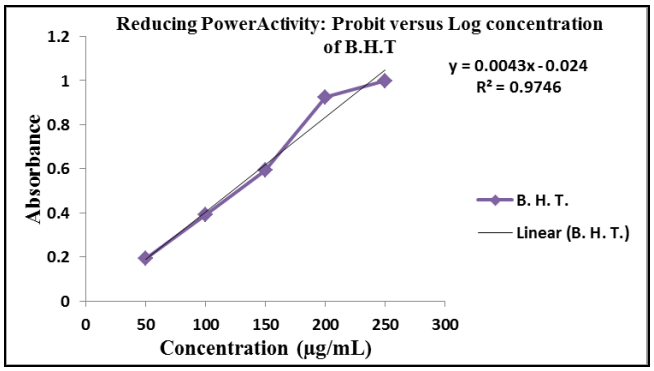

Fig 1: Probit versus Log concentration of B.H.T

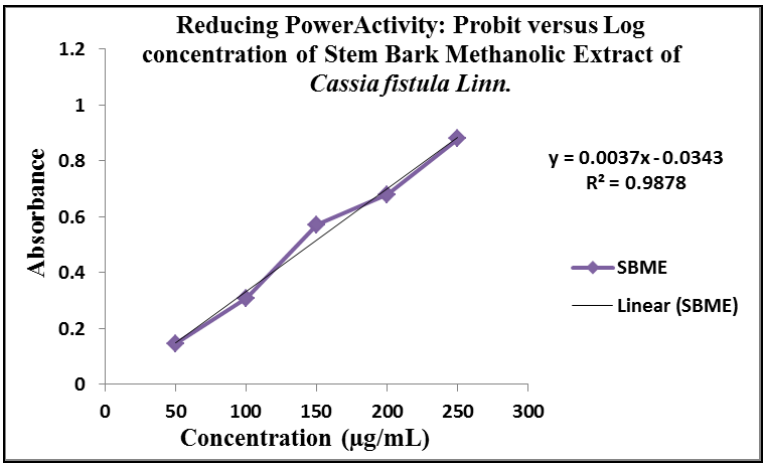

Fig 1: Reducing Power Assay of Stem Bark Methanolic Extract of *Cassia fistula Linn.* With Reference to BHT

The absorbance of these assay mixtures was measured at 700 nm. Increased absorbance of reaction mixture indicated the increased reducing power.

Result of Hematological Parameters

Table 3

Groups	Day of Sample Collection			
Body Weight	**First**	**10th**	**20th**	**30th**
Control	24.7 ±0.35	26.02± 0.50	27.95± 0.51	32.44± 1.01
Radiation only	25.1± 0.31	24.21± 0.42	20.26± 0.12	22.01± 0.32
Radi. + 100mg/kg MESB	25.02 ± 0.32	26.2 ± 0.54	27.51 ± 0.41	29.97 ± 0.92
Radi. + 200mg/kg MESB	24.98 ± 0.25	25.44 ± 0.35	26.01 ± 0.45	27.51 ± 0.85
Radi. +250mg/kg MESB	25.02± 0.32	25.44± 0.51	26.94± 0.50	29.32± 1.07
Hb g/dL				
Control	15.24 ± 0.24	15.16± 0.54	15.03± 0.24	15.36± 0.62
Radiation only	15.02 ±0.54	12.67 ± 0.24	10.62± 1.04	10.02± 0.30
Radi. + 100mg/kg MESB	15.02± 0.20	13.42± 0.63	13.18± 0.56	13.01± 0.64
Radi. + 200mg/kg MESB	14.98± 1.22	14.62± 0.54	13.92± 0.36	14.72± 0.34
Radi. +250mg/kg MESB	15.01± 0.40	13.78± 1.11	14.98± 0.54	14.99± 0.02
R.B.C× 10^6 mm^3				
Control	9.88±0.32	9.33±0.30	9.42±0.26	9.41±0.29
Radiation only	9.43±0.02	8.98±0.21	7.62±0.01	6.33±0.11
Radi. + 100mg/kg MESB	9.28± 0.24	9.23± 0.10	9.04± 0.44	8.14± 0.24
Radi. + 200mg/kg MESB	9.26±0.34	8.86±0.26	8.94±0.19	8.96±0.24
Radi. +250mg/kg MESB	9.23± 0.21	9.03± 0.32	8.98± 0.51	9.02± 0.03
W.B.C×10^3/ mm				
Control	6.95 ± 0.34	6.9 ± 0.53	6.35 ± 0.43	6.43 ± 0.48
Radiation only	6.95 ± 0.10	5.88 ± 0.31	5.01 ± 0.18	5.01 ± 0.02
Radi. + 100mg/kg MESB	6.89 ± 0.43	6.37 ± 0.40	6.34 ± 0.11	5.96 ± 1.00
Radi. + 200mg/kg MESB	6.76 ± 0.43	6.09 ± 0.11	5.04± 0.20	5.82± 0.51
Radi. +250mg/kg MESB	6.82 ± 0.71	6.8 ± 0.18	6.09 ± 0.11	5.22 ± 0.24

Note: All values are expressed as mean ± SD of 6 animals.

1. Results for preliminary screening of phytocompounds support the presence of alkaloids, glycoside, anthraquinone, saponins, tannin, protein, sterol and terpenoids, protein, carbohydrate and flavonoids while absence of anthocyanin in the stem bark extract of *Cassia fistula* Linn
2. The reducing power of bark extract of *Cassia fistula* Linn. Was found to increase with the increasing concentration of the extract. Increased absorbance of reaction mixture indicated the increased reducing power with R^2 value obtained by this method for Cassia bark extract was 0.9878.
3. Hematological parameters WBC, RBC count, Hb indicate the status of health. Any changes in the parameters/ range of blood indices associated with the indication of any disease or physiological pathological conditions. When the concentrations of all hematological parameter are low in blood is known as Anemia. This condition induced may be due to decrease production of RBC or may be rapid destruction of RBC. Hb closely related with concentrations of erythrocytes. Due to oxidative damage of RBC value decreased rapidly. In the present study the obtained maximum value of Hb is 15.36g/dL at the day of 30th and minimum value is 10.02g/dL in group II at the day of 20th. Values of Hb in Group III is minimum as compared to group IV and V but higher than group II. Maximum values of Hb 14.98± 0.54 and 14.99± 0.02g/dL was obtained at the dose of 250mg/kg of MESB on the 20th and 30th day. RBC values in the present study observed with highest value are 9.88 ±0.32 × 10^6 mm^3 on the 1st day and 9.41±0.29× 10^6

mm^3 at 30^{th} day of experiment in the control group. Minimum are 7.62 ±0.01 × 10^6 mm^3 and 6.33±0.11 × 10^6 mm at 20^{th} and 30^{th} day in the experimental group II in which only radiation treated mice which is significantly lower than other groups. of 100mg/kg and 250mg/kg body weight shows the effective effect as compared to dose 200 mg/kg body weight. In the present study leukocytes values observed in between 5.01± 0.18 ×10^3/ mm to 6.95± 0.34×10^3/ mm^3. Gradual lessen in the values of WBC observed in group II after exposure of radiation.

Conclusion

A considerable increase in Erythrocytes values are observed in the group III, IV and V as compared to II. Group II only radiation treated mice showed significant lessen in R.B.C and Hb, is suggested that animal suffering from anemia after exposure of radiation. Report of leukocytes count indicates besides group II only radiation treated mice, changes in the parameter of WBC in groups III, IV and group V observed also. Low value R.B.C, Hb in radiation treated mice may be due to; iron deficient anemia, lack of Fe in diet and trauma. These are necessary for production of blood. It seems that *Cassia fistula* has protected skin from gamma rays and X-rays. *Cassia fistula* stem bark is found to be good as an herbal, natural defensive source against radiation. After further investigation of its dose dependent protective potential, it can be given to the patients who suffer from cancer during radiotherapy or chemotherapy, to lessen the side effect of therapy.

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