MODULATORY INFLUENCE OF *EMBLICA OFFICINALIS* LINN. ON RADIATION AND CADMIUM INDUCED BIOCHEMICAL ALTERATIONS IN THE BRAIN OF SWISS ALBINO MICE

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Protective efficacy of *Emblica officinalis* against radiation and cadmium induced biochemical alterations has been investigated in the present study. Six to eight weeks old male Swiss albino mice were selected for the purpose and divided into seven groups: - Group I (Sham-irradiated), Group II (treated with cadmium chloride solution 20 ppm), Group III (Irradiated with 2.0 Gy gamma rays), Group IV (both irradiated and treated with cadmium chloride solution), Group V (Cadmium and *Emblica* treated), Group VI (radiation and *Emblica* treated), Group VII (radiation, cadmium chloride and *Emblica* treated). The animals were sacrificed at post treatment intervals of 1, 2, 4, 7, 14 and 28 days. The brain was taken out and quantitatively analyzed for different biochemical parameters such as total proteins, glycogen, cholesterol, acid phosphatase activity, alkaline phosphatase activity, DNA and RNA. The value of cholesterol, glycogen, RNA, acid phosphatase activity and alkaline phosphatase activity increased up to day-14 in non drug-treated groups and day-7 in *Emblica*-treated groups and thereafter decreased upto the last autopsy interval studied. The value of total proteins and DNA decreased up to day-14 in non drug-treated groups and day 7 in the drug treated groups then increased in all the groups. In only cadmium chloride (without and with drug) treated animals (Groups II and V) the value of cholesterol decreased during early intervals
(days-14 and 7 respectively) and increased thereafter. Severe changes were observed after combined exposure to radiation and cadmium chloride showing synergistic effect. *Emblica* reduced the severity of changes and made the recovery process earlier and faster. These results indicated that when radiation and cadmium were given simultaneously, cumulative effects were observed. At all the corresponding intervals the drug treated animals showed less severe biochemical changes and an earlier and faster recovery, which may be due to protection provided by *Emblica.*

Key Words: Radiation, Cadmium, *Emblica*, Brain, Mice.

Introduction

Our universe contains diverse kinds of radiation. These radiations have influenced the formative processes of our earth's biological regime and enabled it to diversify its fauna and flora in various ways. When radiations fall upon any object on the earth's surface, they give away some or whole of their energy to the atoms of the material through which they travel.

Apart from ionizing radiation human beings are continuously exposed to a wide range of metallic pollutants, which are released into the environment by mining, smelting, discharging industrial, agricultural and domestic waste, burning fossil fuel

and using pesticides. Cadmium is reported as one of the most toxic heavy metals in the environment and its rapid uptake and accumulation in food chain contribute to its being a potential environmental hazard [(1)].

The nervous system plays a central role in an organism's life and any adverse change in it affects the organism greatly and that is why various studies have been done on chemical, histological and functional alterations of the nervous system in the event of exposure to small doses of radiation and heavy metal.

The wide variety of tissues constituting the brain together with its importance and accessibility has made it a favourite site of study. Amongst numerous problems pertaining to the biological effects of environmental hazards, which have been carefully investigated in recent years in many countries, injury to the brain occupies a special place. Proper knowledge of the response of brain to ionizing radiation and cadmium appears as a problem of great clinical and biological importance.

Radiation exposure causes damage to biological systems and this is mediated by the generation of free radicals and reactive oxygen species targeting vital cellular components such as DNA and membranes. DNA repair systems and the endogenous cellular biochemical defense mechanisms against reactive oxygen species and antioxidant enzymes like reduced Glutathione (GSH), Super oxide dismutase, Glutathione peroxidase, catalase etc. fail upon exposures to higher as well as chronic radiation doses leading to alterations in cell functions, cell death or mutations. Radioprotectors prevent these alterations and protect cells and tissues from the deleterious effects of radiation. Radioprotectors are of great importance due to their possible and potential application during planned radiation exposures, such as radiotherapy, diagnostic scannings, clean up operations in nuclear accidents, space expeditions etc. and unplanned radiation exposures such as accidents in nuclear industry, nuclear terrorism, natural background radiation etc. Many of the available synthetic radioprotectors are toxic to mammalian system at doses required to be effective as radioprotector. The present work pertains to studies on protective effect of *Emblica* against radiation and cadmium induced biochemical changes in the brain of Swiss albino mice.

Emblica officinalis Gaertn (syn. *Phyllanthus Emblica* Linn.) family-Euphorbiaceae is being extensively used in traditional Indian system of medicine and is a constituent of several poly-herbal preparations. Brahma Rasayan, which contains *Emblica*, is reported to have an excellent radioprotective activity in animal models as well as in human volunteers undergoing radiotherapy.[2-3]

Emblica is an excellent antioxidant and a free radical scavanger. It helps in protecting the skin from damaging effect of UV radiation[4]. *Emblica* was found to be hepatoprotective[5], anti-diabetic[6] and reported to reduce the ulcer of the stomach[7]. *Emblica* was found to be non-toxic to human and experimental animals. Many polyphenols such as ellagitannins, phyllemblin, ellagic acid, trigalloylglucose, phyllantidin, nucleic acid, *Emblicannin* and furosin are reported to be present in *Emblica*[8].

Since the brain is the central controlling organ of the body, many investigations have been carried out to study the effect of cadmium and radiation administered individually, but only a few reports on the effect of the simultaneous exposure to these two factors upon brain are available.

Also noteworthy is the fact that many studies have been done to evaluate the protective effect of various drugs, but very few researches have been

conducted to study the effect of *Emblica* on mice brain previously exposed to cadmium and radiation simultaneously.

Materials and Methods

Animals

Six to eight weeks old Swiss Albino mice were brought from an inbred colony maintained in the animal house of C.C.S.University, Hissar. The animals were kept in polypropylene cages in the departmental animal house and maintained on standard mice feed and tap water *ad libitum*. The animals were kept at temperatures between 20 to 25^0 celsius.

Source and procedure of irradiation

A cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada was used to expose the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate of 0.96 Gy/min. The dose was calculated at the mid point by multiplying dose rate and tissue-air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Cadmium chloride treatment

Aqueous solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of glass distilled water, giving a concentration of 20ppm and given as drinking water.

Emblica

Emblica officinalis Linn. juice was procured from Vrtika herbotech, Jaipur (India). The drug was fed orally at the dose rate of 0.01 ml/animal/day. The drug was given from seven days prior to cadmium chloride treatment or/and irradiation and continued upto the last autopsy interval.

Plan of Experimentation

The animals were divided into different control groups according to the treatment given to them, i.e., given cadmium chloride solution as drinking water (Group-II) or exposed to 2.0Gy gamma radiation (Group-III) or both (Group IV). Biochemical changes in the brain of the animals of these groups were compared with those which were given no reatment (Group-I). Against these the animals of the experimental groups were simultaneously given *Emblica* besides the same treatments (Group-V, Group-VI andGroup-VII respectively). Biochemical changes in the brain of the experimental groups were compared with those of the corresponding control groups.

Autopsy of animals

Five animals from each group were sacrificed by cervical dislocation at 1, 2, 4,7,14 and 28 days post-treatment intervals. Immediately after autopsy, the brain was taken out, blotted and weighed on electrical monopan balance. It was kept at -20^0 celsius for various biochemical estimations viz., total proteins, glycogen, cholesterol, acid and alkaline phosphatase activities, DNA and RNA [9-13].

Results and Discussion

In the present study, Group II showed significant decline in the total proteins in the brain, which continued upto 14th day of experiment. This observation indicates that the amount of total proteins is adversely affected by cadmium. Toxic chemicals may impair protein synthesis pattern. The total proteins content decreased in both non-drug treated groups II, III and IV as well as *Emblica*-treated groups V, VI and VII. The value decreased upto day-14 in non drug treated groups II, III and IV and upto day-7 in *Emblica*-treated groups V, VI and VII. Thus in *Emblica* treated groups, an early and fast recovery was observed showing protection by *Emblica* (Fig.1)

The synthesis of proteins depends on the activity of DNA and RNA[14]. Decrease in the protein content of the brain in various groups may be either due to decline in the rate of protein synthesis or increase in protein consumption. Reduced rate of protein synthesis may be due to unfavorable conditions like unavailability of one or more essential enzymes and /or reduction in the sites of protein synthesis[15]. Increase in protein consumption may be due to increased demand of proteins in repair process or increased activity of lysosomal enzymes [16]

The value of glycogen increased in all the experimental groups as compared to the normal. In non-drug treated groups II, III and IV the value increased from day-1 to day-14. Thereafter, the value decreased on day-28. In *Emblica* treated groups V, VI and VII the value rose upto day-7, then declined on day-14 and continued so till day-28. After combined treatment synergistic changes were noted. Thus an early and fast recovery was seen in *Emblica* treated experimental group (Fig.2)

Glycogen accumulation in brain is the expression of a radiation induced biochemical lesion. The most likely mechanism by which glycogen accumulates in irradiated nervous tissue is the inhibition of glycolysis. An increase in glycogen content has been shown in rat and monkey brains following exposure to 250 Kv roentgen radiations and 32 Mev photons respectively[17].

Reversibility of glycogen accumulation during recovery suggests that a reparative process is operating, since at this time tissue apparently recovers the ability to metabolize glycogen deposits. Disappearance of glycogen surplus occurs at approximately the same time when recovery of brain functions occur following roentgen irradiation[18-19].

Increase in glycogen content of the postnatally developing mouse brain after continuous exposure to tritium has also been reported[20]. This increase was observed from 1 to 5 weeks of age and returned to its normal value in 6th week. These findings are in accordance with the present results.

In the present investigation, cholesterol content declined upto day-14 in cadmium chloride treated group II and till day-7 in cadmium chloride and *Emblica*-treated group V. Thereafter, an increase in the value was seen upto day-28 in both the groups. An increase in the value was noted upto day-14 in non-drug treated groups (III, IV) and till day-7 in *Emblica*-treated groups (VI and VII). Thereafter the value declined. After combined treatment with radiation and cadmium chloride synergistic changes were observed. In *Emblica* treated groups the decrease or increase was on the same pattern, but less severe indicating protective effect of *Emblica*(Fig.3).

Other workers have also seen an increase in cholesterol content of the animals exposed at 2 days of age and assayed at 9-10 days of age. There occured a specific increase in the proportion of cholesterol, which seems to be synthesized at a relatively more rapid rate in irradiated brain and since it apparently cannot be broken down in this tissue, the increase in readily detected[21].

An elevation in the value of acid phosphatase activity was observed upto day-14 in groups II, III and IV, thereafter it decreased on day-28. But the value increased only upto day-7 in *Emblica*-treated groups V, VI and VII, then it declined on day-14 and continued so upto day-28. An early and fast recovery in groups V, VI and VII was due to protective effect of *Emblica* (Fig.4).

Acid phosphatase is a lysosomal enzyme and is non-specific phosphomonoestrase. It helps in the autolysis of cells after death. It hydrolyses various phosphate esters and liberates phosphates. Heavy metals induce cellular damage in the tissue that in turn releases lysosomal enzymes thereby increasing the acid phosphatase activity[22]. Irradiation and heavy metal toxicity might cause rupture of lysosomes and hence phosphatase activity increases.

Cell damage resulting from exposure to ionizing radiation and cadmium chloride may be due to disruption of cellular organization, so that the enzymes come in contact with their substrates. Lysosomes contain many powerful hydrolytic enzymes such as cathepsin, phosphatases and nucleases, which upon release cause great damage. It has been suggested that irradiation induces physical and functional changes in the lysosomal membranes, permitting the release of these hydrolytic enzymes and indirectly causing destruction[23-24].

An increase in the value of alkaline phosphatase activity in the brain of Swiss albino mice was observed upto day-14 in non-drug treated groups II, III and IV, thereafter it decreased on day-28. In *Emblica*-treated groups V, VI and VII the value increased upto day-7, then it declined on day-14 and continued so upto day-28. This early recovery in drug treated groups may be due to protection provided by *Emblica* (Fig.5).

An increase in the activity of alkaline phosphatase at early intervals after irradiation has previously been reported[25-26].

The probable reasons for increase may be: -

(a) Increase in the synthesis of the enzyme[27].

(b) Increase in the functional cell population relative to the proliferative epithelial cells, as the latter decrease rapidly due to radiation induced cell death[28].

(c) Disruption of lattice like structure of phospholipids and hydrogen bonds that separate enzymes from their intracellular substrates and

(d) Altered physiological conditions, such as liver function, mediated by alkaline phosphatase activity.

Decrease in DNA content was noted upto day-14 in non-drug treated groups II, III and IV, thereafter it increased on day-28. In *Emblica*-treated groups V, VI and VII the value declined upto day-7 after which it increased on day-14 and day-28. After combined treatment with radiation and cadmium, synergistic changes were observed.

In *Emblica*-treated groups a less prominent decrease was observed showing protection provided by *Emblica* (Fig.6).

DNA is the critical target of radiation damage in living cell, which may lead to alteration in the functional state of cell and further to cell death. Damage caused to DNA molecules by irradiation leads to other metabolic alterations also[29-32]. A fall in DNA content of rat testis after partial body gamma irradiation has also been reported[33]. Their work on testis supports our findings in brain.

Concentration of RNA increased in the brain of all the groups. RNA content increased on day-1 and continued so significantly ($P<0.001$) upto day-14 in non drug-treated groups II, III and IV. The value declined on day-28 but did not reach the normal level. In *Emblica*-treated groups V, VI and VII, RNA content increased up to day-7 significantly ($P<0.001$), thereafter it declined on day-14 and continued so upto day-28 but the difference with the normal value was significant ($P<0.001$). In *Emblica*-treated groups less severe increase was observed showing protection by *Emblic* (Fig.7)..

Effects of ionizing radiation on *in vivo* synthesis of nucleic acids in a mammalian radiosensitive tissue depends to a great extent on two important factors[15]:

(i) More or less rapid cytolysis of large proportion of cells, and

(ii) Change in cell population after irradiation.

RNA metabolism may be influenced by a number of factors. Increase in RNA content after irradiation could be due to an increase in RNA concentration of the surviving cells. Causes of this increase in cellular RNA may be:-

1. Ability of DNA to transcribe RNA is not affected quantitatively[34-36], but the length of the chain of RNA molecules reduces[37].

2. Increase in nuclear RNA polymerase activity[37-38]may contribute to post-irradiation increase in cellular RNA[39].

3. Increased gonadotropin secretion after irradiation has been reported[40]. This increased gonadotropin secretion may accelerate RNA synthesis after higher doses of radiation[41].

Protective mechanism of *Emblica officinalis*

The probable protective mechanisms of action of *Emblica* may be as under:

(1) Radiation has been shown to induce DNA strand breaks and mutation and brings about peroxidative changes in lipids and proteins. *Emblica* exract has been shown to have significant antioxidant activity, which reduces the oxidative changes induced by radiation.

(2) *Emblica* extract has also been found to inhibit mutagenesis by direct binding to certain mutagens as well as by inhibing carcinogen activation.

(3) It stimulates haemopoiesis, thus reducing the myelosuppression induced by radiation.

(4) Moreover, it forms a protective layer in stomach thereby reducing mucosal damage to gastrointestinal lining during irradiation.

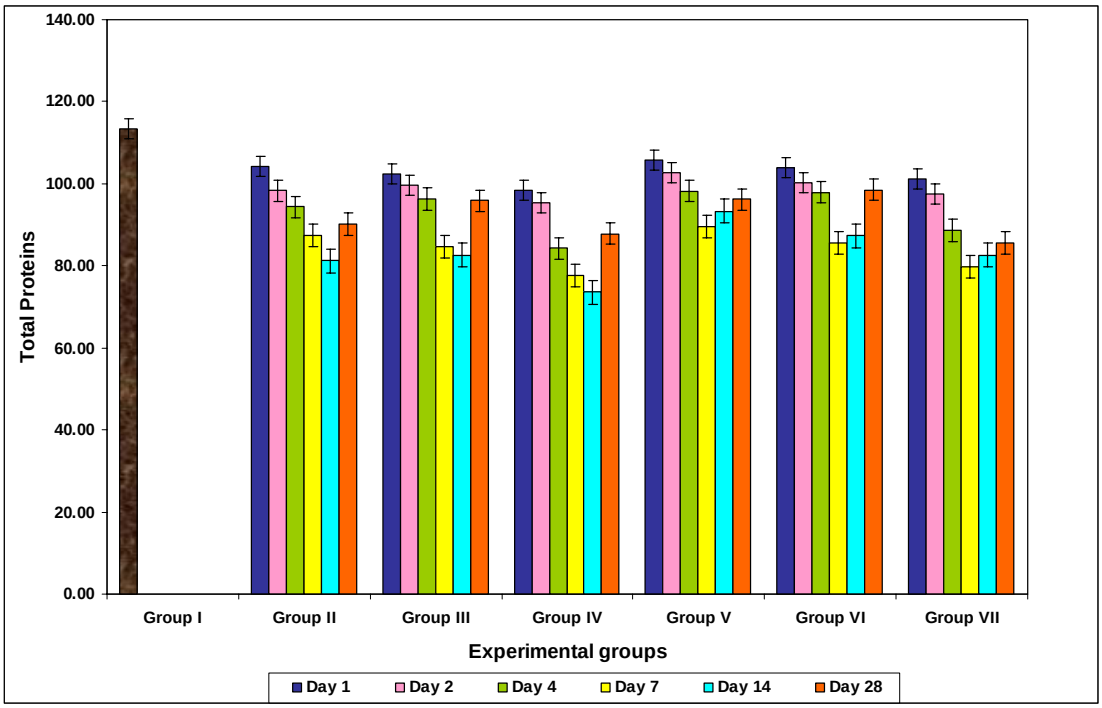
(5) Presence of a variety of polyphenols is reported in *Emblica*. These polyphenols are excellent scavangers of oxygen radicals produced in the body by radiation thus affording protetion to the body[42].

(6) Administration of *Emblica* extract increases GSH level. *Emblica* shows excellent antioxidant activity *in vitro*[43] and the present study also reveals its antioxidant potential.

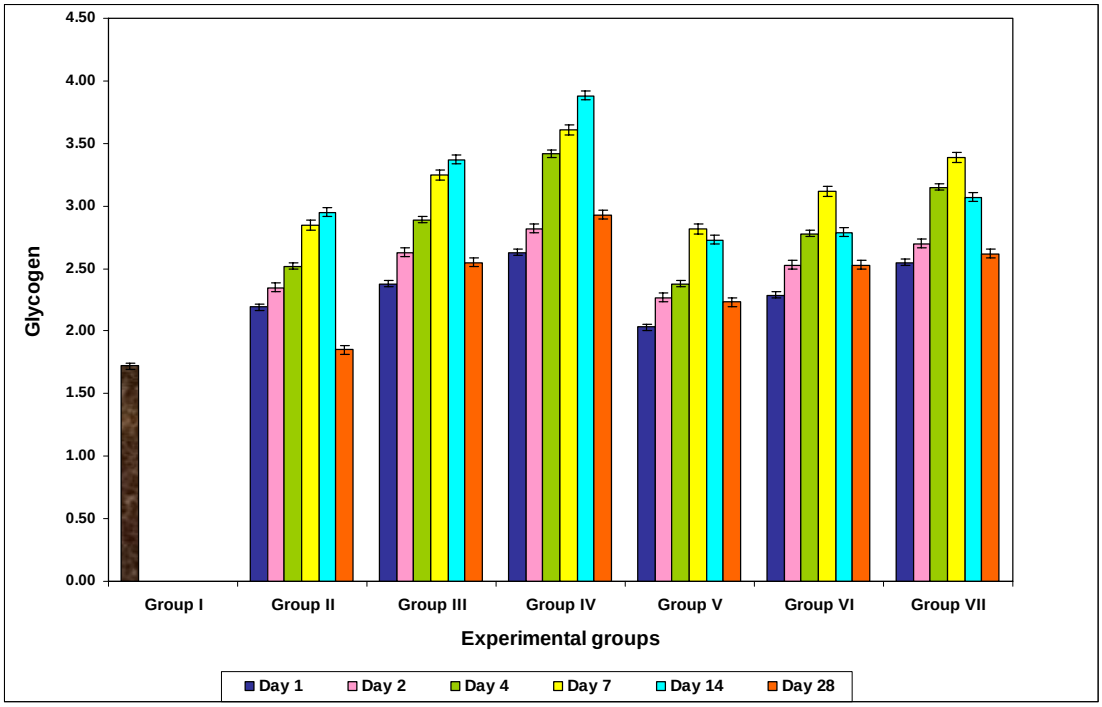
(7) It can be hypothesized that antioxidant activity, potent stimulation of haemopoietic system, non-toxicity as well as easy availability of *Emblica* make it an excellent choice for further development as a natural radioprotector[44].

HISTOGRAM-1

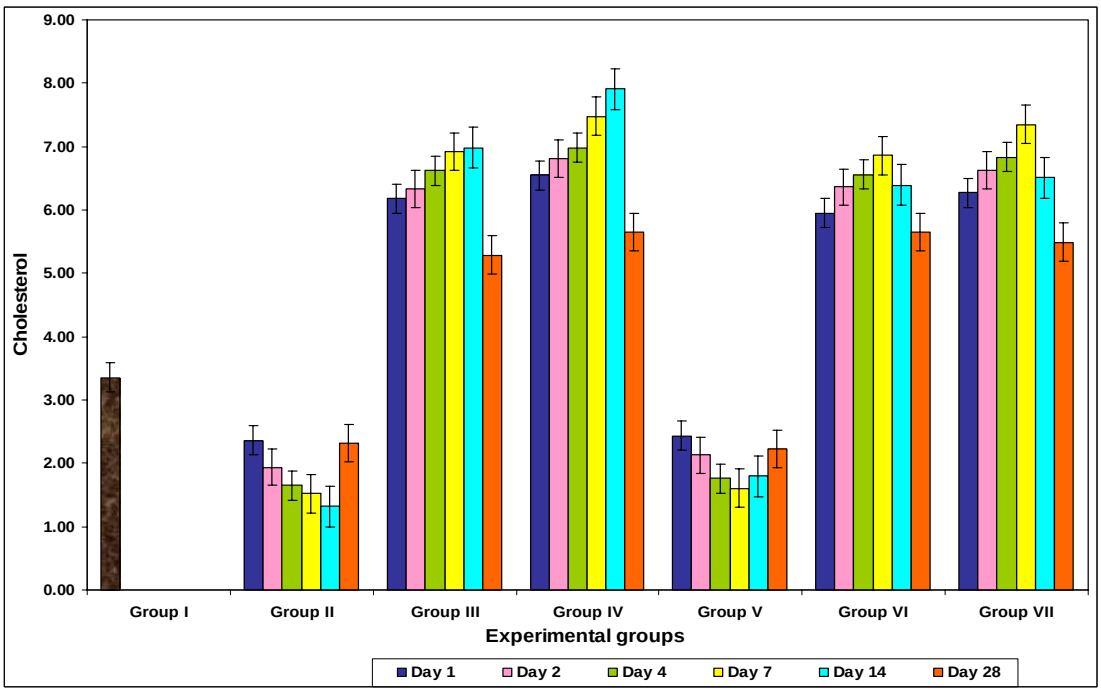
Variations in the Total proteins content in brain of mice in various groups (mg/gm of tissue weight)



HISTOGRAM-2
Variations in the Glycogen content in brain of mice in various groups (mg/gm of tissue weight)

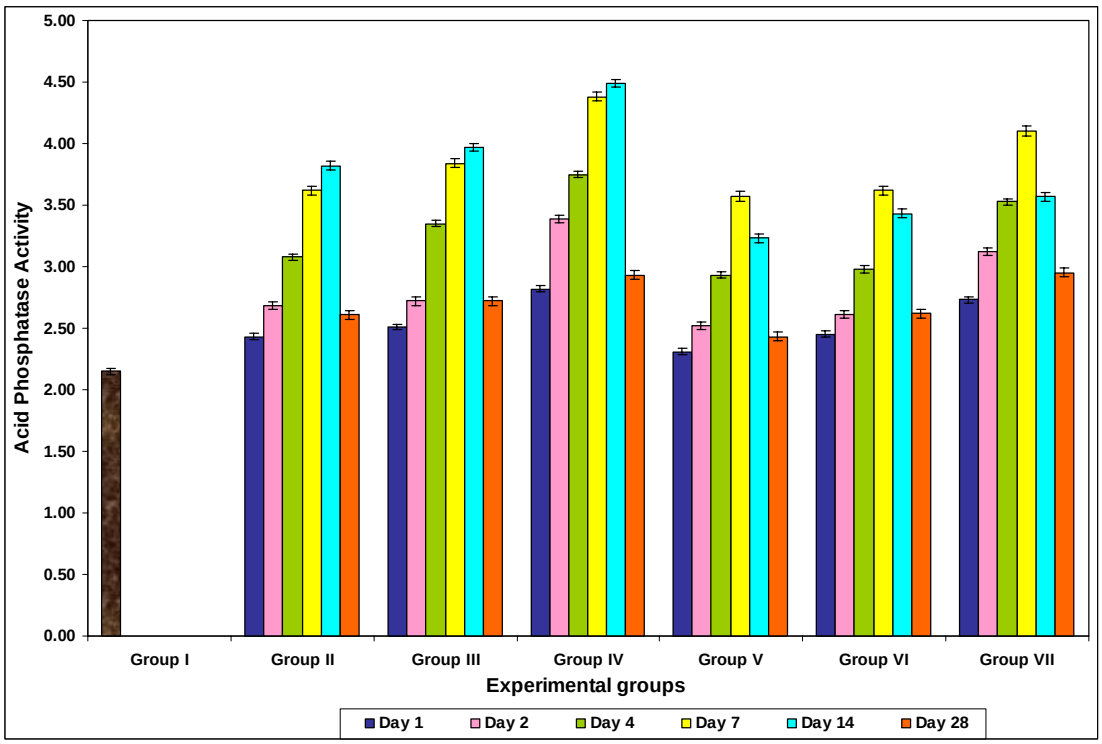


HISTOGRAM-3
Variations in the total Cholesterol content in brain of mice in various groups (mg /gm of tissue wt.)



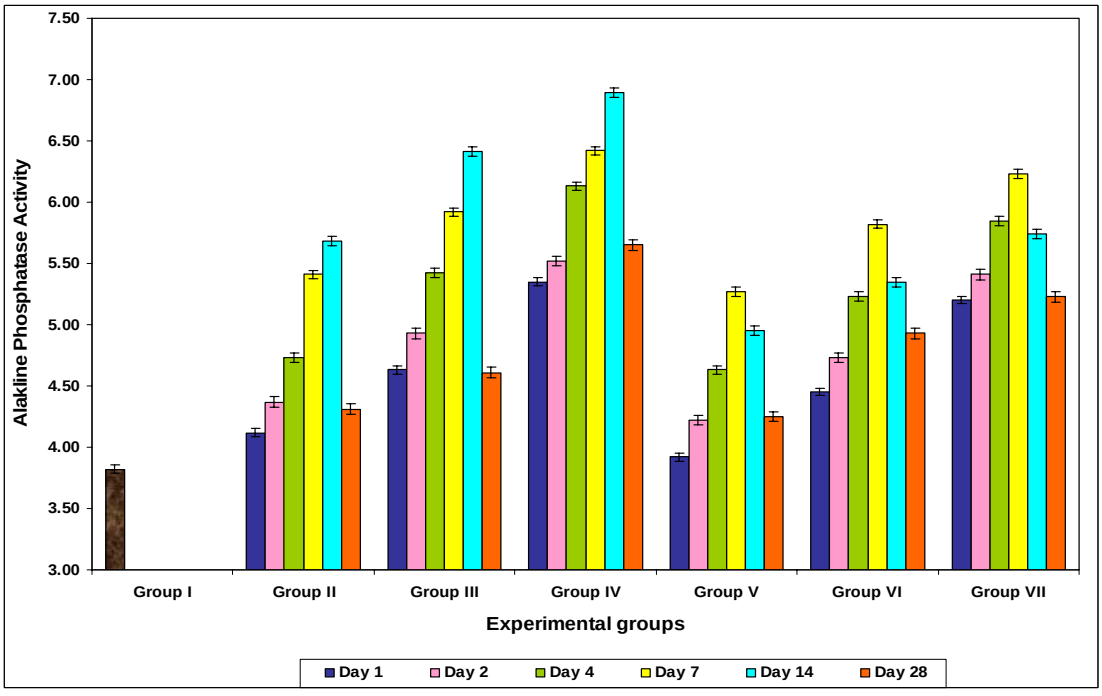
HISTOGRAM-4

Variations in the Acid Phosphatase Activity (mg pi/gm/hr.) in the brain of mice in various groups



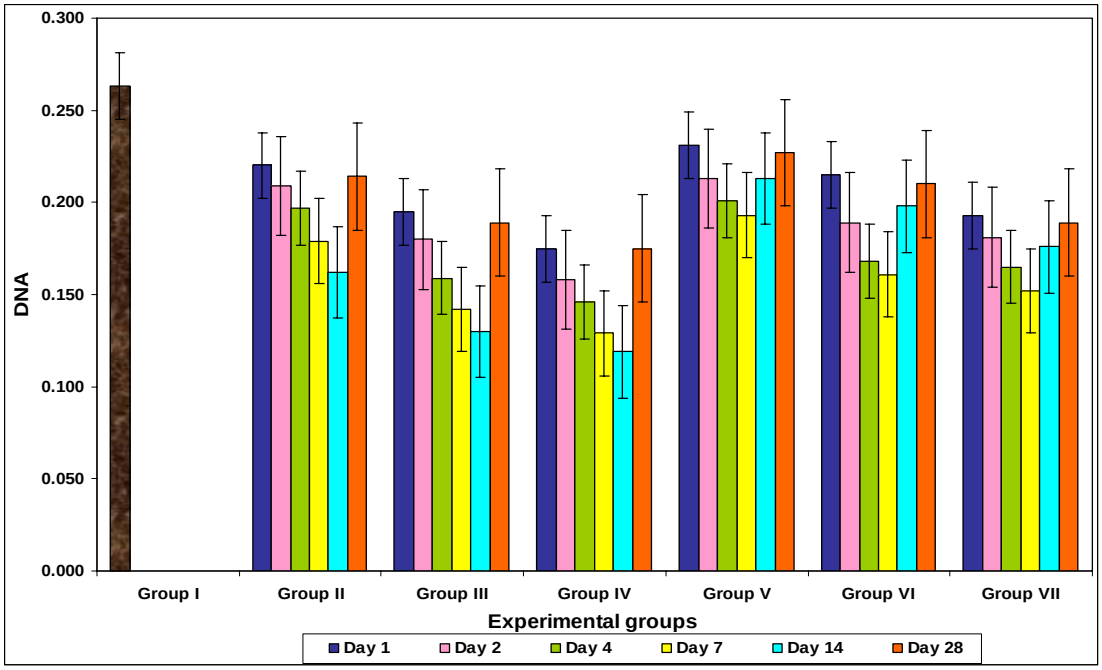
HISTOGRAM-5

Variations in the Alkaline Phosphatase Activity (mg pi/gm/hr.) in the brain of mice in various groups



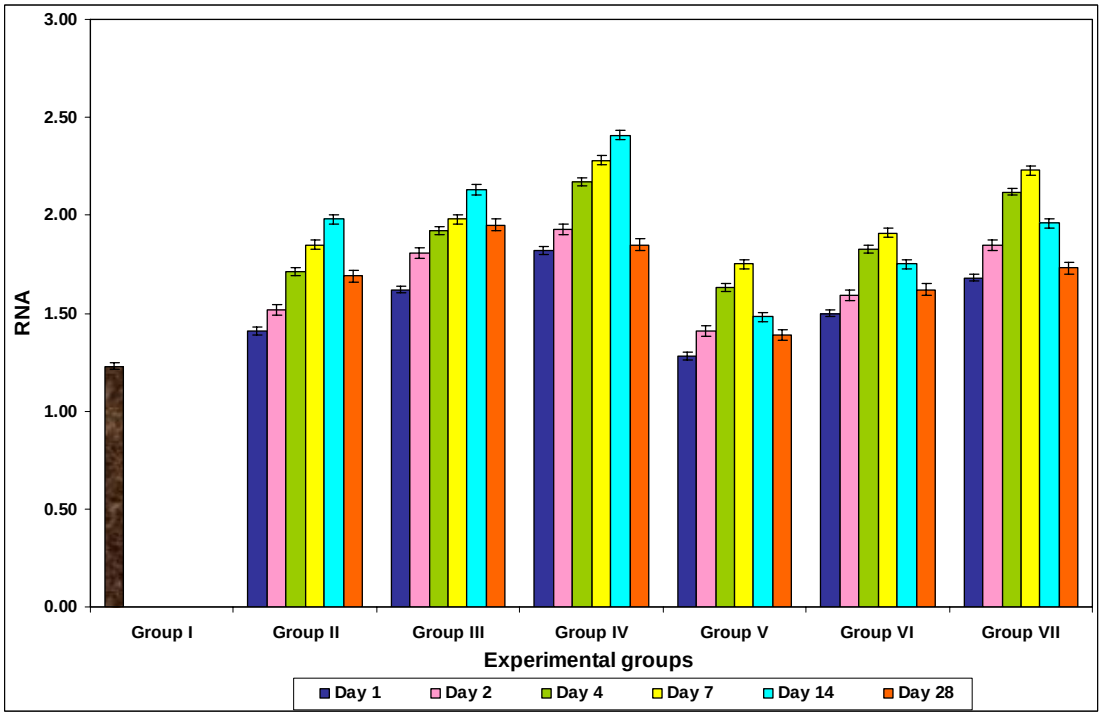
HISTOGRAM-6

Variations in the DNA content (mg/gm tissue weight) in the brain of mice in various groups



HISTOGRAM-7

Variations in the RNA content (mg/gm tissue weight) in the brain of mice in various groups



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