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MODULATORY INFLUENCE OF *ALOE VERA* AGAINST RADIATION AND CADMIUM INDUCED BIOCHEMICAL CHANGES IN THE BRAIN OF SWISS ALBINO MICE

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Abstract: Humans are exposed to ionizing radiations during diagnostic, therapeutic and industrial purposes. Apart from these human also get exposed to ionizing radiations during air and space travel, background radiation, nuclear accidents and use of electronic devices. Therefore it is essential to protect humans from ionizing radiation. Majority of plant and herbs have medicinal properties and they protect against the radiation induced damage by scavenging free radicals and increasing antioxidant status. *Aloe vera* has a long history of its use in medicinal, cosmetic and therapeutic, properties including anticancer effects. In this context, the present study will shed light upon the protective influence of *Aloe vera* against the deleterious effects induced by simultaneous exposure of radiation and cadmium in the brain of Swiss albino mice. For the purpose, six to eight weeks old male Swiss albino mice were selected and divided into seven groups:- Group I (Sham-irradiated), Group II (treated with cadmium chloride 20ppm), Group III (Irradiated with 3.0 Gy and 6.0 Gy gamma rays), Group IV (Both irradiated and treated with cadmium chloride solution), Group V (Cadmium chloride and *Aloe vera* treated), Group VI (radiation and *Aloe vera* treated), Group VII (radiation, and cadmium chloride and *Aloe vera* treated). The animals were sacrificed at each 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 *Aloe vera* treated groups and thereafter decreased up to 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 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. *Aloe vera* reduced the severity of damage and made the recovery process earlier and faster. 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 the protection provided by *Aloe vera*.

Keywords: Mice, Brain, Radiation, Cadmium, *Aloe vera*

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INTRODUCTION

Irradiation has been used increasingly in medicine to help in diagnosis and treatment. Its deleterious effects on biological systems are mediated through the generation of reactive oxygen species (ROS), oxidative stress, induced cytotoxicity, as well as metabolic and morphological impairments[1,2]. Ionizing radiation passing through living tissues generates reactive free radicals. These free radicals can interact with critical macromolecules, such as DNA, proteins or membranes and leads to depletion of endogenous antioxidants resulting in the development of cell damage and potentially cell dysfunction and death[3].

Cadmium is a rare element and found in the environment. Cadmium and its salts are used in industries such as electroplating, production of nickel cadmium batteries, paint, alloys, photocells, insectides and fungicides. Cadmium chloride is used in the production of cadmium yellow which finds its use in dyeing and printing.

Cadmium is released into the environment by human activities and finds its way into the soil, air and water. Cadmium has been found in foods, beverages, fish, meat, milk, eggs, cereals and vegetables[4]. Cadmium is an important environmental and industrial pollutant which accumulates in the body and has an extremely long half life. Chronic and acute cadmium exposure produce well documented pathological syndromes in humans exposed in certain industrial settings and through the environment[5]. Of the hazards associated with exposure to cadmium, anorexia has been reported in smelter employees[6]. Cadmium also produces learning disability and over-reactivity in children[7]. Cadmium exposure causes alterations in the brain neurotransmitter levels and behavior in both neonatal and adult animals[8]. A decreased spontaneous neural firing after administration of cadmium into cerebral cortex or brain stem[9] and a blocked synaptic transmission at peripheral cholinergic and synapses *in vitro* have also been demonstrated[10].

Membrane damage caused by the reactive species H_2O_2 and OH^-ions generated due to the exposure of living tissues to heavy metals may allow the entry excess calcium into cells with subsequent biochemical or micro molecules cellular degradation and necrosis. Calcium channel blockers act on ion conducting cell membrane channels; hence this class of agents may be included in a search for protector with a more favorable therapeutic index. Combined effect of ionizing radiation and other agents are of potentially great importance, because there are many occasions where interaction might occur in our environment.

Efforts were devoted to discover natural and synthetic radioprotectors which may be of great help for human application. Potential radioprotectors were tested to find more effective, less toxic drugs that

reduce the lethality induced by irradiation. Recently, focus has shifted to test the radioprotective potential of plants and herbs[11].

Aloe vera barbadensis, which belongs to the family Liliacae and consists of about more than 250 species. It is commonly called "Guar-patha" or Ghee-Guar. It is rich in vitamins A, E and C, zinc and selenium. It is reported to have antioxidant, antitumor, and anti-inflammatory properties[12].

Brain tissue is highly susceptible to oxidative damage due to its high utilization of oxygen and its poorly developed antioxidative defense mechanism[13]. Therefore, present study was planned to evaluate the modulatory influence of *Aloe vera against* radiation and cadmium induced biochemical changes in the brain of Swiss albino mice.

Materials and Methods

Animals

Healthy male Swiss albino mice {6-8 weeks old} were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar and maintained at 20-25 degree celsius.

The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum.*

Source of irradiation

The animals used in the experiment were irradiated at The Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner [Rajasthan] by Theratron, a Cobalt^{60} beam therapy unit which was a source, procured from Atomic Energy Agency Ltd., Canada . The animals were irradiated at the dose rate of 0.69 to 1.35 Gy/min.

***Aloe vera* Extract**

Fresh fruits of the *Aloe vera* were cleaned, cut into small pieces, air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hrs. (12 hrs. x 3). The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in DDW just before oral administration. An approximate 38% yield of the extract was obtained. The drug was given from seven days prior to Cadmium chloride treatment or irradiation.

Plan of Experimentation

The animals were divided into seven groups:

Group I: Sham-irradiated (normal

Group II: Cadmium chloride treated animals

Group III: Animals treated with radiation

Sub-group IIIa: 3.0Gy

Sub-group IIIb: 6.0Gy

Group IV: Both irradiated and treated with Cadmium chloride

Sub-group IVa: 3.0Gy+$CdCl_2$

Sub-group IVb: 6.0Gy+ $CdCl_2$

Group V: Animals treated with Cadmium and *Aloe vera*

Group VI: Animals treated with Radiation and *Aloe vera*

Sub-group VIa: 3.0Gy+Aloe vera

Sub-group VIb: 6.0Gy+ Aloe vera

Group VII: Animals treated with Radiation , Cadmium Chloride and *Aloe vera*

Sub-group VIIa: 3.0Gy+ $CdCl_2$+ Aloe vera

Sub-group VIIb: 6.0Gy+ $CdCl_2$+ Aloe vera

Autopsy of animals:

A minimum of five animals from each group was sacrificed after 1, 2, 4, 7, 14 and 28 days of treatment. Five Sham- irradiated mice were also be autopsied. The animals were sacrificed by cervical dislocation. Prior to autopsy the animals were weighed.

After sacrificing the animal the brain (cerebral cortex) was taken out it was blotted, weighed and kept at -20^{o}C for biochemical studies.

Biochemical Estimation: The following biochemical parameters were taken into consideration:

1. Total Proteins[14]
2. Glycogen[15]
3. Cholesterol[16]
4. Acid phosphatase activity[17]
5. Alkaline phosphatase activity[17]
6. RNA[18]
7. DNA[19]

Results

Radiation sickness

Animal exposed to 3.0 Gy and 6.0 Gy gamma radiations exhibited signs and symptoms of radiation sickness. These mice were found as lethargic and week. Food and water consumption was reduced, although general activities of such animals were apparently normal during all 20 days post-irradiation. Animals pretreated with *Aloe vera* extract (EOE) and later exposed to 3.0Gy and 6.0Gy gamma radiation did not show any sign and symptoms of radiation sickness. Moreover, a significant weight gain in these animals was observed as compared to control with normal food or water consumption.

Protein content- The value decreased up to day-14 in non drug treated groups II,III and IV and up to day-7 in *Aloe vera* treated groups V, VI and VII .Thus in *Aloe vera* treated groups, an early and fast recovery was observed showing protection by *Aloe vera* .

Glycogen content- 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 *Aloe vera* treated groups V, VI and VII the value rose up to day -7, then declined on day-28. After combined treatment synergistic changes were noted. Thus an early and fast recovery was seen in *Aloe vera* treated experimental groups.

Cholesterol content- Cholesterol content declined up to day-14 in Cadmium chloride treated group II and till day-7 in Cadmium chloride and *Aloe vera* treated group V. Thereafter, an increase in the value was seen up to day-28 in both groups. An increase in the value was noted up to day-14 in non-drug treated groups (III, IV) and till day-7 in *Aloe vera* treated groups (VI and VII). Thereafter an increase in the value was noted up to day-28 without reaching to the normal level.

Acid phosphatase activity- An elevation in the value of Acid phosphatase activity was observed up to day - 14 in groups II, III and IV, thereafter it decreased on day - 28. But the value increased only up to day - 7 in *Aloe vera* treated groups V, VI and VII, then it declined on day - 14 and continued so up to day - 28.

Alkaline phosphatase activity- An increase in the value of alkaline phosphatase activity in the brain of Swiss albino mice was observed up to day-14 in non drug treated groups II, III and IV, thereafter it decreased on day-28. In *Aloe vera* treated groups V, VI and VII the value increased upto day-7, then it declined on day-14 and continued so up to day-28.

RNA content - RNA content increased on day -1 and continued so significantly ($P<0.001$) up to 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 *Aloe vera* 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 up to day -28 but the difference in the value was significant($P<0.001$) as compared to the normal.

DNA content- Decrease in DNA content was noted up to day-14 in non-drug treated groups II, III and IV, thereafter it increased on day-28. In *Aloe vera* treated groups V, VI and VII the value declined up to day-7 after which it increased on day-14 and day-28.

Discussion

The decrease in protein synthesis in the brain is not due to malnutrition but resulted from direct toxic effect of heavy metals. On the other hand, it is believed that heavy metals cause the death of ribosomes by acting on membranes and also the cellular death, thus decreasing protein synthesis[20].

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 250Kv roentgen radiation and 32Mev photons respectively[21].

Reversibility of glycogen accumulation during recovery suggests that a reparative process is operating, since at this time tissue apparently the ability to metabolize glycogen deposits. Disappearance of glycogen surplus occurs at approximately the same time when recovery of brain functions occurs following roentgen irradiation[22-23].

Increase in glycogen content of the post-natal developing mouse brain after continuous exposure to tritium has also been reported[24]. 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[25].

The decrease in cholesterol level in present study might be due to the stress response caused by irradiation to stimulate synthesis of steroid hormones via hypothalamic-pituitary system. The decreased concentration of cholesterol might be due to increased ACTH secretion by pituitary leading to decreased cholesterol concentration[26] . Neurons appear to produce enough cholesterol to survive and to grow, but require external cholesterol to form a sufficient number of synaptic contacts. Since cells in the brain cannot access the cholesterol supply in the blood, they need to synthesize cholesterol by themselves. Glial cells have been found to produce surplus cholesterol and deliver it to nervous tissue via lipoproteins[27-28]. The decrease in cholesterol level in present study may be because of radiation induced free radicals that can damage the glial cells leading to depletion in cholesterol level.

A significant increase over normal in acid phsphatase activity was scored soon after $CdCl_2$ intoxication. However, the activity of such enzyme was later found to be declined significantly but the level remained above normal even at the last autopsy interval (i.e. 28th day). Similar elevation in ACP level has also been recorded after lead (Pb) or mercury (Hg) intoxication by others. Cadmium damage to lysosome by membrane lipid peroxidation, that causes release of hydrolytic enzymes like nucleases and acid phosphatase, which may account for an increase in the enzyme activity. Another possible reason for elevation in ACP may be the damage of liver after $CdCl_2$ intoxication[29].

Maximum depletion from normal in alkaline phosphatase enzyme activity was observed after 12 hrs. of irradiation. Alkaline phosphatase plays an important role in maintenance of cell membrane permeability and acts on monophosphoesters. The damage to cell membrane caused by radiation may be one of the reasons for decrease in activity

of alkaline phosphatase. Such decline in level may be attributed to the several lysosomal enzymes. Post-irradiation reduction in ALP may be due to damage of brush border cells and increased permeability of villi cells[30-32]. Such changes in ALP level in intestine can make changes in brain ALP level through blood. Lynn and Skinner observed a non-exponential loss of activity in alkaline phosphatase at centers of secondary importance for the enzymatic activity and there is a notable destruction of component amino acid residue during radiolysis[33]. Although the activation of lysosomal enzymes in tissues with interphase death is well documented, however information on lysosomal enzymes in liver, kidneys and brain are merged and often contradictory[34] . It is known that lysosomes from different cell types or even from the some tissue vary greatly in their susceptibility to damage by radiation[35].

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

1. More or less rapid cytolysis of large proportion of cells, and

2. 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

Ability of DNA to transcribe RNA is not affected quantitatively, but the length of the chain of RNA molecules reduces.

Increase in nuclear RNA polymerase activity may contribute to post-irradiation increase in cellular RNA.

Increased gonadotropin secretion after irradiation has been reported. This increased gonadotropin secretion may accelerate RNA synthesis after higher doses of radiation[36].

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. Ionizing radiations induce damage to cellular DNA, which is of prime biological significance. The type of damage includes strand breaks, base damage, elimination of bases and sugar damage [37].

Conclusion

Man is exposed to a number of toxic substances in the environment including radiation as well as to toxic metabolites and ROS generated within the body. From the present study it is obvious that *Aloe vera* prevent the toxic effects of ROS, there is likelihood that *Aloe vera* may exert an anti radiation influence in the body. So, it

would further pave way to the formulation of medicine against radiation and toxicity induced during radiotherapy. Owing to this property, the *Aloe vera* known for its functional properties can be further extended to exploit its possible application for various health benefits as nutraceuticals and food ingredient in radiotherapy to protect the normal tissue.

Aloe vera, a potent protein tyrosine kinase inhibitor increased protein, DNA content and maintained the normal levels of other biochemical parameters against the oxidative stress produced by radiation in normal tissue of mice. The results indicate that *Aloe vera* against radiation effect may pave way to the formulation of medicine in radiotherapy for normal tissue and possible against radiomimetic dry induced toxicity.

Result obtained from the present study indicate that the natural medicines found in *Aloe vera,* including antioxidant and other phytonutrients, substantially protect the cerebrum from radiation damage. However, further research is needed especially regarding the mechanistic aspect of this protection.

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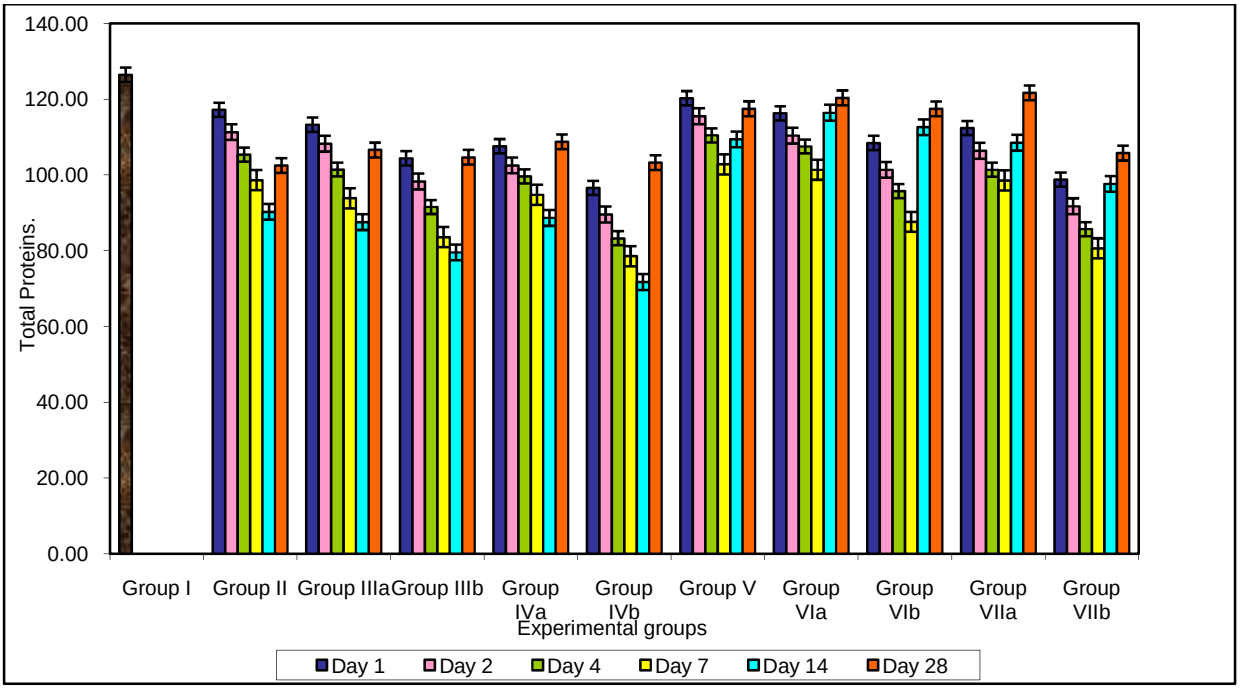
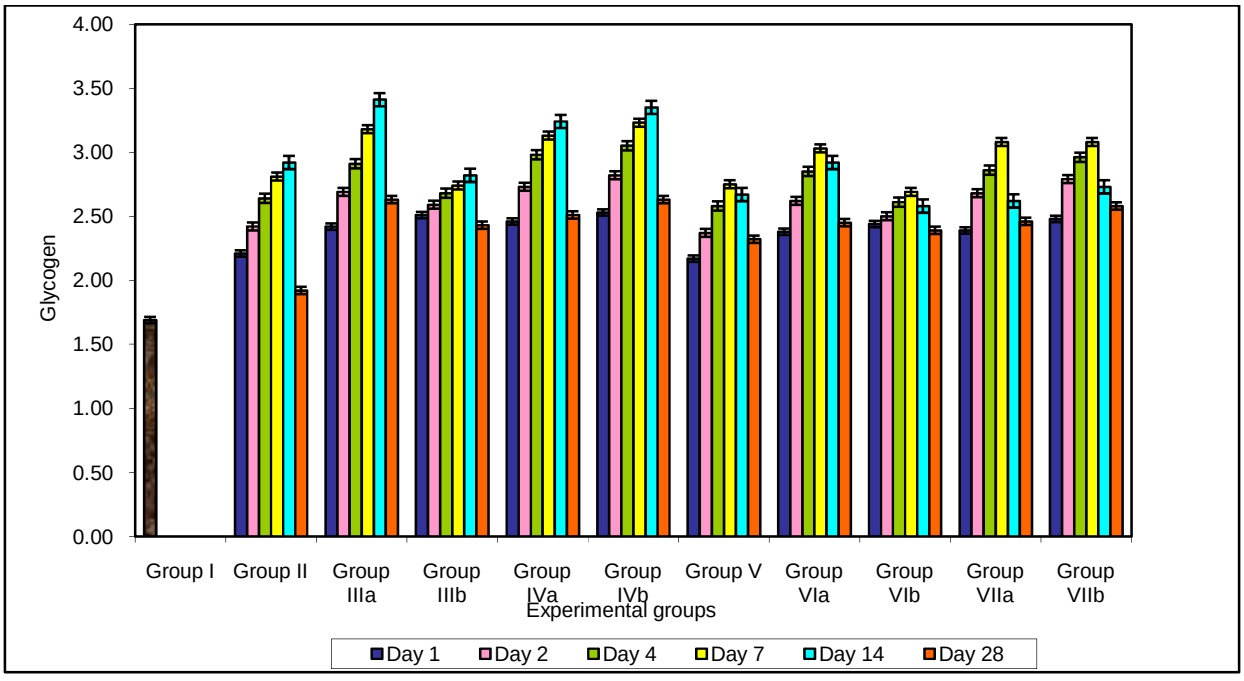
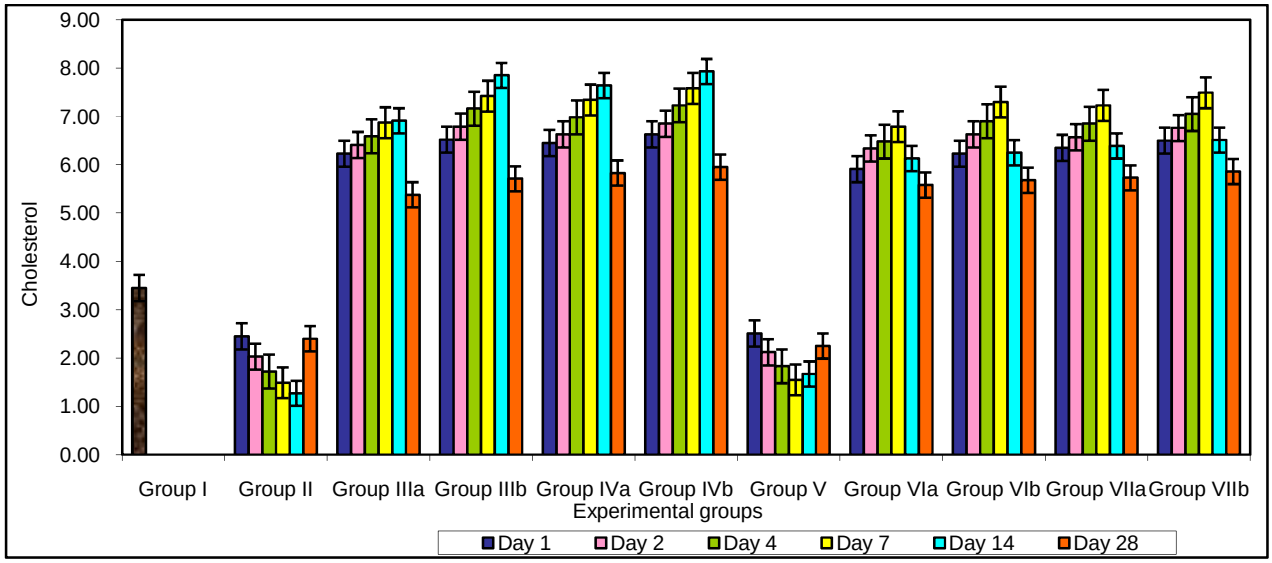
Histogram 1: Variations in the values of Total Proteins (mg/gm of tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



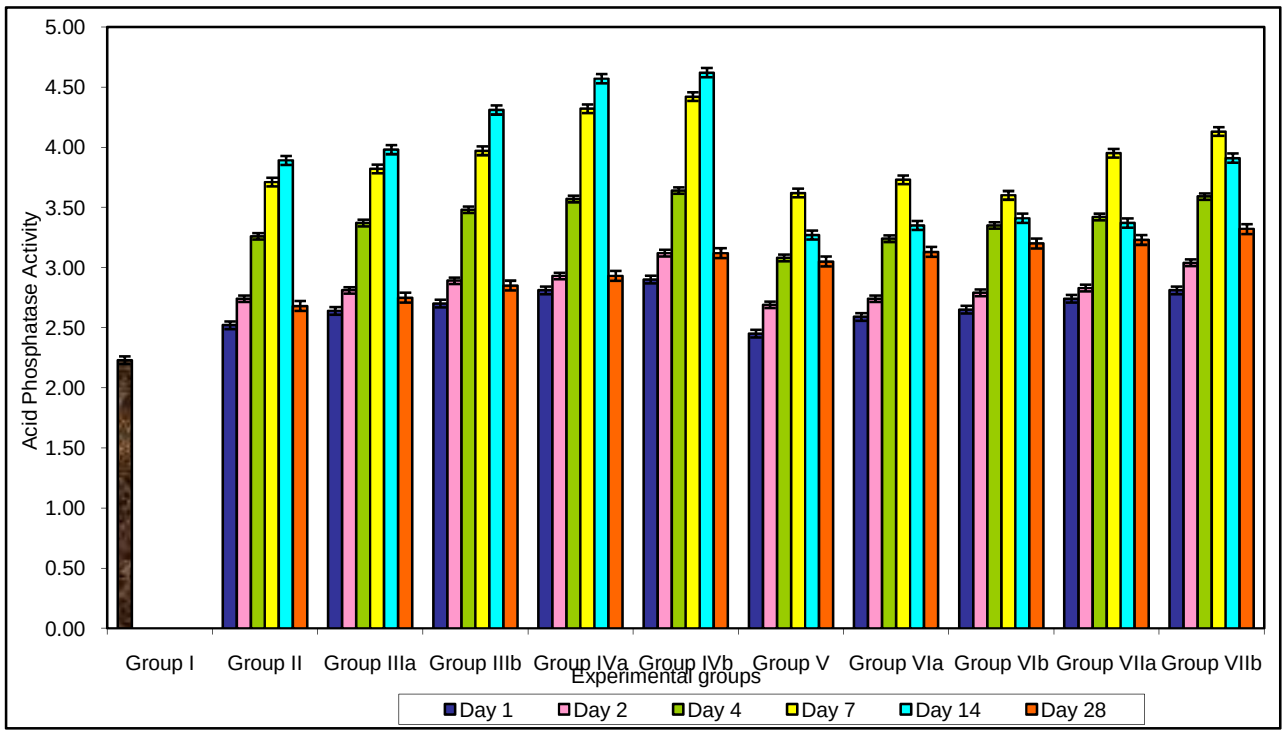
Histogram 2: Variations in the values of Glycogen (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



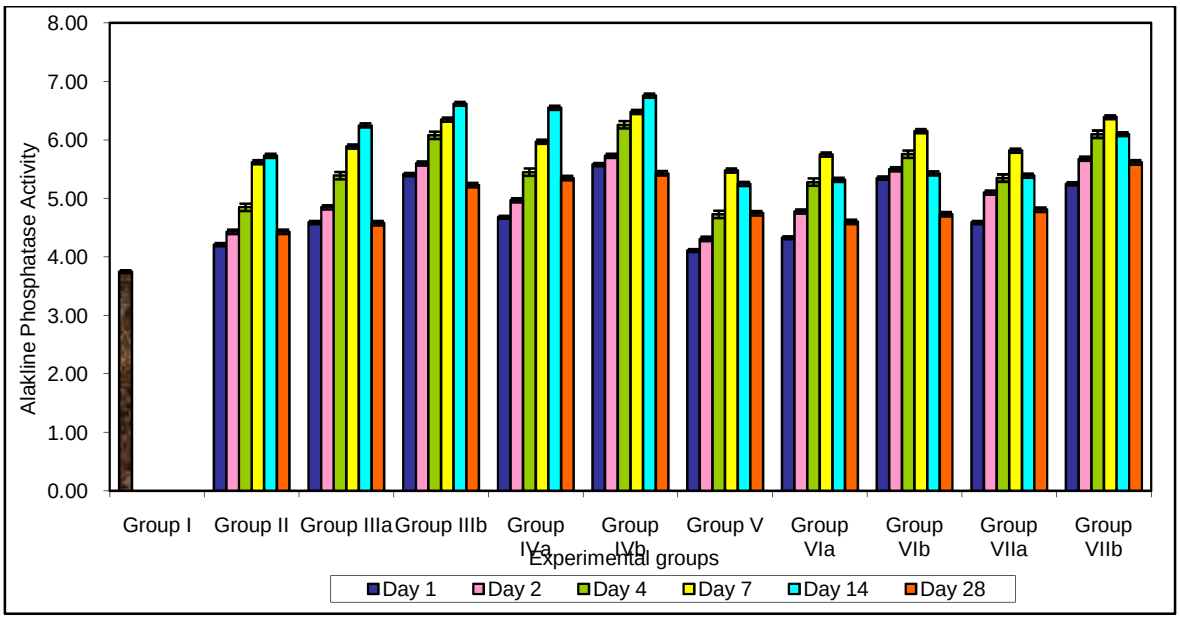
Histogram 3: Variations in the values of Cholesterol (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



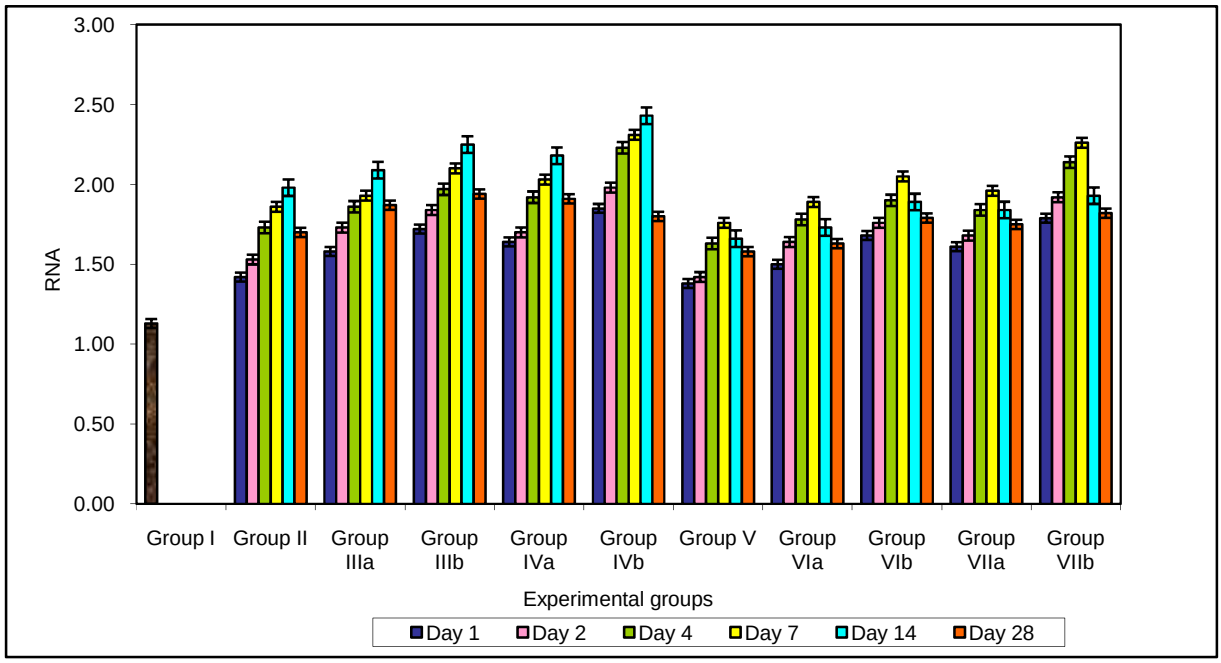
Histogram 4: Variations in the values of Acid Phosphatase activity (mg pi/gm/hr) in the brain of mice in various experimental groups (Mean ± S.E.)



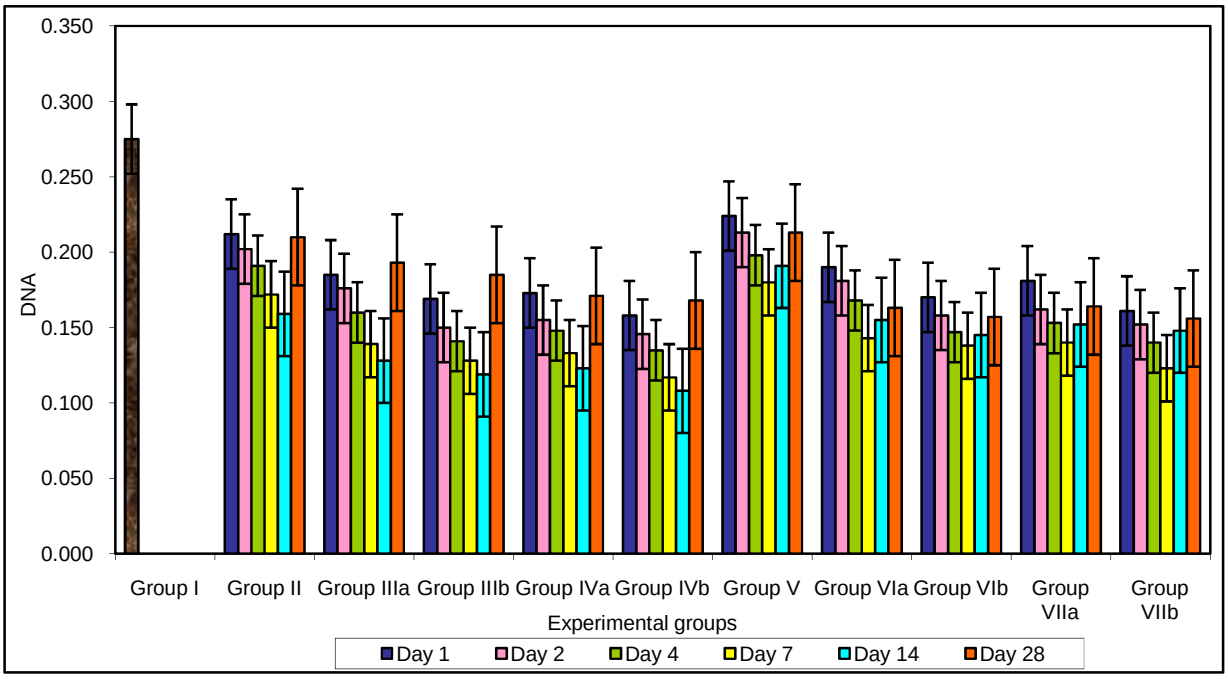
Histogram 5: Variations in the values of Alkaline Phosphatase activity (mg pi/gm/hr.) in the brain of mice in various experimental groups (Mean ± S.E.)



Histogram 6: Variations in the values of RNA (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



Histogram 7: Variations in the values of DNA (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



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