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Studies on the liver histology and biochemistry due to pesticide exposure in rats inhabiting vegetable crop fields

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ABSTRACT

Extensive and indiscriminate use of pesticides on crops poses a health hazard but scarce information is available on the effects of these pesticides on non-target mammalian species in field conditions. Rats were collected from the vegetable fields, sacrificed and the liver was excised for histological and biochemical analysis. The histological observations of the liver, showed slight loosening in the arrangement of hepatic cords around central vein, the loss of radial arrangements of hepatocytes and areas where normal parenchyma was replaced by large blood filled spaces. The mean diameter of central vein of (17.21μm) and (16.15μm) in male rats and (15.70μm) and (17.00μm) in female rats showed significant increase as compared to control during winter and summer seasons respectively. The total protein and total lipid content in the liver of female rats increased significantly, while the level of cholesterol increased significantly in the liver of males during summer season. During the winter season there was a marked increase in Acid Phosphatase (ACP) activity in male and female field rats. ACP activity is essential in the formation of ATP as an energy source, and an alteration in the enzyme may be attributed to cellular leakage causes by chemical induced stress of the tissue. The results indicate that the persistent exposure to a variety of pesticides coupled with environmental conditions caused aberrations in liver structure and function leading to physiological stress through biochemical pathways. There is a strong indication of these variations being gender specific and environmental dependent.

KEYWORDS: Enzymes, Histology, Liver, Microsomal Degranulation, Pesticides, Rat

I. INTRODUCTION

Indian agricultural economy under the impact of Green Revolution witnessed a sharp increased productivity of food grains, leading to a possibility of assured food security and higher income. In the course of time, the need was felt to initiate crop diversification in Punjab [1] with special reference to India is the second largest producer of vegetables in the world, next to China. With 8.495 million hectares under vegetable cultivation, India produced around 146.55 million tons of vegetables [2]. In order to ensure decreased pest damage and subsequent increased crop productivity, farmers use large quantities of chemical pesticides [3]. These pesticides are persistent and contribute variously to environmental degradation, biological magnification and accumulation leading to adverse health effects affecting the nervous system, lungs, reproductive organs, and dysfunction of the immune and endocrine system, birth defects and there are reports of even cancer development in humans [4]. In the present study, rats were collected throughout the year from the fields of vegetable crops and examined the changes in their liver biochemistry and histology.

II. MATERIALS AND METHODS

A. Collection of animals: The present study was carried out on Bandicota bengalensis, which was the predominant species in the vegetable fields of Doraha (30°81'N; 76°01'E), 22 kilometers East of Ludhiana, India. The rats trapped from the field area of Punjab Agricultural University, Ludhiana, were considered as control. Wonder traps were used to collect rats from both the study areas once a

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month during both winter and summer seasons throughout the year and brought to the laboratory. The rats were anaesthetized humanely, sacrificed, liver excised and disposed off safely before the analysis was done.

B. Tissue processing, sectioning and staining: The liver was fixed in alcoholic Bouin's fluid and the tissue was processed for histological sections as per standard procedure. The sections were cut at 5µm thickness and were stained in haemotoxylin – eosin; mounted in DPX as per standard procedure [5].

C. Evaluation of Slides

The liver tissue of both male and female rats of control and from field samples was examined histologically for sinusoidal dilations, mononuclear cell infiltration, pyknosis and necrosis of central vein. The diameter of hepatocytes, hepatic nuclei and central vein was measured using stage micrometer, duly calibrated using an ocular micrometer.

D. Biochemical parameters: The total protein [6], total lipids [7], phospholipids [8], cholesterol [9] were assayed as per the methods mentioned. Enzyme activity of Acid Phosphatase (ACP), Alkaline Phosphatase (AKP) was estimated by [10] method and microsomal degranulation was determined by [11] was also determined.

E. STATISTICAL ANALYSIS: The observations were analyzed by statistical parameters using the Student's t-test and statistical differences with a value of $p \leq 0.05$ were considered significant.

III. RESULTS AND DISCUSSION

A. Organ Weight

The study revealed that there was a significant decrease in weight of liver in female rats as compared to control rats (Figure 1). Loss of weight in liver of control rats during the both agricultural seasons studied throughout the year in both male and female rats (Figure 2).

The compact arrangement of hepatic cords was loosened around central vein which was dilated in all the rats. The loss of radial arrangements of hepatocytes was accompanied with many areas showing hepatocytes with dense and pyknotic nuclei. Areas of haemorrhage resulting in large blood filled spaces where the normal parenchyma was recorded (Plate 1, Figure C-F).

B. Morphometry

The mean diameter of hepatocytes was (3.75µm) and during winter and (3.90µm) and in summer season in male rats (3.95µm) and during winter season while it was (3.98µm) in summer season in female rats. Similarly, mean diameter of hepatic nuclei (1.25µm) during winter season and (1.28µm) during summer season in male rats and in female rats (1.26µm) during winter season and (1.30 µm) during summer season showed non-significant decrease as compared to control but mean diameter of central vein of (17.21µm) and (16.15µm) in male rats and (15.70µm) and (17.00µm) in female rats showed significant increase as compared to control during winter and summer season respectively (Figure 3 and 4).

C. Biochemical Assay

Total proteins and Total lipids

The total protein content in liver showed non-significant decrease in male rats during the year while there was a non significant decrease in protein content during winter season and non significant increase during summer season in female rats (Figure 5).

Total lipid content in liver of male and female rats showed non-significant increase during both seasons (Figure 5) whereas the lipid content of the liver of female control rats showed significant increase in winter season ($p \leq 0.05$) as compared to summer season (Figure 6).

Cholesterol

The concentration of cholesterol was significantly increased during summer season in males and during winter season in female while there was significant decrease in cholesterol content of male field rats of winter from summer season but in females there was significant decrease in cholesterol

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content in control rats and while there was a non-significant decrease in cholesterol level field rats of winter season from that of summer season (Figure 5 and 6).

Phospholipids

A non significant increase in phospholipids was observed in both males and females of the species, but seasonal variations could be a factor. However, the male control rats and field rats of winter season showed significant decrease in phospholipids content from summer season (Figure 5 and 6), indicating effect of environmental conditions.

Acid and Alkaline Phosphatase

There was a marked increase in Acid Phosphatase activity (ACP) in male field rats and a non-significant increase in Rabi season but in control rats of winter season ACP activity decreased from that observed in summer season (Figure 5 and 6). However, the activity of ACP showed a significant decrease in winter season (Figure 5 and 6), again attributable to seasonal variations.

No significant difference was observed in activity of AKP in the liver of male rats as compared to control rats during winter and summer season. The control and field rats showed significant decrease in AKP activity between cropping seasons of winter from summer season (Figure 5 and 6). In females the field rats of winter season showed significant increased in AKP activity. The field rats and control rats of winter showed significant increased in AKP activity from summer season (Figure 6)

Microsomal Degranulation

The microsomal degranulation test conducted on liver of all field rats revealed a seasonal variation in male rats from field having a value more than 5% in winter season, while the females from the field tended towards more than 5% value during the summer season as compared to control rats (Figure 7 and 8)

During the present study it was revealed that there was a decrease in weight of liver in both male and female rats during different seasons throughout the year. Organ weights are usually potential indicator of the effects of diet of the organism and liver is the most important detoxifying organ of the body. The variation in weight of liver is indicative of changed physiology due to a sustained and chronic exposure to pesticides or toxins. Liver plays an important role in metabolism to maintain energy level and structural stability of body. It is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduced toxicity. Any decrease in liver weight is considered as sensitive risk parameter, leading to reduce toxicity as postulated by earlier workers [12, 13 and 14]. An earlier study, [15] reported that there was no significant change in weight of liver in rats at all doses of pyrethroid administered. The present study revealed that there was significant decrease in weight and also there was change in histological and biochemical results are pointers to some physiological function disorders. Effect of environmental conditions and seasonal variation seems to be of importance as the organ weights have considerably decreased in summer as compared to winter season, even in control group of rats which were trapped from areas following good agricultural practices. A decrease in the weight of visceral organs was reported by [16] in rats housed at 28°C. The decrease in weight of liver of female rat during the present study is in agreement with the report of [17] who recorded a significant decrease in weight of liver of female rats when fed cypermethrin at 50 mg/kg body weight continuously for a period of 2 and 4 weeks. Thus the modes of action of pesticide results are in accordance with field and laboratory studies. It was reported by [18] that mice intoxicated with diazinon resulted in hydropic degeneration, necrosis and focal micro vesicular steatosis in liver. The liver of male Wistar rats chronically treated with sub lethal doses of diazinon sustained a form of hepatic injury characterized by cellular lipid accumulation by [19, 20]. Further, [21] showed that diazinon induced liver and kidney damage in ruminant livestock. Hyperplasia of hepatocytes, necrosis, lymphocytic infiltration and steatosis were observed in rats treated with 1/20 LC_{50} of diazinon [22]. [23] injected (150-200gm) with 200mg/kg body weight of carbaryl intraperitoneally, five days a week for thirty days in adult Wistar rats,

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showed a statistically significant increase in the mean long and short diameters in the experimental rats (28.02 ±4.31 and 19.83 ± 3.94 microns, respectively), as compared to that of the controls (22.04 ± 3.64 and 12.29 ± 2.98 microns, respectively), indicative of an increased in cellular activity. Increased in size of hepatocytes and with dense and pyknotic nuclei was observed following administration of 150-200 gm carbaryl with 200 mg/kg body weight intraperitoneally, 5 days a week for 30 days [24].

Decrease in protein content cause delayed development of the male reproductive system and decreased fertility [25]. [26] reported that the male rats treated with chlorpyrifos-ethyl (9.60 mg/kg b.wt.), chlorpyrifos-methyl (300 mg/kg b.wt.) and methomyl (1.70 mg/kg b.wt.) of repetitive oral doses for 90 days showed a significant decrease in total protein content which might be due to an increase in glucose conception by the tissue and an increase in gluconeogenesis as recorded earlier by [27]. The increased in total lipid content might be due to lipogenesis which reflects abnormal carbohydrate metabolism. It led to excessive conversion of pyruvate to free fatty acid. Increased cholesterol is likely to have substantially contributed to the total lipid levels in treated rats [28, 29]. The study by [30] reported that lipid metabolism was enhanced in heat-stressed pigs which were housed at 31°C and showed increase of 26% in lipid content. The present study was in agreement with [31] who reported increase in cholesterol level in liver after administration of Cypermethrin and beta-cyfluthrin orally of LD50 (416.98 mg kg-1 and 354.8 mg kg-1 body weight, respectively) in Wistar rats. Cholesterol increase in the liver might be due to inhibition in the activity of enzymes involved in cholesterol breakup results into deposition of cholesterol in the cell .The increase in cholesterol level indicates inhibitory action of pesticide on Cyt-p-450 enzymes [32, 33, 34] or might be due to high affinity binding [35]. The effect of temperature on phospholipids was studied in an earlier study by [36] and an increase in phospholipids content was observed when rats were exposed to 27°C for 1 or 5 months. Acid phosphatases are enzymes capable of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular acid phosphatase gene is up-regulated by androgens and is down-regulated by estrogens [37]. Activity of marker enzyme acid phosphatase is considered to be a functional indicator of spermatogenesis [38]. Organophosphorus pesticides (Monocrotophos, methyl parathion) are known to cause significant elevation of liver acid phosphatases [39]. The present study was in agreement with the results of [40] who reported that treatment with 48 mg/ kg / day carbosulfan for 20 days caused no change in the activity of ACP in female mice. The change in AKP activity suggested the effect on absorptive or secretory surface of the cell membrane causing cellular leakage as indicated elevated AKP activity as an adaptive rise in enzyme activity to the persistent stress [41, 42].

Degranulation of microsomes value in liver being more than 5%, the chemical having a potential for carcinogenicity as the ribosomes get released from rough endoplasmic reticulum. Rate of metabolism of pesticide is determined by various factors, which determine the rate of entry of pesticides into endoplasmic reticulum of the liver, the major site of detoxification.

The present study has revealed that a population inhabiting vegetable crop fields is being affected by constant, persistent and chronic exposure to insecticides as the reports on experimental animals revealed. It is a mirror to reveal the level of physiological effect of insecticide toxicity on target organisms. The exposure to the pesticide cocktail in field conditions and their residual effect on the food chain are of utmost importance to bring forth the precautions to be undertaken while marketing the product. Despite the mobility of rats, the pesticide application and their widespread usage of pesticides, has a synergetic effect leading to stress conditions in rats.

IV. CONCLUSIONS

The study brings forth the adverse effects of a cocktail of insecticides which have been used on vegetable crops during the winter and summer seasons. The liver is the main organ for detoxification in the body which is seriously affected. The enzyme activity strongly correlated the histological findings. However, there seems to be variations in the enzyme activity of the liver of the rats in

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different seasons and in different gender. The >5% level of microsomal granulation in liver is a positive indicator of the stress caused by insecticides on the organs. This is the first study of its kind on the effect of the insecticides in field conditions in India and it strongly favours the judicious use of insecticides on vegetable crops. Such a study if extrapolated for environmental monitoring in relation to human health could lead to recommendations consequent to policy decisions.

BIBLIOGRAPHY

[1] Karp, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by network of European laboratories. Mol. Breed., 3: 381–390.
[2] Sidhu, K.; Kumar, V.; Dhillon, T.S. 2010. An analysis of vegetable cultivation in Punjab. J. of Life Sci., 2: 37-42.
[3] Dastagiri, M. B.; Chand, R.; Immanuelraj, T. K.; Hanumanthaiah, C. V.; Paramsivam, P.; Sidhu, R. S.; Sudha, M.; Mandal, S.; Singh, B.; Chand, K. 2013. Indian vegetables: production trends, marketing efficiency and export competitivenss. American J. of Agri. and Forestry 1: 1-11.
[4] Goel, A. and Aggarwal, P. 2007. Pesticide poisoning. National Medical J. of India 20: 82-91.
[5] Yadav, S. K. 2010. Pesticide applications- threat to ecosystem. J. of Human Ecol. 32: 37-45.
[6] Humason, G. L. 1972. Animal tissue techniques 3rd edn W H Freeman, San Francisco California.
[7] Lowry, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, A. J. 1951. Protein measurement with folin phenol reagent. J. of Biol. Chem. 193: 265-275.
[8] Folch, I.; Leas, M. and Sloanestansley, G. H. 1957. A simplified method for isolation and purification of total lipids from animal tissue. J. of Biol. Chem. 226: 197-209.
[9] Ames, B. N. 1966. Estimation of phospholipids. In: Neufeld E F and Griessburg V (ed) Methods Enzymol. Pp 11. Academic Press, New York.
[10] Chiamori, N. B. S. and Henry, R. J. 1959. Study of ferric chloride method for determination of total cholesterol and cholesterol esters. American J. of Clinical Pathol. 31: 305-309.
[11] Bessey, O. A.; Lowry, O. H. and Bruck, M. J. 1946. A method for the rapid determination of alkaline phosphatase with five millimeters of serum. J. of Biol. Chem. 164: 321-329.
[12] Hodgson, E. A. 2004. Textbook of modern Toxicol. Pp.203-211. John Wiley and Sons, Inc, New Jersey.
[13] Guyton, A. C. and Hall, J. E. 2006. Textbook of Medical Physiology, 9th Ed., Prism Book (Pvt) Ltd., Bangalore, India. XLIII, 1148.
[14] Gathwan, K. H.; Karkhi, I. H. T. and Yaseen, A. K. 2013. Organophosphororus insecticides induced alterations in liver of male albino rat. Chemical Sci. Transactions 2: 95-98.
[15] Varshneya, C. ; Singh, T.; Sharma, L. D.; Bahga, H. S. and Garg, S. K. 1992. Immunotoxic responses of cypermethrin, a synthetic pyrethroid insecticide in rats. Indian J. of Physiol. and Pharmacol. 36: 123-126.
[16] Sangha G.K, Kaur K, Khera K.S and Singh (2011) Toxicological effects of cypermethrin on female albino rats. Toxicology International 18, 5-8.
[17] El-Shenawy N S, Al-Eisa R A, El-Salmy F and Salah O (2009) Prophylactic effect of vitamin E against hepatotoxicity, nephrotoxicity, haematological indices and histopathology induced by diazinon insecticide in mice. Current Zoology 55:219-26.
[18] Jacqueson A M, Thevenin J M, Warnet J, Claude R and Truhart R (1977) Sex influence on the experimental fatty liver induced by white phosphorus and amanita phallorides in the rat. Acta Pharmacology and Toxicology 4:322-29.
[19] Anthony J, Banister E and Oloffs P C (1986) Effect of sublethal levels of diazinon: Histopathology of liver. Bulletin of Environmental Contamination and Toxicology 37:501-07.
[20] Abdel-Tawab H. Mossa and Moustafa A. Abbassy (2012) Adverse Haematological and Biochemical Effects of Certain Formulated Insecticides in Male Rats. Resesrch Journal Environmental Toxicology 6, 160-68.
[21] Hassan S A, El-Shawaf I M, El-Ghazaly A and El-Azab S M (2007) Ozone administration ameliorates different chemically induced hepatorenal chronic toxicity in rats : a histopathological study. Mansoura Journal of Forensic Medicine and Clinical Toxicology 15:57-67.
[22] Munglang M, Nagar M and Prakash R (2009) Liver in carbaryl treated rats- a morphological and morphometric study. Journal of Anatomical Society of India 58,6-9.
[23] Hamid S, Mahajan R, Singh H (2012) Carbaryl, a pesticide causes “toxic hepatitis” in Albino Rats. Journal of Cytology and Histology 3,149.
[24] Zambrano E , Gonzalez G L, Guzman C, Bercerra G R, Boeck L, Diaz L, Menjivar M, Larrea F and Nathanielsz P W (2005) A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. Journal of Physiology 15, 275–84.
[25] Zama D, Meraihi Z, Boubekrii N, Amrani A, Tebibel S and Baali N (2005) Assessment of changes in some diagnostics enzymes and other parameters in wister albino rats treated with pesticides during gestation. Sci Tech 23:51-6.
[26] Saxena P N and Doneriya R (2004) Hepatobiochemical response in albino rat following oral administration of cybil and hafen. Toxicology International 11, 23-6.

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[27] Saxena P N and Kumar K (2001) Assessment of liver lipid profile in female rats after diazol intoxication. Indian Journal of Environmental Toxicology 11, 71-2.
[28] Kouba M, Hermier D, Dividich J Le (2001) Influence of a high ambient temperature on lipid metabolism in growing pig. Journal of Animal Science 79, 81-7.
[29] Bhushan B, Saxena N P and Saxena N (2013) Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin. Arhiv za Higijenu Rada i Toksikologiju 64, 57-67.
[30] Shivanandappa T and Krishnakumari M K (1981) Histochemical and biochemical changes in rats fed dietary benzene hexachloride. Indian Journal of Experimental Biology 19, 1163-68.
[31] Siddiqui MKJ, Anjum F, Qudri SSH (1987) Some metabolic changes induced by endosulfan in hepatic and extra hepatic tissues of rat. Journal of Environmental Science 22, 553-64.
[32] Stott, Ian, Anupam M, Alex R, Norman W T and Jeffrey R F (1997) Low dose ithiocarbamate attenuates the hepatotoxicity of 1, 3- dichloro- 2- propanol and selectivity inhibits CYP2E1 activity in the rat. Human Experimental Toxicology 16, 262-66.
[33] Zarh J A, Bruschweiler B J and Schlatler J R (2002) Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14α- demethylase and aromatase. Environmental Health Perspect 111, 255-61.
[34] Vaughan A D, Hannon P J, Vaughan N L (1958) Associated effects of diet,environmental temperature and duration of exposure on the major constituents of the livers of rats. American Journal of Physiology 194, 441-45.
[35] Yousef GM, Diamandis M, Jung K, Eleftherios P (2001) Molecular cloning of a novel human acid phoshatase gene that is highly expressed in the testes. Genomics 74, 385-95.
[36] El-Kashoury A A (2009) Influence of subchronic exposure of Profenofos on biochemical markers and microelements in testicular tissue of rats. Nature Science 7: ISSN 1545-0740
[37] Gathwan K H, AL-Karkhi I H T and Yaseen A K (2013) Organophosphororus insecticides induced alterations in liver of male albino rat. Chemical Science Transactions 2, 95-8.
[38] Ksheerasagar R L and Kaliwal B B (2006) Histological and biochemical changes in the liver of albino mice on exposure to insecticide, carbosulfan. Caspian Journal of Environmental Science 4, 67-70.
[39] Murphy SD and Porter S (1966) Effects of toxic chemicals on some adaptive liver enzymes, liver glycogen and blood glucose in fuster rats. Biochemal Pharmacology 15, 1655-76.
[40] Kackar R, Srivastava M K and Raizada R B (1997) Induction of gonadal toxicity to male rats after chronic exposure to mancozeb. Industrial Health 35, 104-11.
[41] Mishra V K, Shrivastava M K and Raizada R B (1998) Testicular toxicity in rat to repeated oral administration of tetramethyl thiuram disulfide (Thiram). Indian Journal of Experimental Biology 36, 3.
[42] Purchase I F H, Jongstoff E, Ahby J, Styles J A, Anderson A, Lefevre P A, Westwood F R (1978) An evaluation of 6 short-term tests for detecting organic chemical carcinogens. British Journal of Cancer 37, 873-903.
[43] Huston D H (1981) The metabolism of insecticides in man. In: Hutson DH and Roberts TR (ed) Progress in pesticide biochem. Vol 7, pp.287-333. John Wiley and Sons, NY.

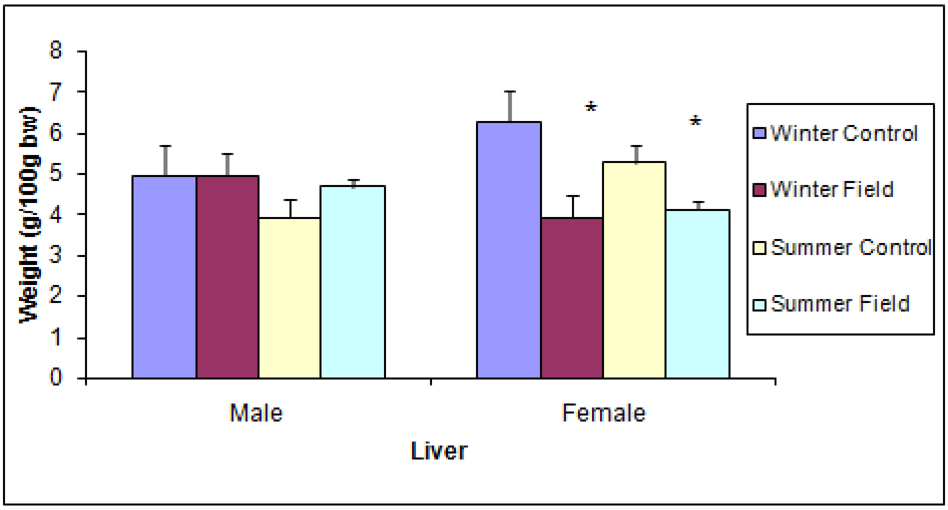

Figure 1: Weight of liver of male and female rats

[*Significant difference at ($p \leq 0.05$) as compared to control]

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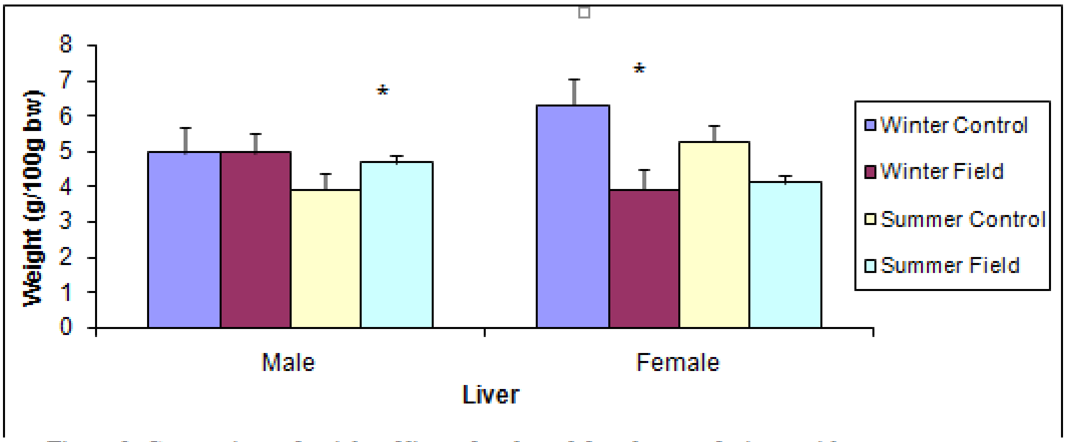

Figure 2: Comparison of weight of liver of male and female rats of winter with summer season

[*Significant difference at (p≤0.05)]

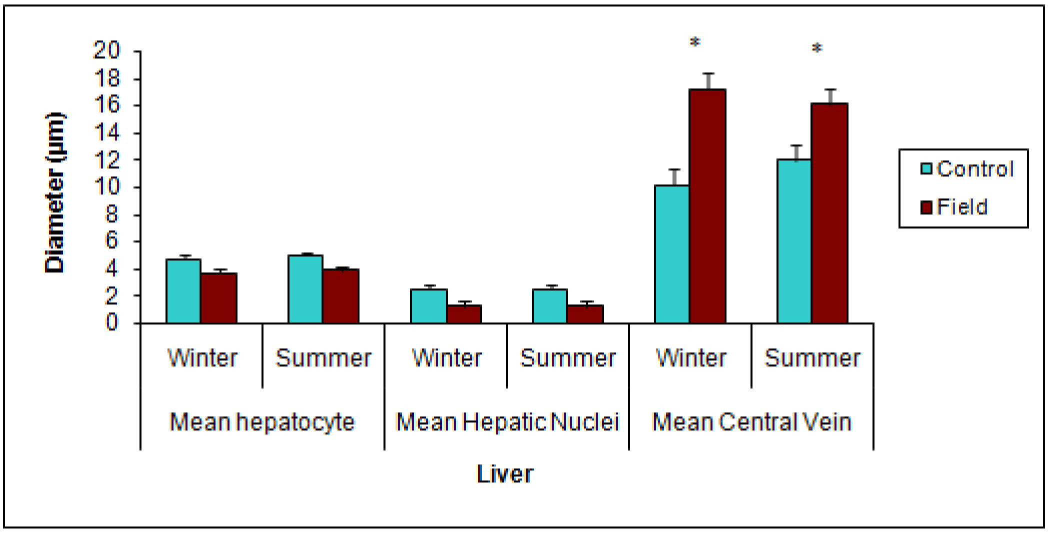

Figure 3: Diameter (µm) of hepatocyte, hepatic nuclei and central vein of male rats [*Significant difference at (p≤0.05)]

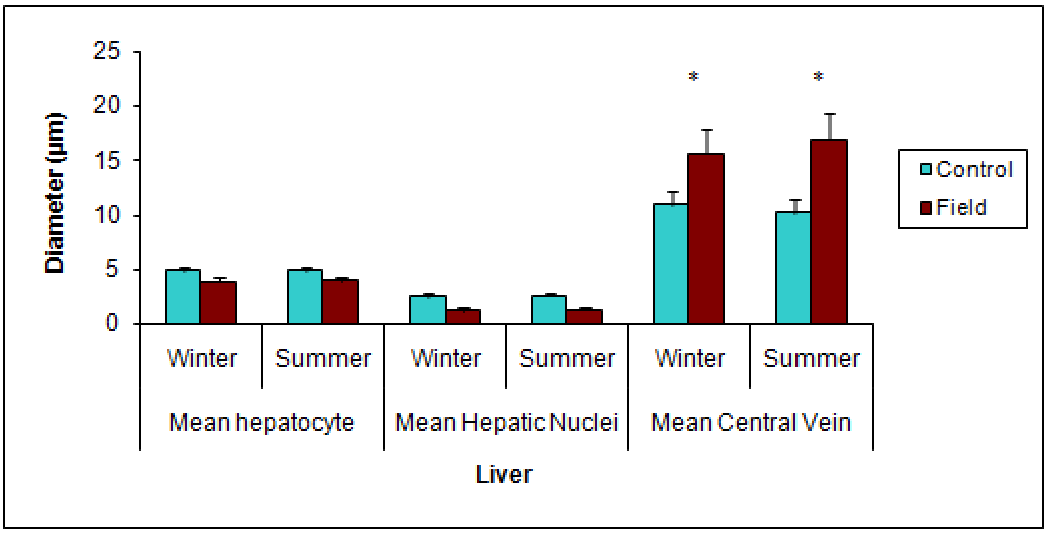

Figure 4: Diameter (µm) of hepatocyte, hepatic nuclei and central vein of female rats [*Significant difference at (p≤0.05)]

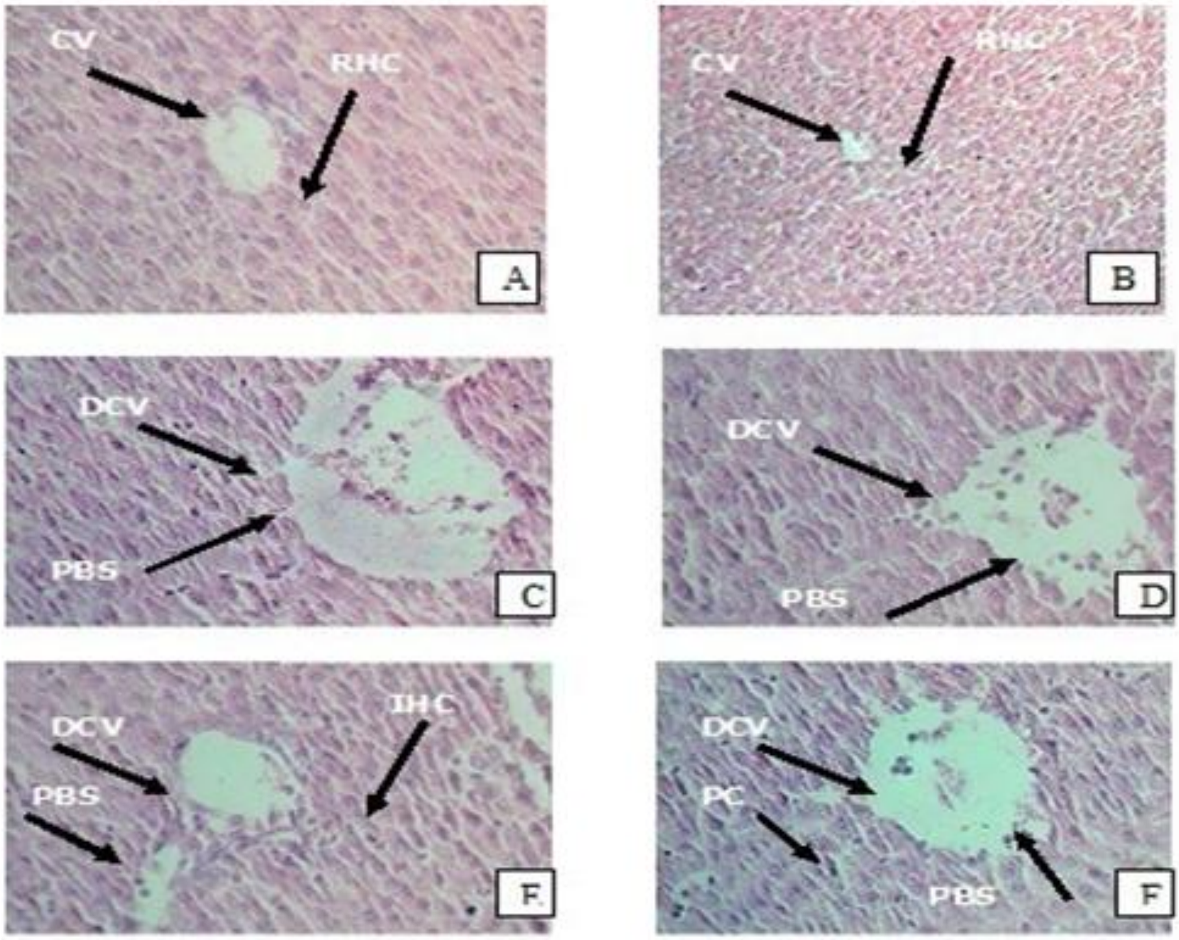

Plate 1, Figure: A-B T.S of liver of control male and female rats showing radially arranged hepatic cords (RHC) around central vein (CV) (X400)
Figure: C-D T.S of liver of male and female of field rats of Winter season showing dilation of central vein (DCV) and normal parenchyma replaced by large blood filled spaces (PBS) (X400)
Figure: E-F T.S of liver of male and female of field rats of summer season showing dilation of central vein (DCV), irregularly arranged hepatic cords (IHC), Pyknotic cells (PC) and PBS (X400)

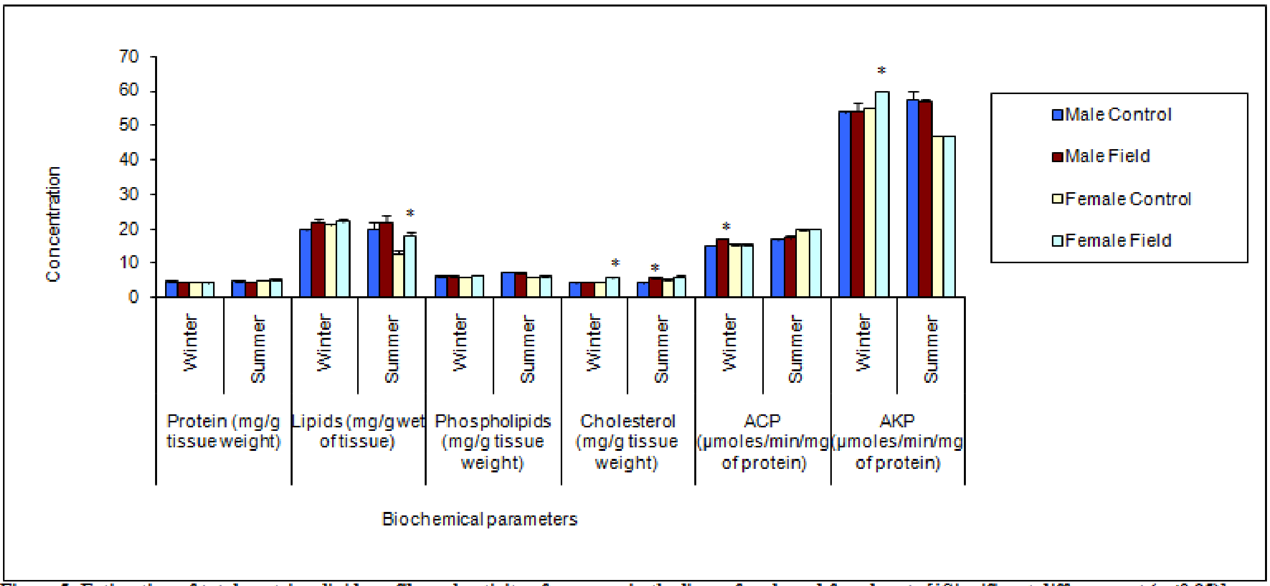

Figure 5: Estimation of total proteins, lipid profile and activity of enzymes in the liver of male and female rats [*Significant difference at ($p \leq 0.05$)]

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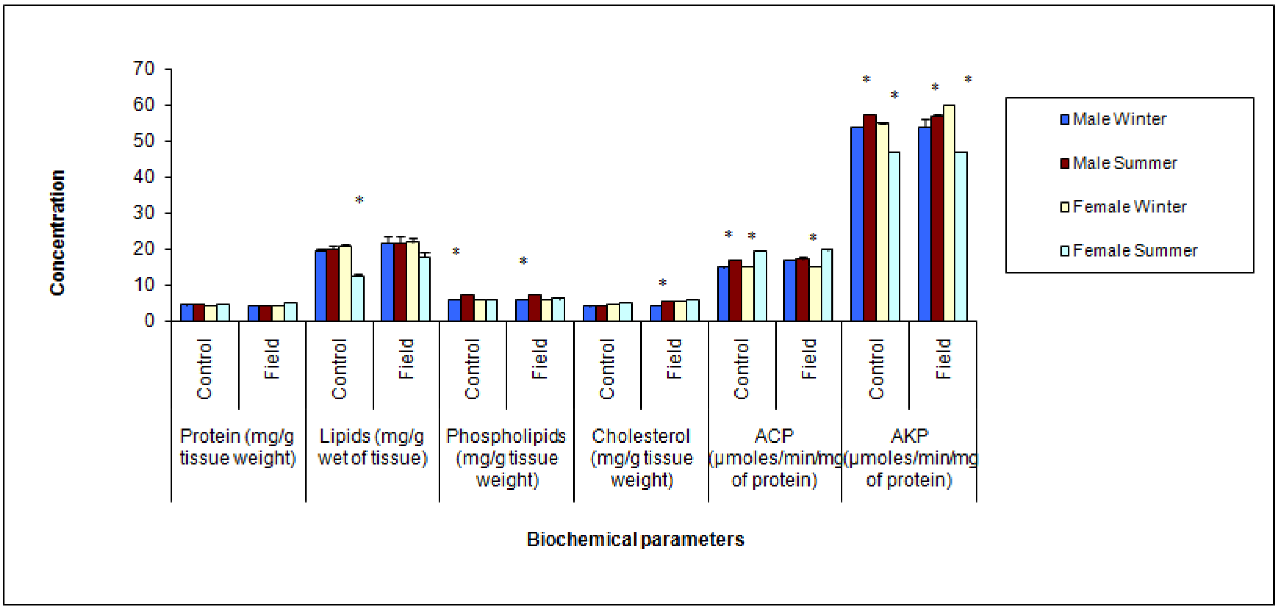

Figure 6: Comparison of concentration of total proteins, lipid profile and activity of enzymes in the liver of male and female rats of winter with summer season.

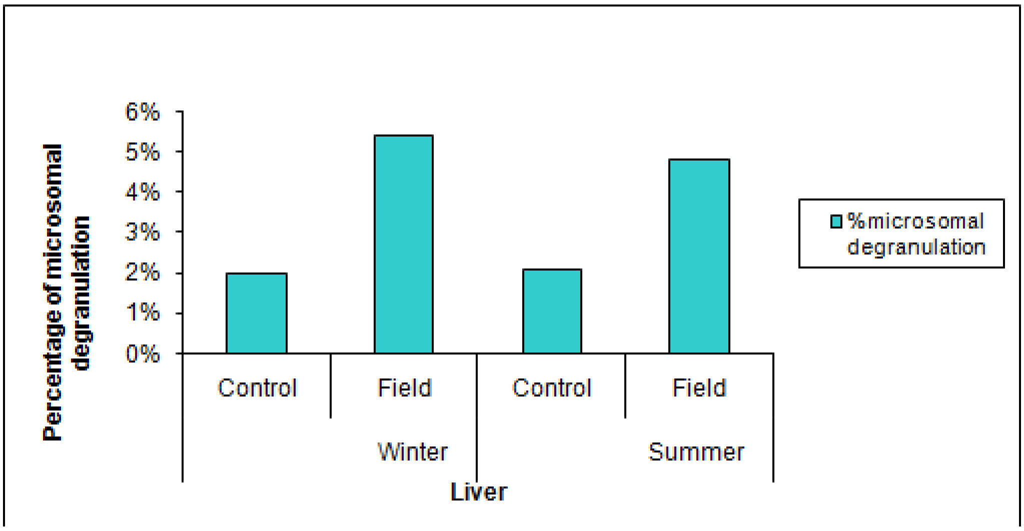

Figure7: Microsomal degranulation (percentage) test in Male rats

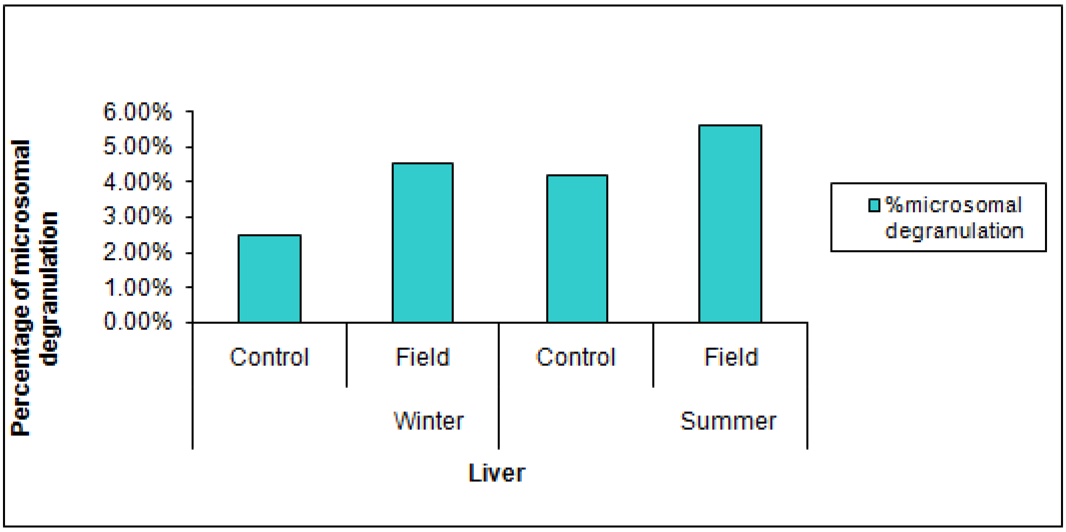

Figure 8: Microsomal degranulation (percentage) test in Female rats

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