Study of Alterations in Enzymatic Antioxidants, Carbohydrate Metabolism and Pancreatic Histomorphology in *Monocrotophos* Exposed Balb/c mice

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Abstract

The present study was undertaken to comprehend the lethal effects of organophosphorus insecticide Monocrotophos exposure on carbohydrate metabolism in Balb/c mice. Mice were procured, acclimatized and segregated in to 3 experimental groups, G-1 (control), G-2 (0.5 LD_{50}) and G-3 (0.75 LD_{50}). Mice were sampled on day 0, 7, 14 and 21 of the experiment. In G-3, blood glucose level was significantly ($p<0.05$) enhanced. Serum enzymes GOT, GPT, LDH were significantly ($p<0.05$) elevated in both Monocrotophos treated groups compared to control. Among treated groups, G-3 showed significantly ($p<0.05$) elevated blood and serum parameters compared to G-2. Serum amylase activity was found stable and no significant ($p>0.05$) alteration was observed in both treated and control groups. Present study concluded that the Monocrotophos has a significant ($p<0.05$) effect on carbohydrate metabolism and pancreatic histology.

Keywords: Monocrotophos, Carbohydrate metabolism, organophosphorus insecticide, pancreatic morphology.

Introduction

In India, agriculture is a major component of the Indian economy as it contributes 22% of the nation's GDP and is the livelihood of nearly 70% the country's workforce. Agriculture in India is solely dependent upon extensive use of pesticides. Despite commendable efforts undertaken to regulate pesticide use in the country, India is still unsure about its use of pesticides. Pesticide management in India is still not in accordance with the standards provided by the FAO who have encouraged countries to phase out perilous pesticides. Uncontrolled use of hazardous pesticides is a consequence of human greed of getting more crops. Human exposure to these lethal pesticides has been and continues to be an important issue to human health. Even though the use of these fatal pesticides is now restricted in many developed countries but they are still used ferociously in many developing countries like India.

Monocrotophos [3 hydroxy-N-methyl-cis-crotonamide dimethylphosphate] is an organophosphorus insecticide is widely used on wide varieties of agricultural crops. It has both systemic and contact properties and has been used against a wide range of insects including mites, Boll worms, sucking insects, leaf eating beetles and other larvae on variety of crops. The toxicity of the insecticidally active organophosphorus compounds to mammals and insects is primarily attributed to their ability to inhibit acetyl cholinesterase (AChE).

Few reports also suggests effect of Monocrotophos on activity through phosphorylation of the active serine hydroxyl group situated in the active centre of acetyl cholinesterase into acetic acid and thus making the enzyme non-available to hydrolyze acetylcholine (ACh) into acetic acid and choline. This results in the accumulation of acetylcholine at all sites of cholinergic transmission, hereby causing continuous stimulation of the muscle or nerve fiber, resulting eventually in the exhaustion and tetany.

Monocrotophos has been most frequently associated with both accidental and intentional fatal pesticide poisonings. Leading Asian countries have banned the use of Monocrotophos because of its unacceptable health risks, but in India, it continues to be produced, used and exported because it is perverted as cheap and necessary. Oral exposure of laboratory animals to Monocrotophos results in a variety of adverse effects including liver effects, neurological effects, reproductive system dysfunction and developmental effects (Karel and Saxena. 1975; Kacew et al.. 1972b; Bhatia et al.. 1972; Stohlman and Lillie 1988). Monocrotophos exposure also induces accumulation of hepatic glycogen in mice administered a single dose of Monocrotophos (Bhatia et al.. 1973). Yarbrwgh (1978) said that exposure of mice or mice to 5–10 mg Monocrotophos /kg/day has resulted in a variety of liver effects including increases in serum alanine aminotransferase activity, necrosis, hepatocytomegaly, hepatitis, and increased liver weights. He further observed alterations in serum liver enzyme activity levels or hepatocytomegaly incidence in individuals exposed to Monocrotophos and Monocrotophos metabolites in contaminated milk products (Yarbrwgh, 1978). Agricultural use of Monocrotophos has also raised concerns regarding its documented ability of bioaccumulation in cultivated products (Matsumura, 1985).

Thus, there is a scanty of information on the immediate toxic effects of Monocrotophos exposure in mice. To comprehend this, Balb/c mice were treated with various doses of Monocrotophos. Alterations in carbohydrate metabolism and pancreatic Histomorphology were studied to evaluate its immediate effects after consumption with agricultural crops.

2. Material and Methods

2.1. Animal

All studies were performed on Balb/c mice (20-25 gms, 2-3 months old, 7-9 cm), obtained from a local animal vendor. All animals were acclimatized in metal cages for two weeks to the laboratory conditions and maintained as per the animal keeping guidelines issued by Department of Zoology, Delhi University. Animals were maintained on pelleted diet (Lipton India Ltd.) and chlorine free tap water adlibitum.

2.2. Chemicals

Analytical grade Monocrotophos and all other used chemicals were procured from Hi-media laboratories, Mumbai, India and used as per standard manufacture's guidelines.

2.3. Experimental Plan

Mice were divided in to three groups of 15 animals each. G-1 (CTRL) received normal pelleted diet and alcohol, G-2 received 0.5 LD_{50} (55 mg/kg bw/day) and G-3 received 0.75 LD_{50} (85 mg/kg bw/day) doses of

Monocrotophos orally in chlorine free tap water twice a day. The experimental period lasted for 21 days. Three mice were sampled on 0, 7, 14 and 21 days at 7.00 am.

2.4. Biochemical analysis and light microscopy

Mice were kept unfed for 12h prior to each sampling. Blood was drawn out from the left ventricle of the heart and was collected in anticoagulant tubes (Tarsons, India) and immediately stored in -20˚C for serum and plasma analysis. Liver and muscles were quickly excised, washed twice with distilled water; blot dried and stored 30% KOH solution in -20˚C until analyzed. Protein content in different preparations was estimated by the method of Lowry et al. (1951) using Folin's reagent with bovine serum albumin (BSA) as protein standard. Blood glucose was analyzed by 'O' toludine method (Copper and Mc Danell, 1970). For glycogen analysis, mice (n=3) were sacrificed. Serum GPT, GOT, LDH and amylase activities were done by using standard kits obtained from Aspen laboratories, Delhi following protocols supplied with kits. For light microscopy, pancreas was quickly excised and fixed in Bouin's fluid for 24h, washed thoroughly and dehydrated with graded alcohol and processed for light microscopy.

2.5. Measurement of Antioxidant Enzymes

In Enzymatic antioxidants, Superoxide dismutase (SOD) activity was analyzed by degree of inhibition of INT (iodo-p-nitro tetrazolium violet) reduction by O_2- generated by xanthine-xanthine oxidase, as described by McCord and Fridovich 1969.

Catalase (CAT) activity was determined as explained by Lartillot et al. (1988). Briefly 2.5 mL of substrate solution made up of 25 mM H_2O_2 in a 75 mM phosphate buffer (pH 7.0) and 20 µL of supernatant which contains 0.2 mg protein/mL were mixed at 25 °C for 2 min and reaction was stopped by adding 0.5 mL of 1 M HCl. OD was taken at 240nm (Shimadzu, UV/VIS, V-530) and CAT activity was calculated as µmol H_2O_2 decomposed/mg protein/min.

GPx activity was assayed as described by Athar and Iqbal (1998). The assay mixture was taken in a total volume of 2 mL which consisted of 1.44 mL phosphate buffer (0.05 M, pH 7.0); 0.1 mL EDTA (1 mM); 0.1 mL sodium azide (1 mM); 0.05 mL GSH reductase (1 IU/mL); 0.1 mL GSH (1 mM); 0.1 mL NADPH (0.2 mM); 0.01 mL H2O2 (0.025 mM), and 0.1 mL of PMS (10%). Oxidation of NADPH was recorded spectrophotometrically (Shimadzu, UV/VIS, V-530) at 340 nm at room temperature. The GPx activity was calculated as nanomoles NADPH oxidized/min/mg of protein. The GR activity was determined using reduced NADPH (Sigma, France) and oxidized glutathione (GSSG) (Sigma, France) as substrates (4 min at 20 °C) by a technique as described by Knörzer et al.1996.

2.6. Statistical analysis

All experiments were done in triplicates and the mean was taken as parameters. All data were analyzed by SPSS version 11 and the level of significance was assessed at $P \leq 0.05$.

3. Result

3.1. Behavioral

8 treated mice showed clinical signs of hyper excitability. They exhibited a distinct loss of appetite and body weight accompanied by a reduction in general activity. Survival of mice was 100, 81 and 73 % in control, 0.5 LD_{50} and 0.75 LD_{50} groups, respectively on day 21.

3.2. Carbohydrate Metabolism

Monocrotophos treated mice showed stress hyperglycemia aka diabetes of injury hyperglycemia which was observed from significantly ($p<0.05$) enhanced blood glucose level (Fig.2 A) throughout the experiment. Unaltered glucose level in control group indicated that hyperglycemia in treated group was due to pesticide side effects. Total protein was also observed significantly less in Monocrotophos treated groups compared to CTRL group (Fig.2 B).

Liver and muscle glycogen levels (Fig.2 C and D) showed a marked ($P<0.05$) decrease on day 7 in both the treatment groups compared to day 0. It was further significantly decreased ($P<0.05$) on day 14 and 21 in both treated groups compared to control. This glycogenolysis could be attributed to physical stress caused by Monocrotophos exposure.

3.3 Serum Enzymes

In control group, no significant ($P>0.05$) alteration was observed in serum GOT and GPT activities throughout experiment. Among treated groups, 0.5 LD_{50} fed group showed significantly ($P<0.05$) low SGOT activity (Fig.1.B) on day 0, 7 and 14 of the experiment however it was not significantly different ($P>0.05$) on day 21. There was no significant ($P>0.05$) difference was observed in SGPT activity (Fig.1.A) on day 0. However on 7^{th} day, it was declined significantly in CTRL and 0.50 LD_{50} group with no significant ($P>0.05$) alteration in 0.75 LD_{50} group. On day 14, it was significantly ($P<0.05$) lowered compared to day 7 and there was no alteration ($P>0.05$) in SGPT activity on day 21 in all the groups.

LDH activity (Fig.1.D) was significantly ($P<0.05$) altered in control group throughout experiment. In 0.50 LD_{50} group, it increased ($P<0.05$) significantly on day 7 compared to day 0 and on 14^{th} day it was stable as on 7^{th} day. On 21^{st} day, LDH activity was declined ($P<0.05$) significantly compared to 14^{th} day. In 0.75 LD 50 fed group, LDH activity was increased ($P<0.05$) significantly on day 7 and it decreased ($P<0.05$) gradually on day 14 and 21 (Fig.6). In Serum amylase activity (Fig.1.C) no significant ($P>0.05$) alteration was observed throughout experiment though it showed a decreasing trend from day 0 to 21 (Fig.7).

3.3. Histomorphology of pancreas

In control group, islets were not damaged and no membrane ruffling was observed. It was viewed that all three A, B and D type cells were intact and no irregularity in shape and size was noticed (Fig.3. A and B). In treated

groups, Monocrotophos exposure induced significant histomorphological changes in the structure of pancreatic. In 0.50 LD_{50} Monocrotophos exposed group islets were significantly damaged and a severe membrane ruffling was observed. Further all three A, B and D type cells were found to be significantly loose and shapeless (Fig.3. C and D). In 0.75 LD_{50} Monocrotophos fed group, Histomorphology showed significant damage to the islets in pancreas. It severely damaged the shape, size of islets and their membrane was severely ruffled. It was further observed that all three A, B and D type cells were significantly damaged with a severe damage to their membranes (Fig.3. E and F).

4. Discussion

Monocrotophos toxicity is manifested by the data showed by the present study. In present study, hyperglycemia was induced and glycogen was found to be accumulated in liver and muscle of mice fed Monocrotophos for 21 days. Similar results were obtained by Stehr-green et al. (1986) who have reported the hyperglycemia in mice when exposed to Monocrotophos contaminated food products. Observed trend is well supported in many studies viz. when a single dose of chlordane to Indian desert gerbils; DDT and dieldrin to mice and acute and chronic poisoning of rabbits with DDT produced hyperglycemia (Karel and Saxena. 1975; Kacew et al.. 1972b; Bhatia et al.. 1972; Stohlman and Lillie 1988). Organochlorine pesticides have been reported to induce all kinds of variations in liver glycogen; accumulation of hepatic glycogen has been reported in mice administered a single dose of dieldrin (Bhatia et al.. 1973). In mirex treated mice, Kendal (1974) observed depletion of liver glycogen whereas Robinsm and Yarbrwgh (1978) reported that it remained unaltered after the treatment. Levels of liver glycogen observed are very close with those observed by Bhatia et al., (1973) and tally with those observed by Altman and Dittmar, (1974) and Dau (1991).Muscle glycogen value is comparable with that reported in bandicoots by Raghukumar and Awanshi, (1980) and in mice by Daoo (1992). The hyperglycemia and increased liver glycogen observed in the present investigation may be attributed to increased glycogen and glucose production by the liver due to increased gluconeogenesis. Probably, the pesticide stress is responsible for stimulation of pituitary adrenocortical axis and as a consequence of this gluconeogenesis is enhanced. This assumption is well supported by the findings of other researchers. Plasma and adrenal corticosterone levels increased in mice after a single or sub lethal dose of DDT (Szot and Murphy. 1970) and a single dose of dieldrin (Bhatia et al, 1971, 1972). The degradation of glycogen in the mammalian system is regulated by the action of enzyme phosphorylase and the deficiency of this enzyme has been reported to cause accumulation of liver glycogen (Steinitz 1967; Field 1968). Onikieno (1963, 1968) has also observed an inhibition of phosphorylase in the liver, kidney and spleen of mice chronically exposed to dieldrin and Monocrotophos. Thus, the inhibition of liver phosphorylase may be another reason for increased liver glycogen. Moreover increased stress due to the high levels of Monocrotophos toxicity could lead to gluconeogenesis which means a reason for accumulation glycogen and later breakdown of it in times of energy needs. Further, the histological observations reveal an unaffected endocrine pancreas. Therefore, the other probable reason for hyperglycemia could be that Monocrotophos inhibits the secretory activity of B cells.

Alterations in plasma enzyme activities are a manifestation of pathologic changes in several organs or tissues (Hess 1983).Cellular necrosis and fatty tissue changes; particularly in the liver, kidney, cardiac and skeletal

muscles are associated with leakage of tissue enzymes into the blood (Altland and Higman 1981). Lots of variations are noticed in the values of serum enzymes of male Balb/c reported by various workers. The mean value of GOT estimated in the present study is higher than the findings of Chatterjee et al., (1973), but is comparable to the value reported by Ghosh (1993). GPT level is higher than that observed by Sarkar et al. (1982) and Chatterjee et al, (1973) but is quite similar to that reported by Daoo (1992). Serum amylase and LDH values agree well with the findings of Ghosh (1993) and Sarkar et al., (1992) respectively. The lower dose of the pesticide produced a marked increase in SGPT, decrease in SGOT, insignificant increase in SLDH and no variation in serum amylase. Agarwal et al., (1978) reported an increase in the serum amylase of rhesus monkeys fed with DOT. Higher dose of the pesticide brought about significant elevation in serum transaminases and LDH but it failed to alter the serum amylase. Increase in the serum transaminases and LDH on exposure to various organochlorine s like DDT, dieldrin, aldrin, DDE have been reported in Coturnix quail, albino mice and rhesus monkey (Dieter 1973, Lucken and Phelps 1969; Agarwal et al., 1978). Mirex failed to alter the levels of serum transaminases and LDH in mice (Robinson and Yarbrough 1977). Altland and Higman 1961, showed an increase in serum GOT and LDH activity in albino mice exposed to BHC. Though early signs of hepatic damage were registered in rabbits fed with DDT, there were no alterations in SGOT and SGPT (Chugh et al., 1990). Organochlorines are found generally but not necessarily to enhance the activity of serum GOT, GPT and LDH. They are also known to inhibit or not affect the levels of the above serum enzymes. In present study, different staining techniques revealed the presence of three types of cells. This is, in accordance with, the observations, in other vertebrates (Bloom 1931., Lacy 1957, Yolk and Lazarus. 1962, Pentakova et al. 1974; Raghukumar and Suryawanshi, 1980, Deshmukh, 1983). The number of B cells is comparatively more than A cells in the present study. This might changes with age, sex and any kind of stress (Hellman 1959) which is well depicted in the present study.

Conclusion

Monocrotophos was found to be toxic in the present study and it caused the hyperglycemia in blood. There was a marked storage of glycogen in liver and muscle in mice in response to Monocrotophos. Serum enzymes were significantly altered in response to high doses of Monocrotophos compared to control. Pancreatic Histomorphology was not much altered in response to Monocrotophos though there were slight alterations in size and no of all the A, B and D type cells.

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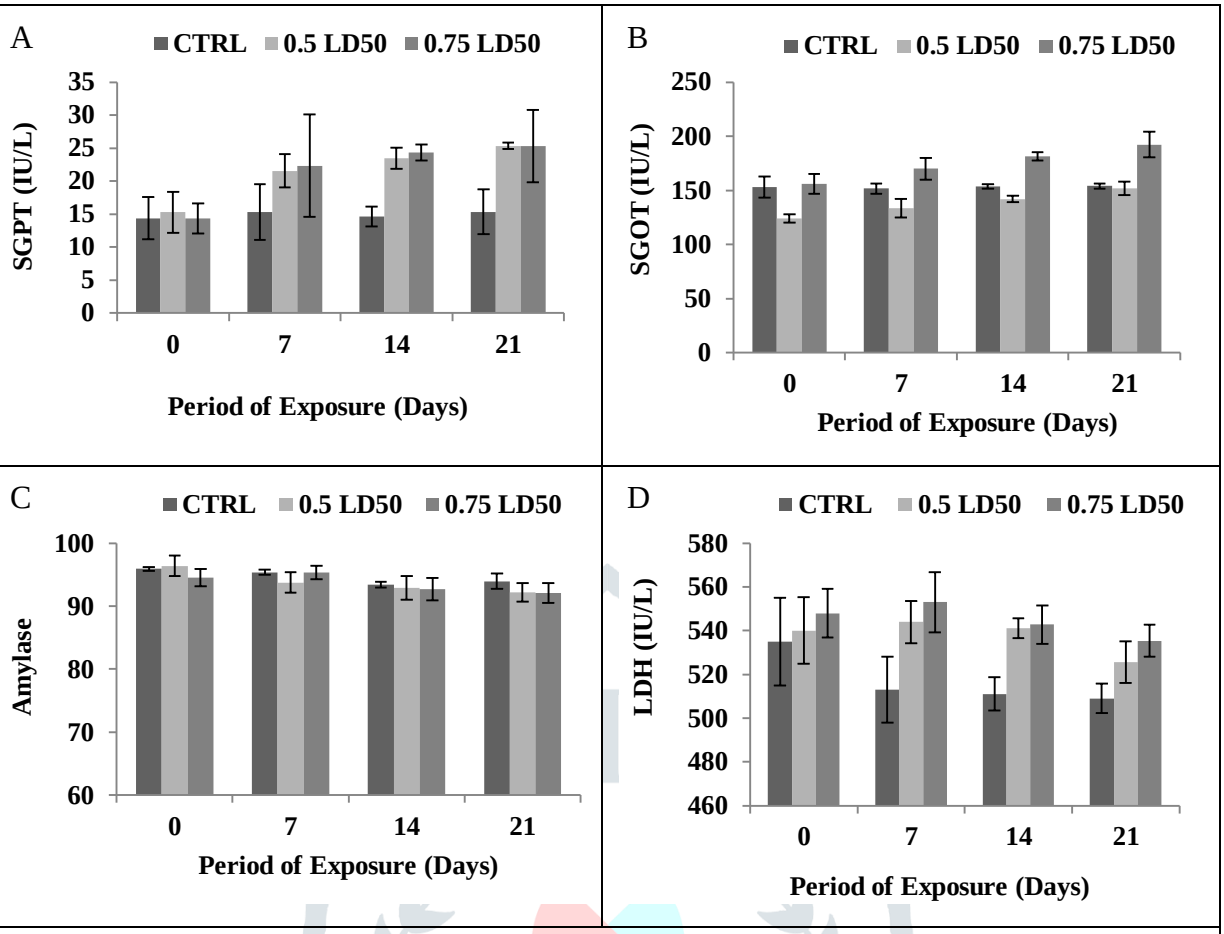

Fig.1. **A**. SGPT level in mice in control and Monocrotophos treated groups. On various days of sampling. **B**. SGOT level in control (CTRL) and treated groups (0.5 LD_{50} and 0.75 LD_{50} of Monocrotophos in male Balb/c on various days of sampling. **C.** Serum amylase activity in CTRL and treated groups **D.** Serum LDH content in control (CTRL) and Monocrotophos treated groups (0.5 LD_{50} and 0.75 LD_{50}) in male Balb/con various days of sampling.

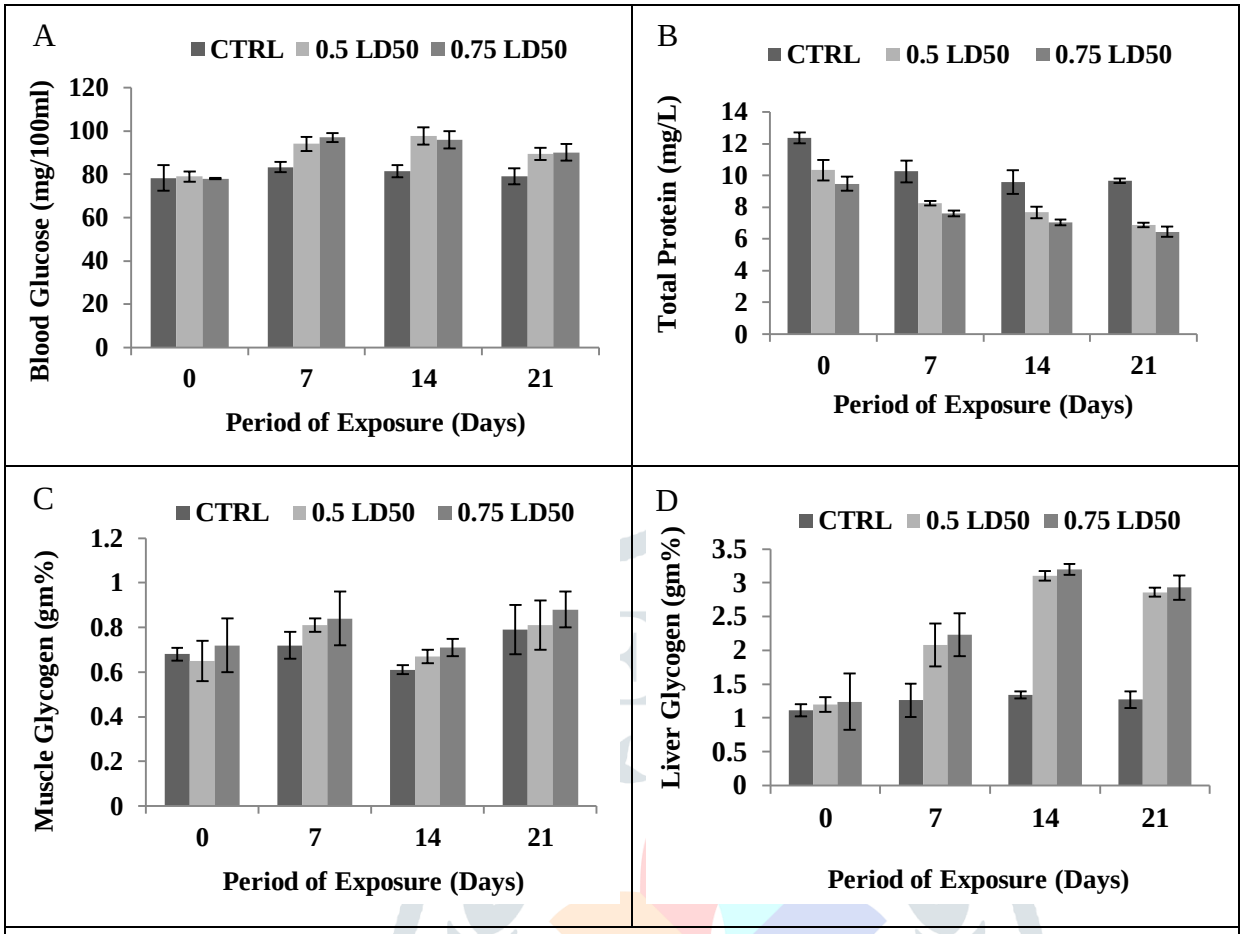

Fig.2. A. Blood Glucose level in mice in control and Monocrotophos treated groups. On various days of sampling. **B.** Total protein content in control (CTRL) and treated groups (0.5 LD_{50} and 0.75 LD_{50} of Monocrotophos in male Balb/c on various days of sampling. **C.** Muscle glycogen activity in CTRL and treated groups **D.** Liver glycogen content in control (CTRL) and Monocrotophos treated groups (0.5 LD_{50} and 0.75 LD_{50}) in male Balb/con various days of sampling.

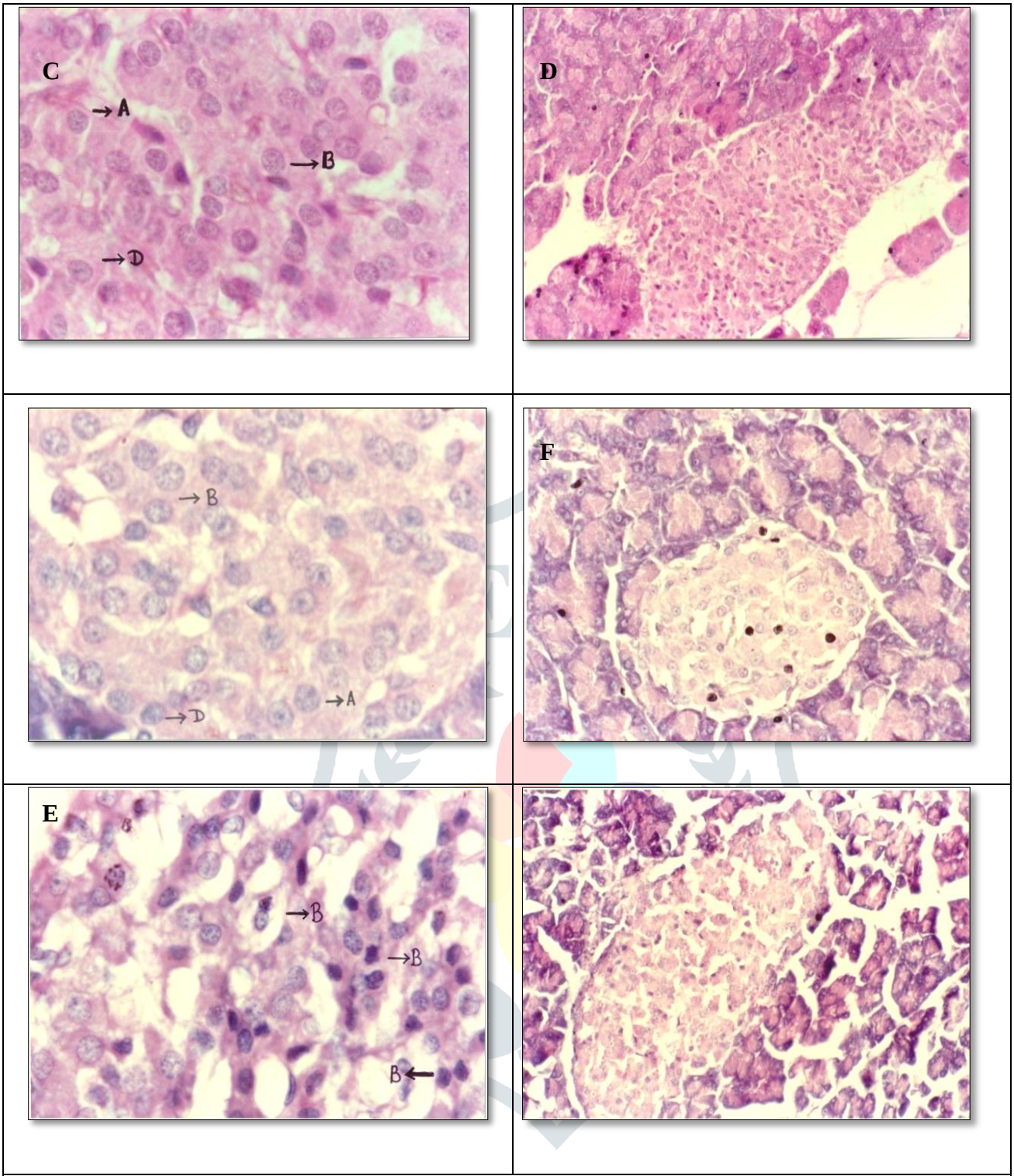

Fig.3. A and B. **P**ancreatic histo-morphology is visualized normal in mice in CTRL group after 21 days of experiment. No membrane ruffling was observed and Islets are not damaged and there is no irregularity in shape and size. C and D. Micrographs showing pancreatic histo-morphology of mice in 0.50 LD_{50} Monocrotophos exposed group after 21 days of experiment. In fig. C. arrow shows Islets were significantly damaged and lost its shape completely and a severe damage to membrane was observed after 21 days if Monocrotophos exposure. N fig. D. It shows all three type of A, B and D cells were significantly damaged and there was severe damage to their membranes was observed E and F. Micrographs showing pancreatic histo-morphology of mice in 0.75 LD_{50} Monocrotophos exposed group after 21 days of experiment. E. Arrow shows Islets were significantly damaged and lost its shape completely and a severe damage to membrane was observed after 21 days if Monocrotophos exposure. F. It shows all three type of A, B and D cells were significantly damaged and there was severe damage to their membranes was observed.