CHAPTER II

Susceptibility of rat pancreas to OPI-induced biochemical alterations
CHAPTER II

SUSCEPTIBILITY OF RAT PANCREAS TO OPI-INDUCED BIOCHEMICAL ALTERATIONS

PREFACE

From our earlier studies (Chapter I), it is clearly evident that both dimethoate and dichlorvos possess the potential to induce oxidative stress in rats. Our studies also showed that while a single dose of DM increased blood glucose levels in rats, single dose of DDVP failed to increase the blood glucose levels. However, administration of repeated, sublethal doses of DDVP significantly elevated the blood glucose levels in rats. Hence, in the present study, it was planned to investigate the oxidative stress associated biochemical perturbations in pancreas of rats subjected to repeated oral doses of DM and DDVP. Another important question addressed in the present study was to determine the impact of DDVP pre-treatment on hyperglycemia and associated biochemical alterations induced by streptozotocin, a diabetogenic agent, in order to obtain evidence whether OPI exposure would predispose experimental animals to STZ-induced diabetes.

This Chapter has been presented under two sections.

Section A describes investigations related to the potency of DM and DDVP to impair glucose homeostasis and associated pancreatic dysfunction/s following their repeated oral exposure in rats.

Section B describes the effect of streptozotocin (STZ) in rats pre-treated with repeated oral doses DDVP.
SECTION A

BIOCHEMICAL PERTURBATIONS IN RAT PANCREAS ON EXPOSURE TO DIMETHOATE AND DICHLORVOS IN VIVO

1.0 INTRODUCTION

OPI are currently responsible for more poisonings than any other single class of pesticides (Sultatos, 1994; Gulr et al., 1996) and various complications have been reported in OPI intoxication cases (Hsiao et al., 1996). Hyperglycemia has been widely reported as one of the adverse effects in poisoning by OPI in humans and animals (Namba et al., 1971; Ramu and Drexler, 1973; Meller et al., 1981; Shobha and Prakash, 2000; Hagar et al., 2002, Seifert, 2001; Abdollahi et al., 2003). Although the precise mechanism/s of OP-induced hyperglycemia is not known, it is speculated to be due to inhibition of acetylcholinesterase of central and peripheral synapses that act in the endocrine regulation of glucose metabolism (Kant et al., 1988, Matin and Siddiqui, 1982). Acute pancreatitis is also a well known complication of OP poisoning (Dressel et al., 1979; Frick et al., 1987; Hsiao et al., 1996), and epidemiological findings indicate that the incidence of pancreatitis is high in OPI intoxication based on various pathophysiological reports (Gokalp et al., 2005). The precise mechanisms underlying OPI-induced pancreatitis are still undefined, although it is believed to involve obstruction of pancreatic ducts and/or enhanced reactive oxygen species (Dressel et al., 1982, Sevillano et al., 2003, Sultatos, 1994). Involvement of oxidative stress following acute exposure to OPI has been reported recently (Banerjee et al., 2001) and it has been demonstrated unequivocally that lipid peroxidation is one of the molecular mechanisms involved in OPI-induced cytotoxicity (Akhgari et al., 2003; Ranjbar et al., 2002; Abdollahi et al., 2004b).
In view of the above, in the present study, we have focused our attention to understand the potential of repeated oral doses of DM and DDVP to cause alterations in glucose homeostasis and the associated biochemical alterations in pancreas of rats. Further, we have examined the various oxidative impairments in pancreas in terms of lipid peroxidation, generation of ROS, response of antioxidant enzymes and examined its correlation with pancreatic acetylcholinesterase activity.

2.0 MATERIALS AND METHODS

2.1 Chemicals

Thiobarbituric acid (TBA), xanthine oxidase, glutathione reductase (GR), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), 2',7'-Dichlorofluorescin diacetate (DCFH-DA), 2',7'-Dichlorofluorescein (DCF), p-nitrophenyl acetate (PNPA), p-nitrophenol (PNP), butyrylthiocholine iodide, streptozotocin and cytochrome 'C' were procured from M/s Sigma Chemical Co., (St. Louis, MO, USA). Ethylenediamine tetraacetic acid (EDTA), hydrogen peroxide (H$_2$O$_2$), xanthine, nicotinamide adenine dinucleotide phosphate–reduced (NADPH), nicotinamide adenine dinucleotide–reduced (NADH), nicotinamide adenine dinucleotide phosphate (NADP), trichloroacetic acid (TCA), reduced glutathione (GSH), oxidized glutathione (GSSG), 1-chloro-2,4-dinitrobenzene (CDNB), sucrose, glucose-6-phosphate, glucose-1-phosphate, sodium fluoride, γ-glutamyl p-nitroanilide, glycyl glycine, N-ethylmaleimide, α-phthalaldehyde, adenosine-5'-monophosphate (AMP), adenosine-tri-phosphate (ATP), L-tyrosine, α-ketoglutaric acid, pyridoxal-5'-phosphate, diethyl dithiocarbamic acid, anthrone, thiourea, glucose-6-phosphate dehydrogenase, β-mercaptopethanol, glucose, sodium pyruvate, 2,6-dichlorophenolindophenol (DCPIP) and acetylthiocholine iodide were procured from M/s Sisco Research Lab, (Mumbai, India). Dimethoate (Technical grade, 97.4 % pure) and Dichlorvos (Technical grade, 94 % pure) were gift from M/s Hyderabad Chemical Supplies Ltd., (Hyderabad, India). Glucometer ("Accu-Check") was procured from M/s Roche
diagnostics (GmbH, Mannheim, Germany). Amylase kit was procured from M/s Span diagnostics (Mumbai, India). All other chemicals used were of analytical grade.

2.2 Animals and care

Adult male rats (CFT-Wistar strain, 12-14 week old, 280 ± 5g) were randomly drawn from the stock colony of our institute animal house facility and were housed individually in polypropylene cages under standard housing conditions (controlled atmosphere with 12:12-hour light/dark cycles, 50% ± 5% humidity, and ambient temperature of 25± 2° C). The rats were acclimatized for 1 week prior to the start of the experiment. They were maintained on commercial pellet diet (M/s Saidurga Feeds and Foods Pvt. Ltd., Bangalore, India) ad libitum and had free access to water. All procedures with animals were conducted strictly in accordance with approved guidelines by the 'Institute Animal Ethical Committee', regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept at a minimum.

2.3 Pesticide solution

Stock solution of Dimethoate (DM: 20mg/ml) and Dichlorvos (DDVP:10mg /ml) was prepared in saline and used for intragastric (oral) administration to rats.

2.4 Streptozotocin solution

Streptozotocin solution (STZ: 10mg/ml) was freshly prepared in citrate buffer (0.1M, pH 4.5) and used for intraperitoneal injection.
3.0 EXPERIMENTAL PROCEDURE

3.1 Effect of repeated oral doses of DM

Adult male rats were grouped by randomized design into three groups (n = 6). Rats of the first group (negative control) received saline daily, while rats (non-fasted) of the treatment groups were orally administered daily (0900 to 1100 hours) Dimethoate (DM) at dosages of 20 or 40mg/kg b.w./day (corresponding to 1/20 and 1/10 of LD₅₀ value: 400 mg/kg b.w., determined in a preliminary study) for 30 days. Both, control and DM treated rats were subjected to oral glucose tolerance test at the end of 30 days and were subsequently killed. Body weights were recorded weekly, and terminally the rats were sacrificed and blood was collected for separation of serum. Pancreas and other vital organs (viz., liver, kidney and adrenals) were excised and their weights were recorded. The biochemical assays included determination of blood glucose, AChE activity in pancreas, amylase and lipase activity in serum and pancreas, oxidative stress parameters and xenobiotic metabolizing enzymes in pancreas.

3.2 Effect of repeated oral doses of DDVP

Adult male rats were grouped by randomized design into three groups (n = 6). Rats of the first group received saline daily, while rats (non-fasted) of the treatment group were orally administered daily (0900 to 1100 hours) DDVP at 20mg/kg b.w./d (corresponding to 1/5 of LD₅₀ value: 100 mg/kg b.w., determined in a preliminary study) for 10 days. Both control and DDVP treated rats were subjected to oral glucose tolerance test at the end of 10 days. Subsequently, the rats were sacrificed and blood was collected for separation of serum. Pancreas and other vital organs (viz., liver, brain and adrenals) were excised, rinsed in ice-cold saline, blotted and stored at 4°C until use. The biochemical assays included determination of blood glucose, AChE activity in pancreas, amylase, lipase, GGT and LDH activity in serum and pancreas as
biochemical indices of pancreatic damage, oxidative stress parameters and xenobiotic metabolizing enzymes in pancreas, and carbohydrate metabolizing enzyme activities in liver and pancreas, as well as histology of pancreas.

3.3 Oral glucose tolerance test

Oral glucose tolerance test was conducted in control and treated rats 24 h after the last dose of DM or DDVP. Blood samples were collected from tail vein of rats that were fasted overnight to obtain baseline blood glucose levels. Subsequently, rats of both control and treated groups were orally administered a bolus of glucose (3 g / kg b.w.). Blood was collected from tail vein of these rats at intervals of 30 min up to 3 h for estimation of glucose.

3.4 Preparation of tissue homogenates

10 or 20 % (w/v) homogenates of the tissues were prepared in various buffers as per the requirement of a given assay.

3.5 Serum

Blood was drawn by cardiac puncture into tubes and then allowed to clot at room temperature for 20min. It was then kept at 4°C for 4 h and centrifuged at 3500 rpm for 10min at 4°C. The supernatant serum was decanted and frozen until use.

4.0 ASSAY METHODS

4.1 Blood glucose

Blood glucose was estimated using glucometer as described earlier (Section 4.1, Chapter IB).
4.2 Lipase (EC 3.1.1.3) (Young et al., 1978)

Lipase activity was estimated by monitoring the hydrolysis of p-nitrophenyl acetate to p-nitrophenol. The assay involved incubation of 100 μM p-nitrophenyl acetate with 10 μl sample (serum / pancreatic homogenate) in a final volume of 2ml at 37°C for 10min. The resultant colored product was read at 420nm against water. Enzyme activity was calculated from PNP standard graph and was expressed as nmoles of PNP released/min/mg protein.

4.3 Amylase (EC 3.2.1.1) (Street and Close, 1956)

The enzyme activity was measured using a commercial kit. The assay is based on the degradation of starch by the enzyme into reducing dextrins and small oligosaccharides with which iodine solution is reacted to give a blue coloration, which is read at 620 nm. The relative decrease in blue color of test is the measure of enzyme activity. The enzyme activity was expressed as Street-Close units.

4.4 \( \gamma \)-glutamyltranspeptidase (GGT) (EC 2.3.2.2) (Novogrodsky et al., 1976)

20 % homogenate of pancreas was prepared in Tris buffer (0.01M, pH 8.0) containing 0.15M NaCl and centrifuged at 10,000g for 10min at 4°C and the supernatant was used for the assay. Assay solution contained 122 μl of the supernatant, 2.5mM \( \gamma \)-glutamyl p-nitroanilide, 30mM glycyl glycine (adjusted to pH 8.0 with NaOH), 0.05 M Tris-HCl (pH 8.0) and 0.075 M NaCl. The contents were mixed and scanned at 410 nm for 1 min. The enzyme activity was calculated based on molar extinction coefficient: 11.3mM at 410nm. Results were expressed as nmoles of product (p-nitroanline or S-acetophenone-cysteinylglycine) formed/min/mg protein.
4.5 Lactate dehydrogenase (LDH) (EC 1.1.1.27) (Kornberg, 1974)

Pancreatic homogenate (10% w/v) was prepared in Tris-HCl buffer (82.4mM, pH 7.2) containing 210mM NaCl, and then centrifuged at 10,000 rpm for 10min at 4°C. 1μl of the supernatant/serum was placed in a cuvette and the reaction was started by addition of 0.8ml NADH (in Tris-HCl. 0.25mM, pH 7.2), 0.15 ml of sodium pyruvate (10.66 mM) and 49μl of distilled water. The reaction was monitored over a period of 5 min at 340nm. The results were expressed as nmoles of NADH oxidized/ min/ mg protein.

4.6 Acetylcholinesterase (AChE) (EC 3.1.1.7) (Ellman et al., 1961)

AChE activity in serum and other tissues viz., brain, liver, adrenal and pancreas was estimated as described earlier (Section 4.2, Chapter IB).

4.7 Butyrylcholinesterase (BuChE) (EC 3.1.1.8) (Ellman et al., 1961)

100 μl of serum / tissue homogenate was taken in 2.83 ml of phosphate buffer (0.1M, pH 8.0) with 50μl of DTNB solution (10mM) and 20 μl of butyrylthiocholine iodide (1mM). The absorbance was read at 412 nm immediately after addition of the substrate and the absorbance was recorded for 5min. The change in absorbance per minute (ΔA) was calculated and the enzyme activity was expressed as nmoles of substrate hydrolyzed/min/mg protein.

4.8 Reactive oxygen species (ROS) (Keston and Brandt, 1965)

Tissue homogenate (10%) was incubated with 5μM DCFH-DA in a final volume of 2ml for 45min at room temperature. The intensity of the fluorescence was measured at 530nm following excitation at 485nm. The amount DCF (resulting from the ROS mediated oxidation of DCFH, which is produced by hydrolytic cleavage of DCFH-DA by cellular esterases) was calculated from the DCF standard graph. Results were expressed as pmoles of DCF/min/mg protein.
4.9 Lipid peroxidation (LPO) (*Buege and Aust, 1978*)

10% (w/v) homogenate of tissue was prepared in KCl (1.15%, w/v) and centrifuged at 3500 rpm for 10 min. 250 µl of the supernatant was added to 2ml of TBA-TCA-HCl (0.374%-15%-0. 25N) and the tubes were placed in boiling water bath for 15min. After cooling and centrifugation, the color of the supernatant was read at 535nm. The amount of thiobarbituric acid reactive substances (TBARS) in the supernatant was calculated using the molar extinction coefficient of 1.56X 10^5 M^-1 cm^-1 and the results were expressed as nmoles of MDA/g tissue.

4.10 Reduced glutathione (GSH) (*Benke et al., 1974*)

Reduced glutathione was estimated in tissue homogenate as described earlier (*Section 4.3, Chapter IB*).

4.11 Oxidized glutathione (GSSG) (*Hissin and Hilf, 1976*)

Tissue homogenate (10% w/v) was prepared in phosphate buffer (0.1M, pH 7.4), centrifuged and 0.5ml of the supernatant was incubated with 200µl of 0.04 M N-ethylmaleimide for 30 min at room temperature to interact with GSH present in the tissue. 100 µl aliquot from this mixture was diluted with 1.8 ml of 0.1 N NaOH and incubated with 100 µl of the o-phthalaldehyde (OPT) (1mg/ml in alcohol) at room temperature for 15 min. The fluorescence of the mixture was measured at 420nm with the excitation at 350nm. Oxidized glutathione concentrations were calculated from standard graph obtained with GSSG and the results were expressed as pmoles of GSSG/mg tissue.

4.12 Protein carbonyls (*Levine et al., 1990*)

Tissue homogenate (10% w/v) was prepared in 20mM Tris-HCl, 0.14 M NaCl buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 min at 4°C. 0.1ml of the supernatant was precipitated with an equal volume of 20% TCA and centrifuged. The pellet was resuspended in 1ml of
2,4-dinitrophenylhydrazine solution (10mM, in 2M HCl) and allowed to stand at room temperature for 60min with occasional vortexing. Proteins were then precipitated by adding 0.5ml of 20 % TCA. The resulting mixture was centrifuged and the pellet obtained was washed 3 times with 1ml acetone and 1ml of 2% SDS (in 20mM Tris-HCl, 0.1M NaCl, pH 7.4) was added to dissolve the pellet. The absorbance of the solution was read at 360nm and the carbonyl content was calculated using a molar extinction coefficient of 22,000 M⁻¹cm⁻¹. Results were expressed as nmoles of carbonyl/mg protein.

4.13 Catalase (CAT) (EC 1.11.1.6) (Beers and Sizer, 1952)

Tissue was homogenized (10% w/v) in phosphate buffer (0.1M, pH 7.4), centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was used for the assay. Varying volumes of supernatant along with 25μl of H₂O₂ (3%) was added to 3ml phosphate buffer (50mM, pH 7.4) and the decrease in absorbance due to H₂O₂ degradation was monitored at 240nm for 5min. The enzyme activity was calculated based on molar extinction coefficient at 43.6M⁻¹cm⁻¹ and the results were expressed as μmoles of H₂O₂ consumed / min/mg protein.

4.14 Superoxide dismutase (SOD) (EC 1.15.1.1) (Flohe and Otting, 1984)

Tissue was homogenized (10% w/v) in potassium phosphate buffer (0.1M, pH 7.4) and centrifuged at 10,000 rpm for 10 min 4°C, and the supernatant was used for the assay. To a semi-micro cuvette, 2.9 ml of solution A (5μl xanthine in 0.01M NaOH + 2μl cytochrome 'c' + 50mM phosphate buffer and 0.1mM EDTA, pH7.8) and 0.1ml of solution B (an equal volume of xanthine oxidase and 0.1 mM EDTA) were added. Reaction mixture without enzyme was used as blank. After adding various volumes of supernatant (enzyme source), inhibition of cytochrome 'c' reduction was monitored at 560nm for 5min. Results were expressed as units of SOD/mg protein. One unit was defined as the amount of enzyme that decreases the initial rate of cytochrome 'c' reduction to 50% of its maximal value for the particular sample being analyzed.
4.15 Glutathione reductase (GR) (EC 1.6.4.2) (Carlberg and Mannervik, 1975)

The reaction was started by adding 25μl of homogenate to the reaction mixture (final volume 1ml) containing 0.2 M sodium phosphate buffer (pH 7.0), 2 mM EDTA, 1mM oxidized glutathione (GSSG) and 0.2 mM NADPH. The enzyme activity was measured indirectly by monitoring the oxidation of NADPH following decrease in OD/min for a minimum of 3 min at 340 nm. One unit enzyme activity was defined as nmoles of NADPH consumed/min/mg protein based on Molar extinction coefficient of 6.22 mM⁻¹cm⁻¹.

4.16 Glutathione peroxidase (GPX) (EC 1.11.1.9) (Flohe and Gunzler, 1984)

Tissue homogenate (10% w/v) was prepared in phosphate buffer (0.1M, pH 7.0) containing 0.5mM EDTA and centrifuged at 10,000 rpm for 10min at 4°C. 5 or 10μl of supernatant was incubated with 0.575ml phosphate buffer (0.1M, pH 7.0 containing 0.5mM EDTA), 100 μl of glutathione reductase (2.5U/ml), 100μl of GSH (10mM) and 100 μl of NADPH (1% in sodium bicarbonate solution) at 37°C for 10 min. Finally, 100μl of 12mM t-butylhydroperoxide was added to the above mixture and the oxidation of NADPH over 5min was recorded at 340nm. Results were expressed as nmoles of NADPH oxidized/min/mg protein.

4.17 Glutathione transferase (GST) (EC 2.5.1.18) (Jakoby and Habig, 1980)

To 0.5ml of potassium phosphate buffer (0.2M, pH 7.2) were added 0.1ml of GSH (10mM), 0.01ml of 1-chloro-2,4-dinitro benzene (CDNB, 0.1M) and 390μl of distilled water and mixed. 1-2.5 μl of the tissue homogenate was then added to the above mixture and the formation of the adduct of CDNB (S-2,4-dinitrophenyl glutathione) was monitored by measuring net increase in absorbance at 340nm. The enzyme activity was calculated based on absorption coefficient of 9 mM⁻¹ cm⁻¹. Results were expressed as μmoles of adduct formed/min/mg protein.
4.18 NADPH-Cytochrome P<sub>450</sub> reductase (EC 1.6.2.4) (*Omura and Takesue, 1970*)

The reaction mixture containing 0.3M potassium phosphate buffer (pH 7.5), 0.2mM potassium ferricyanide and tissue homogenate in a final volume of 1 ml was incubated with 0.1mM NADPH at 25°C. The enzyme activity was calculated using extinction coefficient 6.22mM<sup>−1</sup>cm<sup>−1</sup>. One unit of enzyme activity was defined as that causing the oxidation of one mole of NADPH / min. The enzyme activity was expressed as μmoles of NADPH utilized/min/mg protein.

4.19 NADH-Cytochrome b<sub>5</sub> reductase (EC 1.6.2.2) (*Mihara and Sato, 1972*)

NADH-Cytochrome b<sub>5</sub> reductase was assayed by measuring the rate of reduction of potassium ferricyanide. The reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 1 mM potassium ferricyanide and tissue homogenate in a final volume of 1ml was incubated at 25°C with 0.1 mM NADH. The enzyme activity was calculated using extinction coefficient of 1.02 mM<sup>−1</sup> cm<sup>−1</sup>. One unit of enzyme activity was defined as that causing the reduction of one mole of ferricyanide/min. The enzyme activity was expressed as μmoles of NADH utilized/min/mg protein.

4.20 DT-diaphorase (DT-D) (EC 1.6.99.3) (*Ernestr et al., 1962*)

This method involves measurement of reduction, at 550nm using NADH as the electron donor and 2, 6-dicholorphenolindophenol as the electron acceptor. The reaction was started by adding 50 μl of the homogenate to the reaction mixture containing 2.73ml of phosphate buffer (50mM, pH 7.6), 50 μl of 0.3 mM NADH, 50 μl of 0.4mM DCPIP, 100 μl of 33μM cytochrome ‘c’ and 20 μl 0.07% bovine serum albumin. The enzyme activity was calculated using the extinction coefficient 21mM<sup>−1</sup>cm<sup>−1</sup> and expressed as μmoles of NADH utilized/min/mg protein.
4.21 NADPH Diaphorase (NADPH-D) (EC 1.6.99.1) (Huennekens et al., 1957)

This method is based on reduction of methylene blue to leukomethylene blue during the transfer of hydrogen from NADPH to NADPH-diaphorase. The reaction mixture containing 830μl of phosphate buffer (50mM, pH 7.6), 100 μl of NADPH (2mM) and 20 μl sample was incubated at 37°C for 10 min. To this 50μl of methylene blue (0.8mM) was added. Enzyme activity was assayed by following the decrease in absorbance of NADPH at 340nm. The enzyme activity was calculated using extinction coefficient 6.22mM⁻¹cm⁻¹ and expressed as nmoles of NADPH utilized/ min/ mg protein.

4.22 Glycogen (Nicholas, 1956)

Liver was homogenized (5 % w/v) in TCA (5%) for 3 min at 4°C. The homogenate was spun and to an aliquot of the supernatant, 5 volumes of cold 95% EtOH was added and left overnight to precipitate glycogen. After removal of EtOH, the glycogen precipitate was dissolved in 2 ml of distilled water. Blank and standard were prepared with distilled water and glucose solution (0.5 mg/ml) respectively. 10 ml of Anthrone reagent was added to all the tubes and the tubes were placed in a boiling water bath for 15 min. After cooling, the absorbance was measured at 620 nm against the blank. Glycogen content was measured using glucose standard and the results were expressed as mg of glycogen /g tissue.

4.23 Glycogen phosphorylase (EC 2.4.1.1) (Niemeyer et al., 1961)

The enzyme was assayed by measuring the release of orthophosphate from glucose-1-phosphate in the presence of glycogen and AMP. A stock solution containing 25 mM glucose-1-phosphate, 75 mM NaF, 2.5 mM AMP, and 1% glycogen was prepared in 67 mM citrate buffer, pH 6.0. 1.2 ml of the above solution was incubated for 2 min at 30°C, further 0.2 ml of liver homogenate was added and immediately 0.5 ml of the assay mixture was withdrawn into 10% TCA. After incubation for 5 min at 37°C, an aliquot (0.5 ml)
of the assay mixture was transferred into 10% TCA. The TCA extracts were spun and the iP in an aliquot of supernatant was determined. Results were expressed as nmoles of Pi released/ min / mg protein.

4.24 Inorganic phosphate (Fiske and Subbarow, 1925)

300 μl of the supernatant was added to 3ml of solution containing 2% ammonium molybdate, 6 N H₂SO₄, and 10% ascorbic acid mixed in 1:2:3:4 ratio. The resultant mixture was incubated at 37°C for 1h and read against blank at 820nm. The amount of inorganic phosphate liberated was calculated using potassium dihydrogen phosphate as the standard.

4.25 Glucose-6-phosphatase (G-6-P) (EC 3.1.3.9) (Hers, 1959)

0.1 ml of 1 % liver homogenate prepared in 250 mM sucrose, containing 1 mM EDTA (pH 7.0), was incubated with 50 mM glucose-6-phosphate at 37°C for 30 min. The reaction was stopped by addition of 1ml 10 % TCA, and the phosphate released by the enzymatic activity was measured as described earlier. Results were expressed as nmoles of Pi released /min /mg protein.

4.26 Glucokinase (GK) (EC 2.7.1.2) (Crane and Sols, 1955)

The assay mixture in a volume of 2 ml, consisted of 0.2 IU of G-6-PDH, 0.25 mM NADP, 5 mM ATP, 5 mM MgCl₂ , 5 mM β-mercaptoethanol, 25 μl of liver homogenate and either 0.05 mM glucose or 100 mM glucose. The rate of reduction of NADP by G-6-PDH, which is directly proportional to formation of G-6-P, was monitored at 340 nm. Activity of glucokinase was obtained by subtracting the rate of reduction of NADP with 0.5 mM glucose from that with 100 mM glucose and the results were expressed as μmol of NADP utilized/min/mg protein.
4.27 Glutamate dehydrogenase (GDH) (EC 1.4.1.3) (Bryla et al., 1994)

A solution containing final concentration of NADPH (100 μM), α-ketoglutarate (5 mM), ammonia (50 mM), EDTA (0.1 mM), and bovine serum albumin (1 mg/ml) in 0.1 M phosphate buffer (pH 7.6) was incubated in a 1 cm path length quartz cuvette at 25°C and the reaction was started by adding homogenate (10 μl). The decrease in absorbance at 340 nm was monitored for 1 min. One unit of activity was defined as that amount of enzyme required to oxidize 1 μmol of NADH per minute at 30°C and the results were expressed as moles of NADPH utilized/min/mg protein.

4.28 Protein estimation (Lowry et al., 1951)

Protein content of tissue homogenate and serum was estimated as described earlier (Section 4.4, Chapter I B).

4.29 Histopathology

Pancreas was excised, and blotted. A portion of the organ was fixed in 10% neutral buffered formalin, embedded in paraffin, processed by standard histological techniques (Lillie and Fullmer, 1976), stained with hematoxylin/eosin and examined by light microscopy.

4.30 Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± S.E from six rats in each group. The results were processed by the Programme: Microsoft Office Excel 2002. Paired Students t-test was applied to the results obtained in the two groups of rats: control and DDVP treated. In all cases, P values lower than 0.05 were considered to be statistically significant. Tukey test for multiple comparisons was performed on data of DM toxicity study to determine the significant differences among the groups and ‘p’ values < 0.01 were considered significant.
5.0 RESULTS

5.1 Biochemical perturbations induced in rat pancreas by repeated oral doses of DM

5.1.1 Growth and organ weights

Repeated oral doses of DM failed to induce any distinct clinical signs of toxicity or mortality. Data on the body weights along with the relative organ weights are presented in Table 2.1. While the weight gain in rats of control group at the end of 30 days was 17.9%, rats treated with the higher dose of DM showed only 7.6% weight gain. There was a marked increase (32%) in pancreatic weight in rats administered higher dose of DM (170.73mg ± 6.74 vs. 129.05mg ± 6.43). However, only marginal increases in liver and kidney weights were observed in rats administered with higher dose of DM.

5.1.2 Oral glucose tolerance

Data on blood glucose levels monitored in control and DM treated rats following glucose overload over a 3h period is presented in Fig.2.1. Fasting blood glucose levels in rats of all the three groups were similar. There was no significant difference in blood glucose levels measured at 30 min. However DM treated rats showed higher blood glucose level (135 mg/dl) at 60 min and beyond. The glucose levels in control rats returned to normal level (90 mg/dl) at the end of 3h suggesting normal glucose tolerance while on the other hand DM treated rats showed higher levels of blood glucose (125 and 129 mg/dl) at the end of 3h indicating altered glucose tolerance.

5.1.3 Blood glucose, acetylcholinesterase and reduced glutathione

Data on blood glucose levels, pancreatic AChE activity and reduced glutathione levels in control and DM treated rats are presented in Table 2.2 & Fig. 2.2. The blood glucose levels in DM treated rats were elevated in a dose-dependent manner and the percent increase in glucose levels was 15 and 51 % at the low and high dose respectively. The activity of AChE in pancreatic tissue
was markedly reduced (40 and 90 %) among DM treated rats. While the decrease in the reduced GSH was marginal in the lower dose (11%), it was 18 % at the higher dose.

5.1.4 Pancreatic damage

The activity of enzymes viz., amylase and lipase in serum and pancreatic tissue following DM treatment is presented in Fig. 2.3 & Fig. 2.4. There was a significant increase in serum lipase activity (20 and 38 %) in DM treated rats compared to the activity in control rats (5.79 ± 0.26 nmol PNP/mg protein/min). However, pancreatic tissue lipase activity was significantly decreased in rats administered with DM (18 and 63%) with respect to control (37.1 nmol PNP/mg protein/min). While serum amylase activity was increased (by 2-3 folds) in DM treated rats, pancreatic amylase activity was marginally decreased.

5.1.5 Oxidative stress in pancreas

ROS and TBARS levels determined in the pancreatic tissue are presented in Fig. 2.5. There was a dose-related elevation in ROS levels among treated rats. While the increase at the lower dose was 66%, a dramatic (150%) increase was evident at the higher dose. Concomitantly, a dose-related increase in TBARS levels was observed in DM treated rats. There was 2.5 and 3.7 fold increase in TBARS level at lower and higher doses of DM respectively.

5.1.6 Activities of antioxidant enzymes and phase II enzymes in pancreas

Data on the activities of various antioxidant enzymes in pancreas following DM treatment is presented in Table 2.3 & Fig. 2.6. In general, activities of SOD, CAT, GR and GST were significantly elevated in DM treated rats compared to the control rats. At the higher dose, activities of SOD, CAT, GR and GST were increased by 112%, 64%, 45% and 100% over the control. However, the GPX activity was diminished (50%) at the higher dose.
5.1.7 Activities of phase II enzymes in pancreas

Data on the activities of phase II enzymes such as NADPH-diaphorase and DT-diaphorase are presented in the Table 2.4 & Fig. 2.7. There was a 27% increase in the activity of NADPH-diaphorase and a 16% increase in the activity of DT-diaphorase in the rats treated with higher dose of DM.

Table 2.1 Body weight and relative organ weights of rats administered repeated oral doses of DM for 30 days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DM (mg/kg b.w/d)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>282.67</td>
<td>± 9.04</td>
<td>287.28</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>333.33</td>
<td>± 9.27</td>
<td>327.67</td>
</tr>
<tr>
<td>Pancreas (mg/100g b.w.)</td>
<td>129.05</td>
<td>± 6.43</td>
<td>144.25</td>
</tr>
<tr>
<td>Liver (g/100g b.w.)</td>
<td>3.08</td>
<td>± 0.21</td>
<td>3.25</td>
</tr>
<tr>
<td>Kidney (g/100g b.w.)</td>
<td>0.75</td>
<td>± 0.10</td>
<td>0.74</td>
</tr>
<tr>
<td>Adrenals (mg/100g b.w.)</td>
<td>15.26</td>
<td>± 1.28</td>
<td>15.41</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6);  
<sup>a</sup> Comparison of control and other groups;  
<sup>b</sup> Comparison of DM: 20mg/kg b.w/d treated group with DM: 40 mg/kg b.w/d treated group
Fig. 2.1 Oral glucose tolerance at the end of 30 days in control (CTR) and DM treated rats (DM1: 20 mg/kg b.w/d; DM2: 40 mg/kg b.w/d)

Values are mean ± SEM (n=6)
Table 2.2 Blood glucose, acetylcholinesterase (AChE) and reduced glutathione (GSH) levels in pancreas of rats administered oral doses of DM for 30 days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DM (mg/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Initial blood glucose</td>
<td>85.33 ± 3.85</td>
<td>87.34 ± 5.23</td>
</tr>
<tr>
<td>Final blood glucose</td>
<td>91.33 ± 2.41</td>
<td>105.28 ± 3.57</td>
</tr>
<tr>
<td>AChE</td>
<td>4.96 ± 1.47</td>
<td>2.94 ± 1.75</td>
</tr>
<tr>
<td>GSH</td>
<td>1.11 ± 0.02</td>
<td>0.99 ± 0.05</td>
</tr>
</tbody>
</table>

1 mg/dl; 2 nmoles substrate hydrolyzed/min/mg protein; 3 mg/g tissue
Values are mean ± SEM (n=6);
\* Comparison of control and other groups;
\( \text{a} \) Comparison of DM (20 mg/kg b.w.) group with DM (40 mg/kg b.w.) group

Fig. 2.2 Blood glucose, AChE and GSH levels in pancreas of rats administered oral doses of DM (DM1: 20 mg/kg b.w/d; DM2: 40 mg/kg b.w/d) for 30 days.
Fig 2.3 Activities of lipase in serum and pancreas of control (CTR) and DM treated rats (DM1: 20 mg/kg b.w/d; DM2: 40 mg/kg b.w/d)

Values are mean ± SEM (n=6)

* Comparison of control and other groups (P < 0.01),
Comparer of DM1 with DM2 (P < 0.01)
Fig. 2.4 Activities of amylase in serum and pancreas of control (CTR) and DM treated rats (DM1: 20 mg/kg b.w/d; DM2: 40 mg/kg b.w/d)

Values are mean ± SEM (n=6)

*Comparison of control and other groups (P < 0.01),

†Comparison of DM1 with DM2 (P < 0.01)
Fig. 2.5 ROS levels and extent of LPO in pancreas of control (CTR) and DM treated rats (DM1: 20 mg/kg b.w/d; DM2: 40 mg/kg b.w/d)

Values are mean ± SEM (n=6);
* Comparison of control and other groups (P < 0.01),
* Comparison of DM1 with DM2 (P < 0.01)
Table 2.3 Activities of antioxidant enzymes in pancreas of rats administered oral doses of DM for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD$^1$</th>
<th>CAT$^2$</th>
<th>GPX$^3$</th>
<th>GR$^3$</th>
<th>GST$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>26.42 ± 2.2</td>
<td>9.38 ± 0.31</td>
<td>27.18 ± 5.24</td>
<td>17.50 ± 1.60</td>
<td>0.03 ± 0.004</td>
</tr>
<tr>
<td>DM1</td>
<td>42.72 ± 0.38$^a$</td>
<td>10.24 ± 0.32</td>
<td>25.23 ± 3.89</td>
<td>19.72 ± 2.03</td>
<td>0.04 ± 0.003$^a$</td>
</tr>
<tr>
<td>DM2</td>
<td>56.23 ± 1.18$^{a,b}$</td>
<td>15.44 ± 0.51$^{a,b}$</td>
<td>13.85 ± 2.20$^{a,b}$</td>
<td>25.30 ± 1.30$^{a,b}$</td>
<td>0.06 ± 0.003$^{a,b}$</td>
</tr>
</tbody>
</table>

$^1$ units/mg protein; $^2$ μmol/min/mg protein; $^3$ nmol/min/mg protein; $^4$ μmol/min/mg protein
Values are mean ± SEM (n=6)
$^a$ Comparison of control (CTR) and other groups;
$^b$ Comparison of DM1 (DM: 20mg/kg b.w/d) group with DM2 (DM: 40mg/kg b.w/d) group

Fig. 2.6 Activities of antioxidant enzymes in pancreas of rats administered oral doses of DM (DM1: 20 mg/kg b.w/d; DM2: 40mg/kg b.w/d) for 30 days
Table 2.4 Activities of Phase II enzymes in pancreas of rats administered oral doses of DM for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme Activity</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NADPH-D¹</td>
<td>DT-Diaphorase²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.78 ± 0.27</td>
<td>0.62 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td></td>
<td>9.12 ± 0.37</td>
<td>0.60 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM1</td>
<td></td>
<td>11.17 ± 0.42a,b</td>
<td>0.72 ± 0.02a,b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ nmoles of NADPH utilized/min/mg protein; ² μmoles of NADH utilized/min/mg protein
Values are mean ± SEM (n=6)

* Comparison of control (CTR) and other groups
* Comparison of DM1 (DM: 20mg/kg b.w/d) group with DM2 (DM: 40mg/kg b.w/d) group

Fig. 2.7 Activities of Phase II enzymes in pancreas of rats administered oral doses of DM (DM1: 20 mg/kg b.w/d; DM2: 40mg/kg b.w/d) for 30 days
5.2 Biochemical perturbations induced in rat pancreas by repeated oral doses of DDVP

5.2.1 Body and organ weights

Repeated oral doses of DDVP failed to induce any distinctive clinical signs and symptoms of toxicity or mortality. There was no significant change in the body weights among control and DDVP treated rats. The relative weights of the vital organs of DDVP treated rats were in the same range as that in control rats (data not shown).

5.2.2 Oral glucose tolerance

The blood glucose levels monitored in control and DDVP treated rats following glucose overload over 3h period is presented in Fig.2.8. Fasting blood glucose levels in DDVP treated rats were significantly higher (108 mg/dl) compared to that of control rats (75 mg/dl). DDVP treated rats showed higher blood glucose level at all the measured time points. Blood glucose levels in control rats returned to normal level (99 mg/dl) at the end of 3h suggesting normal glucose tolerance. On the other hand, DDVP treated rats showed higher levels of glucose (133 mg/dl) at the end of 3h indicating altered blood glucose tolerance.

5.2.3 Blood glucose

Data on the blood glucose levels in control and DDVP treated rats is presented in Table 2.5. Blood glucose level in DDVP treated rats was elevated significantly (75%) at the end of 10 days.

5.2.4 Pancreatic damage

The activity of enzymes viz., lipase and amylase in serum and pancreas of rats following DDVP intoxication are presented in Table 2.6 & Fig. 2.9. There was a 35 % increase in serum lipase activity and 12 % increase in serum amylase activity in rats administered DDVP. However, pancreatic lipase activity
was significantly reduced (26%), while pancreatic amylase activity was markedly elevated (47%) in rats administered DDVP. While GGT activity in serum was markedly higher (86%), serum LDH activity was elevated by 45% indicating the generalized tissue damage due to DDVP administration (Table 2.7 & Fig. 2.10). Similarly, pancreatic GGT activity was elevated by 58% while pancreatic LDH activity was reduced significantly (31%) in DDVP treated rats.

5.2.5 ChE activity in tissues

Acetylcholinesterase enzyme activity in various vital organs of rats administered DDVP is presented in Table 2.8 & Fig. 2.11 and BuChE activity is presented in Table 2.9 & Fig. 2.12. AChE activity was reduced in all the vital tissues to varying extent due to DDVP administration while both AChE and BuChE were equally and significantly reduced (30%) in pancreas. Adrenal AChE and BuChE activity were decreased by 27% while liver AChE was not affected and BuChE (34%) was reduced. Serum pseudocholinesterase activity in rats administered DDVP were 35 % and 42% lower than the control values.

5.2.6 Oxidative damage in pancreas

The levels of ROS and LPO in tissues of rats of various groups are presented in Table 2.10 & Fig. 2.13. ROS generation was significantly increased in all the vital organs such as pancreas (51%), adrenals (61%), liver (22%) and brain (37%). A significant increase in the levels of TBARS was also evident in pancreas (38%), adrenals (64%), liver (23%) and brain (50%).

5.2.7 Non-enzymic antioxidant levels in tissues

The levels of reduced glutathione were marginally decreased in adrenals (13%), liver (15%) and brain (16%) while, the levels were increased marginally in pancreas. Oxidized glutathione levels were decreased in pancreas (20%), adrenals (30%) and liver (31%) but increased in brain (70 %) of rats administered DDVP. Protein carbonyl levels in all the organs were increased
significantly with pancreas showing 28% increase over control (Table 2.11 & Fig. 2.14).

5.2.8 Antioxidant enzymes in tissues

Activities of various antioxidant enzymes in tissues of rats subjected to repeated oral doses of DDVP are presented in Table 2.12 & 2.13; Fig. 2.15 & 2.16. The enzymes activities were elevated in tissues of rats treated with DDVP except in brain where the activities of GPX and GST were decreased. In pancreas, increase in the enzyme activities over control were as follows: catalase (44%), SOD (76%), GR (30%), GPX (68%) and GST (27%); in adrenals: catalase (105%) and SOD (28%); in liver: GR (50%) and GPX (50%). In brain, the activities of SOD and GR were significantly increased while, the activity of GPX was significantly reduced compared to that in control rats.

5.2.9 Phase I enzymes in liver and pancreas

Activities of Phase I enzymes in pancreas and liver of rats administered DDVP along with control values are presented in Table 2.14 & Fig. 2.17. In liver, cytochromeB5 reductase activity was elevated marginally (9%) in rats administered DDVP, and there was marginal decrease (13 %) in cytochromeP450 reductase activity. Similarly in pancreas, the activity of cytochromeB5 reductase was increased marginally (12%) while activity of cytochrome P450 reductase enzyme was decreased (22%) in treated rats.

5.2.10 Phase II enzymes in liver and pancreas

DT-diaphorase and NADPH-diaphorase activity were reduced by 16 and 18% respectively in liver of DDVP administered rats while, in pancreas, activity of DT-diaphorase was increased significantly (41%), and the activity of NADPH-diaphorase was decreased (33 %) (Table 2.15 & Fig. 2.18).
5.2.11 Glycogen and carbohydrate metabolizing enzymes in liver

Liver glycogen and carbohydrate metabolizing enzymes in rats subjected to DDVP intoxication are presented in Table 2.16 & Fig. 2.19. Liver glycogen was reduced significantly (40%) due to DDVP administration. Activities of glycogen phosphorylase and glucose-6-phosphatase enzymes were significantly increased (by 100 % and 87% respectively) while a 50 % reduction in the activity of liver glucokinase was evident in liver of DDVP treated rats.

5.2.12 Glucokinase and Glutamate dehydrogenase activities in pancreas

Pancreatic glucokinase and glutamate dehydrogenase enzyme activities in control and DDVP administered rats are presented in Table 2.17 & Fig. 2.20. Both the enzyme activities were reduced significantly (by 52% and 37%) in pancreas of DDVP treated rats.

5.2.13 Histopathology of pancreas

The islets in the pancreas of control rats (Fig. 2.21 A and C) appeared to be mostly intact while, most of the islet tissue in pancreas of DDVP-treated rats (Fig. 2.21 B and D) was atrophied. Pancreatic histology of DDVP administered rats revealed packed cells with mixed inflammatory cells and areas of hemorrhage, suggestive of acute inflammatory lesion.
Table 2.5 Blood glucose levels in rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/dl)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>95.33 ± 3.85</td>
<td>100.14 ± 9.06</td>
<td></td>
</tr>
<tr>
<td>DDVP</td>
<td>87.34 ± 5.23</td>
<td>150.85 ± 5.83</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of six rats in each group.
Values significantly different from controls by Student's t Test are indicated:

* P < 0.01

Fig. 2.8 Oral glucose tolerance at the end of 10 days in control (CTR) and DDVP (20 mg/kg b.w./d) treated rats

Values are mean ± SEM (n=6)
Table 2.6 Activities of lipase and amylase in serum and pancreas of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Lipase^1</th>
<th>Amylase^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Control</td>
<td>35.13 ± 2.92</td>
<td>397.14 ± 35.38</td>
</tr>
<tr>
<td>DDVP</td>
<td>47.32 ± 2.77^a</td>
<td>292.54 ± 15.04^a</td>
</tr>
</tbody>
</table>

^1 nmol PNP/min/mg protein; ^2 Street Close Unit

Values are mean ± SEM (n=6); Values significantly different from controls by Student’s t Test are indicated: ^a P< 0.05; ^b P< 0.01

Fig. 2.9 Activities of lipase and amylase enzymes in serum and pancreas of control and DDVP (20 mg/kg b.w/d for 10 d) treated rats
Table 2.7 Activities of γ-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) in serum and pancreas of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>GGT$^1$</th>
<th></th>
<th>LDH$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Pancreas</td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>49.49 ± 7.07</td>
<td>938.89 ± 146.91</td>
<td>24.00 ± 2.92</td>
</tr>
<tr>
<td>DDVP</td>
<td>91.91 ± 14.14$^a$</td>
<td>1480.46 ± 98.08$^a$</td>
<td>34.83 ± 0.90$^b$</td>
</tr>
</tbody>
</table>

$^1$IU/L; $^2$μmol/min/mg protein
Values are mean ± SEM (n=6); Values significantly different from controls by Student's t Test are indicated: $^a$ P ≤ 0.05; $^b$ P ≤ 0.01

Fig. 2.10 Activities of GGT and LDH in serum and pancreas of control and DDVP (20 mg/kg b.w/d for 10 d) treated rats
Table 2.8 Acetylcholinesterase (AChE) activity in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Pancreas</th>
<th>Adrenals</th>
<th>Liver</th>
<th>Brain</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.77</td>
<td>12.39</td>
<td>7.07</td>
<td>64.19</td>
<td>8.95</td>
</tr>
<tr>
<td></td>
<td>± 1.17</td>
<td>± 0.55</td>
<td>± 0.27</td>
<td>± 9.39</td>
<td>± 0.19</td>
</tr>
<tr>
<td>DDVP</td>
<td>6.80</td>
<td>8.92</td>
<td>6.58</td>
<td>42.71</td>
<td>5.85</td>
</tr>
<tr>
<td></td>
<td>± 0.47(^{a})</td>
<td>± 0.68(^{c})</td>
<td>± 0.28</td>
<td>± 0.82(^{a})</td>
<td>± 0.49(^{a})</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6)
Values significantly different from control by Student's t Test are indicated:
\(^{a}\) P< 0.05, \(^{c}\) P< 0.001

Fig. 2.11 AChE activity in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.9 Butyrylcholinesterase (BuChE) activity in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Adrenal</th>
<th>Pancreas</th>
<th>Liver</th>
<th>Brain</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.01±0.09</td>
<td>5.25±0.33</td>
<td>3.99±0.37</td>
<td>4.78±0.19</td>
<td>0.72±0.04</td>
</tr>
<tr>
<td>DDVP</td>
<td>3.66±0.20a</td>
<td>3.82±0.12a</td>
<td>2.65±0.38</td>
<td>3.31±0.13c</td>
<td>0.42±0.03b</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6)
Values significantly different from controls by Student’s t Test are indicated:

- a P≤ 0.05,
- b P≤ 0.01,
- c P≤ 0.001

Fig. 2.12 BuChE activity in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.10 ROS and TBARS levels in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ROS$^1$</th>
<th>TBARS$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DDVP</td>
</tr>
<tr>
<td>Pancreas</td>
<td>57.50 ± 0.05</td>
<td>86.63 ± 5.72$^a$</td>
</tr>
<tr>
<td>Adrenals</td>
<td>99.76 ± 8.15</td>
<td>160.51 ± 5.70$^b$</td>
</tr>
<tr>
<td>Liver</td>
<td>81.46 ± 3.62</td>
<td>99.66 ± 4.39$^c$</td>
</tr>
<tr>
<td>Brain</td>
<td>220.17 ± 8.03</td>
<td>302.53 ± 22.98$^a$</td>
</tr>
</tbody>
</table>

$^1$ pmol DCF/min/mg protein; $^2$ nmol MDA/g tissue
Values are mean ± SEM (n=6)
Values significantly different from controls by Student’s t Test are indicated:
$^a$ P≤ 0.05; $^b$ P≤ 0.01; $^c$ P≤ 0.001

Fig 2.13 ROS and TBARS levels in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
### Table 2.11 GSH, GSSG and Protein Carbonyl (PC) Levels in Tissues of Rats Administered Oral Doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>GSH&lt;sup&gt;1&lt;/sup&gt;</th>
<th>GSSG&lt;sup&gt;2&lt;/sup&gt;</th>
<th>PC&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DDVP</td>
<td>Control</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.01 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.12 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>716.71 ± 62.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adrenals</td>
<td>1.38 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.20 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9267.81 ± 589.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>1.55 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2180.7 ± 308.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain</td>
<td>1.78 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1993.88 ± 343.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>mg/g tissue; <sup>2</sup>pmol/mg tissue; <sup>3</sup>nmol/mg protein

Values are mean ± SEM (n=6); Values significantly different from controls by Student’s t Test are indicated: <sup>a</sup> P< 0.05; <sup>b</sup> P< 0.01

**Fig 2.14** GSH, GSSG and PC levels in tissues of rats administered oral dose of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.12 Activities of catalase (CAT) and superoxide dismutase (SOD) in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DDVP</th>
<th>Control</th>
<th>DDVP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT^</td>
<td>SOD^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.76 ± 0.26</td>
<td>3.97 ± 0.05^a</td>
<td>13.69 ± 2.06</td>
<td>24.03 ± 3.97^a</td>
</tr>
<tr>
<td>Adrenals</td>
<td>15.47 ± 0.88</td>
<td>31.78 ± 7.94^b</td>
<td>58.71 ± 1.88</td>
<td>75.09 ± 2.09^b</td>
</tr>
<tr>
<td>Liver</td>
<td>522.56 ± 23.49</td>
<td>661.60 ± 92.33</td>
<td>57.58 ± 4.13</td>
<td>80.33 ± 12.48</td>
</tr>
<tr>
<td>Brain</td>
<td>0.32 ± 0.017</td>
<td>0.40 ± 0.048</td>
<td>52.67 ± 4.38</td>
<td>68.86 ± 6.51^a</td>
</tr>
</tbody>
</table>

^1μmol/min/mg protein; ^2units/mg protein
Values are mean ± SEM (n=6); Values significantly different from controls by Student's t Test are indicated: ^a P≤ 0.05; ^b P≤ 0.01

Fig. 2.15 Activities of CAT and SOD in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.13 Activities of glutathione-dependent antioxidant enzymes in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>GR$^1$</th>
<th>GPX$^1$</th>
<th>GST$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DDVP</td>
<td>Control</td>
</tr>
<tr>
<td>Pancreas</td>
<td>23.59</td>
<td>30.66</td>
<td>32.15</td>
</tr>
<tr>
<td></td>
<td>± 1.06</td>
<td>± 1.33$^a$</td>
<td>± 1.99</td>
</tr>
<tr>
<td>Adrenals</td>
<td>30.24</td>
<td>33.38</td>
<td>179.53</td>
</tr>
<tr>
<td></td>
<td>± 1.91</td>
<td>± 1.24</td>
<td>± 29.47</td>
</tr>
<tr>
<td>Liver</td>
<td>58.48</td>
<td>87.71</td>
<td>132.70</td>
</tr>
<tr>
<td></td>
<td>± 1.99</td>
<td>± 3.66$^b$</td>
<td>± 10.9</td>
</tr>
<tr>
<td>Brain</td>
<td>22.09</td>
<td>27.25</td>
<td>80.39</td>
</tr>
<tr>
<td></td>
<td>± 0.64</td>
<td>± 1.68$^b$</td>
<td>± 9.28</td>
</tr>
</tbody>
</table>

$^1$nmol/min/mg protein; $^2$µmol/min/mg protein
Values are mean ± SEM (n=6)
Values significantly different from controls by Student's $t$ Test are indicated: $^a$ P ≤ 0.05; $^b$ P ≤ 0.01

Fig. 2.16 Activities of glutathione-dependent antioxidant enzymes in tissues of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.14 Activities of Phase I enzymes in pancreas and liver of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Control (μmol/min/mg protein)</th>
<th>DDVP (μmol/min/mg protein)</th>
<th></th>
<th>Control (μmol/min/mg protein)</th>
<th>DDVP (μmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytochrome B&lt;sub&gt;5&lt;/sub&gt; Reductase</td>
<td>Cytochrome P&lt;sub&gt;450&lt;/sub&gt; Reductase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>500.33 ± 30.44</td>
<td>560.00 ± 36.47</td>
<td>11.80</td>
<td>1508.33 ± 59.06</td>
<td>1649.00 ± 7.57</td>
</tr>
<tr>
<td>Liver</td>
<td>1508.33 ± 59.06</td>
<td>1649.00 ± 7.57</td>
<td>163.78</td>
<td>142.67 ± 5.27</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6)
Values significantly different from controls by Student's t Test are indicated:

\( ^a \) P ≤ 0.05

Fig. 2.17 Activities of Phase I enzymes in pancreas and liver of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.15 Activities of Phase II enzymes in pancreas and liver of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>DT-Diaphorase 1</th>
<th>NADPH-Diaphorase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control DDVP</td>
<td>Control DDVP</td>
</tr>
<tr>
<td>Pancreas</td>
<td>9.34 ± 0.99</td>
<td>13.16 ± 0.93 a</td>
</tr>
<tr>
<td></td>
<td>13.51 ± 1.87</td>
<td>9.07 ± 0.35 a</td>
</tr>
<tr>
<td>Liver</td>
<td>115.14 ± 2.54</td>
<td>96.46 ± 5.99 a</td>
</tr>
<tr>
<td></td>
<td>128.89 ± 4.27</td>
<td>106.19 ± 5.25 a</td>
</tr>
</tbody>
</table>

1. µmol NADH utilized/min/mg protein; 2. nmol NADPH utilized/min/mg protein
Values are mean ± SEM (n=6)
Values significantly different from controls by Student’s t Test are indicated: a P ≤ 0.05

Fig. 2.18 Activities of Phase II enzymes in pancreas and liver of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.16 Glycogen content and carbohydrate metabolizing enzyme activities in liver of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Glycogen*</th>
<th>Glycogen phosphorylase** (GP)</th>
<th>Glucose-6-phosphatase** (G-6-P)</th>
<th>Glucokinase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63.42 ± 2.21</td>
<td>10.56 ± 0.54</td>
<td>872.00 ± 130.00</td>
<td>71.90 ± 4.75</td>
</tr>
<tr>
<td>DDVP</td>
<td>37.95 ± 7.94\textsuperscript{a}</td>
<td>20.09 ± 3.15\textsuperscript{b}</td>
<td>1631.00 ± 283.00\textsuperscript{b}</td>
<td>35.64 ± 6.10\textsuperscript{b}</td>
</tr>
</tbody>
</table>

* mg/g tissue; ** nmol iP/min/mg protein; \# pmol/min/mg protein
Values are mean ± SEM (n=6)
Values significantly different from controls by Student's t Test are indicated: \textsuperscript{a} P≤ 0.05;
\textsuperscript{b} P≤ 0.01

Fig. 2.19 Glycogen content and carbohydrate metabolizing enzyme activities in liver of rats administered oral dose of DDVP (20 mg/kg b.w/d for 10 d)
Table 2.17 Glucokinase and glutamate dehydrogenase enzyme activity in pancreas of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)

<table>
<thead>
<tr>
<th></th>
<th>Glucokinase*</th>
<th></th>
<th>Glutamate dehydrogenase**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.61 ± 5.13</td>
<td>27.98 ± 3.35</td>
<td></td>
</tr>
<tr>
<td>DDVP</td>
<td>21.98 ± 3.97c</td>
<td>17.53 ± 1.29a</td>
<td></td>
</tr>
</tbody>
</table>

* μmol/min/mg protein; ** mmol/min/mg protein
Values are mean ± SEM (n=6)
Values significantly different from controls by Student’s t Test are indicated:
\[ P \leq 0.05; \quad ^a P \leq 0.001 \]

Fig 2.20 Glucokinase and glutamate dehydrogenase activity in pancreas of rats administered oral doses of DDVP (20 mg/kg b.w/d for 10 d)
Fig 2.21. Hematoxylin-eosin stained sections of pancreas of control rats showing normal islets with clusters of purple stained β-cells (A & C); DDVP-treated (20 mg/kg b.w/d for 10 d) rats showing irregular morphology of islets with degenerative changes and infiltration (B & D); (A & B: 10X; C & D: 40X)
6.0 DISCUSSION

The mechanisms involved in blood glucose alterations following OPI exposure have been under investigation in recent years since hyperglycemia is one of the prominent side effects of OPI poisoning in humans (Namba et al., 1971; Hayes et al., 1978; Meller et al., 1981). The ability of OPI to cause hyperglycemia has also been demonstrated in laboratory animals (Matin and Siddiqui, 1982; Fletcher et al., 1988). OPI are known to impair both, endocrin and biochemical functions of pancreas (Sikk et al., 1985; Hsiao et al., 1996). Accordingly, the present study addressed the potential of repeated doses of dimethoate (DM) and dichlorvos (DDVP) to induce perturbations in glucose homeostasis and also examined whether oxidative stress plays a role in these OPI-induced pancreatic dysfunctions.

Our results showed that oral administration of DM at both the dosages for one month induced significant elevation in blood glucose levels in rats. Further, we observed significant alteration in oral glucose tolerance among DM-treated rats. These data are in agreement with the earlier reports, which have shown elevated blood glucose levels after administration of DM (Hagar et al., 2002). Further, we observed increased blood glucose levels in rats administered repeated oral doses of DDVP for 10 days accompanied with altered glucose tolerance at the end of the treatment period. Earlier, hyperglycemic effect has been reported in experimental animals following both acute and chronic OPI exposures (Begum and Vijayaraghavan, 1999; Sarin and Gill, 1999; Abdollahi et al., 2004a; Pournourmohammadi et al., 2005).

Hyperglycemia has been suggested to occur as a consequence of increased accumulation of acetylcholine (ACh) at the nerve endings in pancreas subsequent to AChE inhibition (Gallo and Lawryk, 1991). In the present study, we observed significant dose-dependent AChE inhibition in pancreatic tissue following administration of DM, which is indicative of OPI intoxication. Administration of DDVP for 10 days resulted in inhibition of AChE in both
pancreas and brain to an equal extent. The possible pathogenetic sequel speculated for the pancreatic insult in OP poisoning is excessive cholinergic stimulation of the pancreas and ductal hypertension (Hsiao et al., 1996, Sahin et al., 2002). Pancreas is a sensitive organ and hence pressure elevation can cause severe tissue damage.

In the present study, repeated oral doses of DDVP significantly inhibited BuChE in pancreas. It is known that butyrylcholinesterase enzyme is more abundant in pancreatic tissue (Sahin et al., 2002; Harputluoglu et al., 2003). Although the real substrate(s) is still unknown, BuChE can hydrolyze hydrophobic and hydrophilic carboxylic or phosphoric acid ester containing compounds. Butyrylcholinesterase (pseudocholinesterase or nonspecific cholinesterase) has no known physiological function but it can be considered as an endogenous scavenger of anticholinesterase compounds. BuChE detoxifies them before they reach to AChE at physiologically important target sites (Cokugras, 2003). Our results indicate the possible extent of protection offered by BuChE against inhibition of AChE in pancreas.

Liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose as glycogen and the release of glucose via glycogenolysis and gluconeogenesis (Hers, 1990; Nordlie et al., 1999; Abdollahi et al., 2003). Liver glycogenolysis and gluconeogenesis might be stimulated, at least to some extent, by the detoxification mechanisms, essential for metabolism or degradation and elimination of the pesticides from the body (Begum and Vijayaraghavan, 1999). Acute intraperitoneal treatment of rats with diazinon has been reported to result in depletion of brain glycogen and increase the activities of glycogen phosphorylase (GP) (Matin and Husain, 1987a, b). Treatment of rats with acute diazinon also resulted in hyperglycemia, depletion of glycogen from brain and peripheral tissues accompanied by increased activity of GP in the brain and liver, and increased activity of the hepatic gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK)
Sublethal single dose of dimethoate an OP insecticide has been shown to increase the hepatic and muscle GP activity of Indian catfish (*Clarias batrachus*; Begum and Vijayaraghavan 1995, 1999). In another study, fish exposed to sublethal doses of malathion showed muscle glycogenolysis with concomitant hyperglycemia (Mishra and Srivastava, 1983).

Chronic exposure to Dichlorvos is also reported to cause a significant depletion in brain glycogen content accompanied with an increase in the activity of GP (Sarin and Gill, 2000). Dichlorvos exposure has also been reported to decrease liver glycogen content and affect glucose homeostasis (Omkar and Shukla, 1985; Verma et al., 1983). Single dose of DDVP (50% LD$_{50}$) has been found to increase the activity of glycogen phosphorylase, while decreasing UDP-glucose pyrophosphorylase activity (Teichert-Kuliszenewska et al., 1981). Recent study of Abdollahi et al., (2004a) reported stimulatory effect of liver GP in rats exposed to Malathion. One possible explanation for stimulated GP and increased glycogenolysis could be the release of catecholamines from adrenal medulla. Therefore one mechanism suggested for increased activities of liver GP in response to OPI exposure might be the induction of metabolic processes to meet the augmented stress conditions, which could finally result in hyperglycemia and diabetes.

Hepatic glucokinase plays an important role in glycogen synthesis (Ferre et al., 1996). Increased glucokinase activity has a potent enhancing effect on glycogen synthesis (Seoane et al., 1996; O'Doherty et al., 1996). In contrast, impaired hepatic glycogen synthesis is observed in glucokinase deficiency (Postic et al., 1999). Stimulatory effect of malathion on activities of GK and GDH was reported by Panahi et al., (2006). In fact, it has been proposed that the glycolysis and gluconeogenesis are stimulated to counteract increased oxidative stress conditions caused by OPI (Begum and Vijayaraghavan, 1999; Abdollahi et al., 2004b). In the present study, DDVP treatment induced a reduction in the activity of both hepatic and pancreatic
glucokinase. Pancreatic beta cells also play a major role in maintaining glucose homeostasis through secretion of insulin in response to changes in the extracellular glucose. Glucose-stimulated insulin secretion is regulated by the rate of glucose metabolism within beta cells (Megalsson and Matschinsky, 1986; Newgard and McGarry, 1995) and a key event in this process is the phosphorylation of glucose by glucokinase (GK). GK, also referred as hexokinase IV, is a member of the mammalian hexokinase (HK) family that catalyses the initial step in glucose metabolism by the most metabolic pathways (e.g. glycolysis, pentose phosphate cycle and glycogen synthesis) (Wilson, 1995). In beta cells, glucokinase plays a key role in regulating insulin secretion in response to glucose (Megalsson and Matschinsky, 1986; Matshinsky, 1996).

In the present study, DM administration significantly increased the activity of lipase in serum. This finding is suggestive of the potential of DM to cause acute pancreatitis, since the increase in lipase activity by two-fold is specific for the diagnosis for pancreatitis. Increased serum lipase activity has also been reported after administration of methidathion, an OPI (Mollaoglu et al., 2003). Several studies have demonstrated acute pancreatitis after oral exposure to various OPI (Dressel et al., 1979, Hsiao et al., 1996, Moore and James, 1988). Our results corroborate with earlier reports of acute pancreatitis in humans after accidental cutaneous exposure to DM (Marsh et al., 1988). Another biochemical marker, often employed to evaluate pancreatitis, is the increased amylase activity. DM and DDVP treatment elevated serum amylase activity supporting the evidence of pancreatic damage. Similar increase in amylase activity has been reported in dogs after diazinon administration (Dressel et al., 1982).

Our histopathological evidences confirmed the pancreatic damage induced by DDVP. The degeneration of the acini observed in the section of pancreas of DDVP treated rats might be considered as a toxic inflammatory process inducing pancreatitis. Chronic dimethoate administration has been
shown to induce degenerative changes in pancreatic islets (Hagar et al., 2002), while several other reports have also suggested that acute pancreatitis may follow oral ingestion of OPI such as mevinphos, malathion, parathion and diazinon (Hsiao et al., 1996; Moore and James, 1989; Dressel et al., 1979).

Another major question addressed in the present investigation was whether the OPI-induced pancreatic dysfunction is mediated through oxidative stress. Active oxygen metabolism plays a vital role in the normal functioning of the pancreas. Several key enzymes in reactive oxygen species (ROS) defense are usually low in pancreatic islets compared to other tissues suggesting that islet cells are uniquely susceptible to oxidative stress-induced damage (Grankvist et al., 1981, Kakkar et al., 1998). Further, the low levels of antioxidant enzyme gene expression may account for exquisite sensitivity of β-cell to ROS and free radical induced damage leading to β-cell death and type-1 diabetes (Ho and Bray, 1999). Oxidative stress has been implicated to be an important component in the toxicity of several OPI (Yamano and Morita, 1993; Bagchi et al., 1995; Yamano and Morita, 1995; Yang and Dettbarn, 1996). In the present study, we obtained a dose-dependent increase in levels of ROS and TBARS in pancreas due to both DM and DDVP treatment. These findings suggest that exposures to both DM and DDVP induce significant oxidative stress in the pancreas thereby leading to its dysfunction.

We also observed increased activities of serum and pancreatic GGT and LDH in DDVP treated rats. γ-glutamyltranspeptidase, a key enzyme in GSH metabolism provides high intracellular levels of GSH required for conjugation by GST (Stark et al., 2002). In the current investigation, the observed increase in the activity of GGT both in serum and pancreas may be due to the tissue response to cope up with the oxidative stress. The biological significance of GGT-dependent LPO in vivo might be multifold. It is conceivable that the prooxidant effects of GGT activity are normally balanced by its established role in favouring the cellular uptake of precursors for GSH re-synthesis, thus
allowing the reconstitution of cellular antioxidant defence. We also observed increased activity of LDH in serum and pancreas of rats administered DDVP. LDH is a pivotal cellular metabolic enzyme between the glycolytic pathway and carboxylic acid cycle (Lehninger, 1984). Lactate dehydrogenase is an oligomeric enzyme and cytoplasmic marker enzyme, which is well known indicator of damage by xenobiotic compounds (Reddy and Lokesh, 1996).

Pesticides that induce free radical generation may also oxidatively modify cellular proteins. Stadtman (1992) reported that some amino acid residues are oxidized to carbonyl derivatives and consequently, the carbonyl content of proteins is being employed as a measure of protein damage. In our study, DDVP significantly elevated the levels of PC in pancreas. This correlated with the pattern of OS response seen in pancreas.

Depletion of GSH, the most abundant cellular non-protein thiol, is associated with oxidative stress and cytotoxicity of pro-oxidant xenobiotic. Glutathione is presumed to be an important endogenous defence against the peroxidative destruction of cellular membranes. GSH can act either to detoxify activated oxygen species such as H₂O₂ or to reduce lipid peroxides themselves (Freeman et al., 1982). Tissue GSH concentration reflects the potential for detoxification and the levels may decrease due to an increased use of glutathione to detoxify. Enhanced status of lipid peroxidation and concomitant depletion of GSH pools in pancreas provides further evidence for the occurrence of the oxidative stress in DM treated rats. Interestingly, we did not observe any alteration in GSH levels in pancreas of DDVP treated rats. This could probably due to the shorter treatment regime or suggestive of lesser oxidative stress in pancreas.

Cytosolic free radicals are removed either non-enzymatically or by antioxidant enzymes such as SOD and GPX, which oxidize GSH to GSSG. GSSG is then reduced back to GSH by GR through oxidation of NADPH to NADP+, which is recycled by the pentose phosphate pathway. Furthermore,
glutathione S-transferase (GST) catalyzes the conjugation of GSH to OPI, leading to its detoxification and elimination (Ziegler, 1985). The level of GSSG was significantly reduced in pancreas of DDVP treated rats.

The results of the present study revealed that levels of all the antioxidant components namely, SOD, CAT, GPX and GR, which take care of ROS generated \textit{in vivo} were enhanced by DM and DDVP. Thus, the superoxide generated is accounted for by the enhanced SOD and converted to \( \text{H}_2\text{O}_2 \), which in turn is converted to \( \text{H}_2\text{O} \) by catalase or GPX. The GSSG generated thereby is again reduced by GR, thus replenishing the depleted pool of GSH. This may be the reason that the level of GSH remained unchanged following DDVP treatment. Our results showed increased activity of SOD and CAT in the pancreas of DM as well as DDVP treated rats. Superoxide dismutase is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays a protective role against ROS-induced oxidative damage. The increased activity of SOD in DM and DDVP treated rats probably indicates an activation of the compensatory mechanism through the effects of DM on progenitor cells. Pancreas also has certain amount of CAT activity and the elevated activity of CAT in DM and DDVP treated rats may be due to an adaptive response to the generated free radicals (Koner et al., 1997). These findings suggest that a state of oxidative stress is induced in pancreas of DM and DDVP treated rats.

We also observed a decrease in the activity of GPX and increase in the activity of GR in pancreas of DM treated rats while, pancreas of DDVP treated rats showed increase in activity of both GPX and GR. Knowing that GR is the enzyme responsible for providing reduced GSH from its oxidized form (GSSG) and that GGT activity is important for the re-synthesis of GSH inside the cell, it is not surprising, therefore, to detect an increase in GR activity as a compensatory mechanism for replenishing the GSH concentration inside the cell. GPX converts \( \text{H}_2\text{O}_2 \) or other lipid peroxides to water or hydroxy lipids, and
during this process GSH is converted to GSSG. To recycle GSSG, the cell utilizes the enzyme NADPH-dependent GSH reductase, the NADPH being supplied to the reaction by glucose-6-phosphate dehydrogenase (Bachowski et al., 1997).

Many environmental chemicals that are not chemically reactive are metabolically activated by phase I and phase II xenobiotic-metabolizing enzymes in order to exert their effects. In normal cells, the reactive intermediates formed by phase I reactions are conjugated with glucuronides, sulfate or glutathione, facilitating their excretion. The conjugation reactions are catalyzed by phase II biotransformation enzymes including glutathione-s-transferase, DT-diaphorase, sulfotransferases and UDP-glucuronyl transferases (Shweita and Timisany, 2003). While Phase I enzymes increase the toxicity of a chemical, Phase II enzymes serve to detoxify the electrophilic metabolites. Imbalances in phase I and Phase II metabolizing enzymes has been reported in various disease conditions (Pelkonen et al., 1999; Subapriya et al., 2005; Williams and Phillips, 2000).

Cytochrome P450, a phase I enzyme catalyses the oxidation of lipophilic chemicals via C- or N-hydroxylation to electrophilic ultimate carcinogens. The catalytic efficiency of cytochrome P450 is enhanced by the ubiquitous electron transport protein cytochrome b5. The reactive metabolites generated by phase I enzymes interact with DNA, including mutations that can initiate oncogene transformation (Modugno et al., 2003; Williams and Philips, 2000). In the present study, DDVP treatment resulted in decreased activity of cytochrome P450 reductase. Pancreatitis represents a manifestation of uncoordinated detoxification reactions between pancreatic cytochrome P450 monooxygenases and phase II conjugating enzymes, resulting in the irreversible consumption of glutathione in the acinar cell (Walling, 1998).

Activities of the GSH-dependent phase II detoxifying enzymes in pancreas, GR and GST were also significantly increased in DM and DDVP-
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treated rats. Among the phase II enzymes, GSTs are a family of enzymes that catalyzes the conjugation of reactive chemicals with GSH and play a major role in protecting cells. After generating conjugated GSH, these are subsequently eliminated via a GSH conjugated-recognizing transporter (Nakamura et al., 2000). The inducers of GST were suggested to induce isoforms of GST, which specifically detoxify products of free radical damage (Fiander and Schneider, 2000). The increase in GST activity in the pancreatic tissue among DM and DDVP treated rats indicate the role played by this system in detoxification of these OPI.

Interestingly, we found increase in activity of DT-diaphorase in pancreas of both DM and DDVP treated rats. DT-diaphorase and NADPH-diaphorase are generally induced in coordination with GST and GGT that are involved in protecting cells against toxins and carcinogens. While DT-diaphorase, a flavoprotein facilitates the 2-electron reduction of quinones, NADPH diaphorase catalyzes hydrogen transfer from NADPH (Begleiter et al., 1997; Huennekens et al., 1957). Phase II enzymes, such as GST, and DT-diaphorase, are considered to be a major mechanism of protection against chemical stress (Gerhauser et al., 1997). They are a class of widely distributed enzymes that detoxify toxins either by destroying their reactive centers or by conjugating them with endogenous ligands, facilitating their excretion. DT-diaphorase is a flavoprotein that catalyses the two electron reduction of quinines to hydroquinones and nitrogen oxide. This reaction prevents the formation of semiquinones by one electron reduction and in turn the generation of free radicals from the autooxidation of semiquinone. Reduction of quinones and nitrogen oxide might also make them available for conjugation with UDP-glucuronic acid, facilitating their excretion (Begleiter et al., 1997; Talalay et al., 1995).

In conclusion, our results indicate that both DM and DDVP have the propensity to significantly alter glucose homeostasis and the associated
oxidative impairments observed in pancreas may wholly or in part contribute towards the development of pancreatitis in adult rats. Our studies also demonstrate the impairment in carbohydrate metabolism in rats due to OPI exposures.

7.0 SUMMARY

1. Administration of multiple oral doses of DM (20 and 40 mg/kg b.w/d for 30 d) caused significant elevation (15 and 51%) in blood glucose levels in rats.
2. DM treated rats subjected to oral glucose tolerance test at the end of 30 d showed significantly higher blood glucose levels (135 mg/dl) at 60 min and beyond which remained high (125 and 129 mg/dl) up to 3 h suggesting altered glucose tolerance.
3. A marked increase (32%) in pancreatic weight was evident in DM treated rats only at the higher dose.
4. DM treated rats showed significant elevation in serum lipase (20 and 38%) and amylase (2-3 folds) activities which were accompanied with marginal decrease in lipase (18 and 63%) and significant decrease in amylase activity in pancreas.
5. Dose-related elevation in ROS (66% and 150%) and lipid peroxide levels were accompanied by marginal decrease in the reduced GSH levels in DM treated rats.
6. At the high dose, increased activities of antioxidant enzymes such as SOD, CAT, GR and GST and altered activities of xenobiotic metabolizing enzymes viz., NADPH-diaphorase (27% increase) and DT-diaphorase were evident.
7. Marked reduction (40 and 91 %) in the activity of AChE was also demonstrable in pancreas of DM treated rats.
8. Administration of repeated oral doses of DDVP (20 mg/kg b.w /d for 10 d) induced significantly higher blood glucose levels in rats.
9. Following glucose overload, DDVP treated rats showed higher glucose levels (149mg/dl) at 60 min and beyond indicating altered glucose tolerance.
10. DDVP also induced increases in serum lipase (35%), serum amylase (12%) and pancreatic amylase (47%) activities, while it significantly reduced pancreatic lipase activity (26%).

11. Serum lactate dehydrogenase and pancreatic \(\gamma\)-glutamyltranspeptidase activities were elevated (45 and 59%) while, pancreatic and adrenal AChE activity was significantly reduced (by 31%) in DDVP treated rats.

12. Enhanced ROS levels and lipid peroxidation were accompanied by depleted glutathione levels in pancreas and other vital organs in DDVP treated rats, concomitant with elevated activities of pancreatic antioxidant enzymes such as catalase (44%), SOD (76%), GR (30%), GPX (68%) and GST (27%).

13. However, varying degrees of increase in the activities of DT-diaphorase (41%) and cytochrome B\(_5\) reductase (12%) and decrease in the activities of NADPH-diaphorase (33%) and cytochrome P\(_{450}\) reductase (22%) were evident in pancreas of DDVP treated rats.

14. Carbohydrate metabolism was markedly affected in rats administered DDVP as evidenced by significantly reduced liver glycogen (40%), increased activities of glycogen phosphorylase and glucose-6-phosphatase enzymes and marked reduction (50%) in liver glucokinase activity.

15. Pancreatic glucokinase and glutamate dehydrogenase enzyme activities in DDVP treated rats were also reduced significantly (by 52% and 37%).

16. Further, histology of pancreas of DDVP administered rats revealed atrophied islets, packed cells with mixed inflammatory cells and areas of hemorrhage, suggestive of acute inflammatory lesion.
SECTION B

IMPACT OF DDVP PRE-TREATMENT ON HYPERGLYCEMIA AND BIOCHEMICAL ALTERATIONS IN STREPTOZOTOCCIN TREATED RATS

1.0 INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated (Kataoka et al., 1983; Paik et al., 1982; Sandler et al., 2000; Shewade et al., 2001). Further, increased oxidative stress has been a widely accepted participant in the development and progression of diabetes and its complications (Ceriello, 2000; Baynes and Thorpe, 1999; Baynes, 1991) since diabetes is usually accompanied by increased production of free radicals (Baynes and Thorpe, 1999; Baynes, 1991; Chang et al., 1993) or impaired antioxidant defenses (Halliwell and Gutteridge, 1990; Saxena et al., 1993).

Diabetes is rapidly emerging as a major health problem in the world (Zimmet et al., 2001; Onkamo et al., 1999) and its prevalence is rising due to the westernized life style, which includes excessive energy intake and physical inactivity (Zimmet et al., 2001). However, several drugs (Ferner, 1992) and environmental factors also contribute towards the development of diabetes (Ferner, 1992; Yoon et al., 1987; Department of Veterans Affairs, 2001). The increasing use of organophosphate pesticides with their adverse effects on glucose metabolism may probably be an additional factor in the speedy expansion of diabetes in the world.

Little information is currently available regarding the toxicity of environmental chemicals in the presence of diabetes. It is also not well known if exposure to certain pesticides will worsen the complications of diabetes.
Retrospective studies have only begun to examine the interaction of environmental agents and diabetes. It has been reported that the metabolic rate and inactivation of drugs are altered in diabetes, especially in type I (Dixon et al., 1961; Thummel and Schenkman, 1990). Based on these findings, diabetic patients may be more susceptible to pesticide toxicity due to changes in the degree of their action, detoxification or elimination or vice versa.

The purpose of the present study was to investigate the effect of DDVP on the expression of diabetic condition in rats using sub-diabetic dose of streptozotocin.

2.0 MATERIALS AND METHODS

Presented under 2.1 to 2.4 of Section A of this Chapter.

3.0 EXPERIMENTAL PROCEDURE

3.1 Animal treatment and experimental protocol

Growing adult male rats were grouped by randomized design into two groups \( n = 12 \). Rats of the first group (control) received saline daily for 10 d, while rats (non-fasted) of the second group were orally administered daily (0900 to 1100 hours) DDVP at 20mg/kg b.w/d (corresponding to 1/5 of LD\(_{50}\) value: 100 mg/kg b.w, determined in a preliminary study) for 10 d. After 10 days, rats of the control group were further divided into two sub groups of six animals each; the first sub group served as control ('untreated control'), while the second sub group of rats was intraperitoneally injected streptozotocin (STZ, 25 mg/kg b.w.) ('STZ'). The group of rats administered with DDVP was also divided into two sub groups; the first sub group of rats served as DDVP control ('DDVP'), while the second sub group of rats was injected with streptozotocin (i.p, 25mg/kg b.w.) ('DDVP+STZ'). Following streptozotocin injection, the rats were given glucose water (5% w/v) for 48 h to prevent initial STZ-induced hypoglycemic mortality. Seven days after streptozotocin administration, all the
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rats were sacrificed under mild diethyl ether anesthesia. Samples of blood were collected by cardiac puncture for serum, and pancreas were excised and stored at 4°C until use. The biochemical assays included determination of blood glucose, liver glycogen, amylase and lipase activity and oxidative stress parameters in pancreas.

3.2 Preparation of tissue homogenates

10 or 20 % (w/v) homogenates of the tissues were prepared in various buffers as per the requirement of a given assay.

3.3 Serum

Blood was drawn by cardiac puncture into tubes and processed separately for obtaining serum as described earlier (Section 3.5, Chapter II A).

4.0 ASSAY METHODS

4.1 Blood glucose estimation

Blood glucose was estimated using glucometer as described earlier (Section 4.1, Chapter I A). Results were expressed as mg glucose/ dl blood. Mean of three such measurements are taken for every rat.

4.2 Glycogen (Nicholas et al., 1956)

Glycogen in liver was estimated as described earlier (Section 4.22, Chapter II A).

4.3 Reactive oxygen species (ROS) (Keston and Brandt, 1965)

ROS in tissue was estimated by DCFH oxidation method as described earlier (Section 4.8, Chapter II A).
4.4 Lipid peroxidation *(Buege and Aust, 1978)*

Lipid peroxidation in pancreas was estimated by measuring the thiobarbituric acid reactive substances as described earlier *(Section 4.9, Chapter II A)*.

4.5 GSH (reduced glutathione) *(Benke et al., 1974)*

Reduced glutathione in pancreas was estimated as described earlier *(Section 4.3, Chapter I A)*.

4.6 Lipase (EC 3.1.1.3) *(Young et al., 1978)*

Lipase activity was estimated by monitoring the hydrolysis of p-nitrophenyl acetate to p-nitrophenol as described earlier *(Section 4.2, Chapter II A)*.

4.7 Amylase (EC 3.2.1.1) *(Street and Close, 1956)*

The enzyme activity was measured using a commercial kit as described earlier *(Section 4.3, Chapter II A)*.

4.8 Protein estimation *(Lowry et al., 1951)*

Protein content of tissue homogenate and serum was estimated as described earlier *(Section 4.4, Chapter I B)*.

4.9 Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± S.E from six rats in each group. The data were analyzed employing analysis of variance (ANOVA) using Statistica software (STATSOFT, USA). Duncan’s multiple regression test for multiple comparisons was performed to determine the significant differences among the groups. ‘p’ values < 0.01 were considered significant.
5.0 RESULTS

5.1 Body and organ weights

Rats administered a single dose of STZ, which were pre-treated with DDVP for 10 days showed no clinical signs of toxicity or mortality. Terminally, there were no significant alterations either in body weights among various treatment groups. The relative weights of vital organs were similar in rats of all four groups (data not shown).

5.2 Blood glucose and liver glycogen levels

Data on blood glucose and liver glycogen levels in rats treated with repeated doses of DDVP and further subjected to a single dose of STZ are given in Table 2.18. DDVP treated rats showed higher (22%) levels of blood glucose compared to normal control rats. As expected, rats injected with STZ alone also showed elevated (37%) level of blood glucose (Fig. 2.22). However, blood glucose levels of DDVP pretreated rats administered STZ showed relatively higher (66%) blood glucose level compared to all the groups.

Liver glycogen levels were significantly lower in rats administered either DDVP (18%) or STZ (19%) alone while, rats administered DDVP followed by STZ revealed further lower levels of glycogen (46 %).

5.3 Oxidative stress in pancreas

Data on ROS, LPO and GSH levels among rats treated with repeated doses of DDVP and further subjected to a single dose of STZ are presented in Table 2.19 & Fig. 2.23. ROS levels were significantly elevated in STZ (40%) and DDVP (55%) groups compared to 'untreated control' group. However, ROS levels were markedly higher (81.23 ± 6.52 pmole DCF/min/mg protein) in 'DDVP+STZ' group of rats. Pancreas of rats administered with either DDVP or STZ alone showed marginally higher levels of the lipid peroxides compared to that in 'untreated controls' while, the levels of lipid peroxides generated in
pancreas of ‘DDVP+STZ’ rats showed significant increase (110%) compared to all other groups. Pancreatic reduced glutathione level in ‘DDVP+STZ’ rats was significantly lower (37%) while, rats administered with DDVP or STZ alone also had significantly lower levels of GSH, although to a lesser extent.

5.4 Pancreatic damage markers

Activities of pancreas specific enzymes such as lipase and amylase in serum and pancreas of rats of the various groups are shown in Table 2.20. STZ treatment alone did not alter serum and pancreatic lipase amylase activities. Both, serum and pancreatic lipase and amylase activities were significantly elevated in rats administered DDVP alone. Rats of ‘DDVP+STZ’ group showed significant increase in activities of both lipase and amylase in serum (30% and 83 % respectively) while in these rats the pancreatic lipase activity was decreased significantly (20 %) and pancreatic amylase activity was increased (35%).
Table 2.18 Blood glucose and liver glycogen levels in rats pretreated with oral doses of DDVP (20 mg/kg b.w/d for 10 d) followed by a single dose of STZ (i.p, 25 mg/kg b.w)

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose(^1)</th>
<th>Liver glycogen(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>113.53(^a) ± 2.31</td>
<td>41.55(^c) ± 2.01</td>
</tr>
<tr>
<td>DDVP</td>
<td>138.37(^b) ± 4.17</td>
<td>34.20(^b) ± 1.42</td>
</tr>
<tr>
<td>STZ</td>
<td>155.03(^c) ± 5.09</td>
<td>33.34(^b) ± 2.23</td>
</tr>
<tr>
<td>DDVP+STZ</td>
<td>188.99(^d) ± 4.44</td>
<td>22.62(^a) ± 3.52</td>
</tr>
</tbody>
</table>

\(^1\)mg/dl; \(^2\)mg/g tissue

Values are mean ± SEM (n=6); Mean in the same column with different superscript differ significantly (p<0.05)

Fig. 2.22 Blood glucose levels in rats pretreated with oral doses of DDVP (20 mg/kg b.w/d for 10 d) followed by a single dose of STZ (i.p, 25 mg/kg b.w)
### Table 2.19 ROS, TBARS and GSH levels in pancreas of rats pretreated with oral doses of DDVP (20 mg/kg b.w/d for 10 d) followed by a single dose of STZ (i.p, 25 mg/kg b.w)

<table>
<thead>
<tr>
<th>Group</th>
<th>ROS(^1)</th>
<th>TBARS(^2)</th>
<th>GSH(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>38.76(^a) ± 4.04</td>
<td>242.76(^a) ± 19.18</td>
<td>1.07(^c) ± 0.03</td>
</tr>
<tr>
<td>DDVP</td>
<td>60.18(^b) ± 4.59</td>
<td>294.94(^a) ± 10.65</td>
<td>0.83(^b) ± 0.01</td>
</tr>
<tr>
<td>STZ</td>
<td>54.27(^b) ± 2.89</td>
<td>283.63(^a) ± 7.27</td>
<td>0.78(^b) ± 0.02</td>
</tr>
<tr>
<td>DDVP+STZ</td>
<td>81.23(^c) ± 6.52</td>
<td>389.38(^b) ± 38.47</td>
<td>0.67(^a) ± 0.02</td>
</tr>
</tbody>
</table>

\(^1\) pmol DCF/min/mg protein; \(^2\) nmol/g tissue; \(^3\) mg/g tissue

Values are mean ± SEM (n=6)

Mean in the same column with different superscript differ significantly (p<0.05)

---

![Graph](image)

**Fig. 2.23** ROS, TBARS and GSH levels in pancreas of rats pretreated with oral doses of DDVP (20 mg/kg b.w/d for 10 d) followed by a single dose of STZ (i.p, 25 mg/kg b.w)
Table 2.20 Activities of lipase and amylase in serum and pancreas of rats pretreated with oral doses of DDVP (20 mg/kg b.w/d for 10 d) followed by a single dose of STZ (i.p., 25 mg/kg b.w)

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipase (nmol PNP/mg protein/min)</th>
<th>Amylase (Street-Close Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Untreated control</td>
<td>52.73&lt;sup&gt;a&lt;/sup&gt; ± 3.51</td>
<td>257.77&lt;sup&gt;b&lt;/sup&gt; ± 13.79</td>
</tr>
<tr>
<td>DDVP</td>
<td>70.96&lt;sup&gt;b&lt;/sup&gt; ± 3.36</td>
<td>178.76&lt;sup&gt;a&lt;/sup&gt; ± 10.06</td>
</tr>
<tr>
<td>STZ</td>
<td>51.43&lt;sup&gt;a&lt;/sup&gt; ± 4.56</td>
<td>255.22&lt;sup&gt;b&lt;/sup&gt; ± 17.37</td>
</tr>
<tr>
<td>DDVP+STZ</td>
<td>68.10&lt;sup&gt;b&lt;/sup&gt; ± 3.28</td>
<td>206.69&lt;sup&gt;a&lt;/sup&gt; ± 6.18</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6)
Mean in the same column with different superscript differ significantly (p<0.05)
6.0 DISCUSSION

The primary objective of the present study was to examine whether pretreatment with DDVP renders rats more susceptible to a single dose of STZ, a diabetogen. Increase in blood glucose and LPO levels with reduction in GSH content were the salient features observed in DDVP as well as STZ-treated rats. Our results clearly showed that a pre-existing disrupted glucose homeostasis and pancreatic damage caused by DDVP increased the STZ-mediated hyperglycemia relative to control rats. Although DDVP and STZ are structurally and mechanistically different, our results indicate the possibility of a common mechanism of potentiation of pancreatic injury in rats.

Streptozotocin is frequently used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells (Yamagishi et al., 2001; Stefek et al., 2002; Kalender et al., 2002). Streptozotocin (STZ) is an antibiotic derived from Streptomyces achromogenes. It has the property of selective destruction of β-cells after injection, while the exocrine part remains unchanged (Richter et al., 1971). STZ enters the β-cell via a glucose transporter (GLUT2) and promotes alkylation of DNA. The potent alkylating property of STZ is the principle reason for its toxicity (Szkudelski, 2001). Because of its highly specific β-cell toxicity and its subsequent diabetogenic effects, STZ has been used to study β-cell damage in a wide variety of in vivo models (Bolaffi et al., 1987). The cytotoxic action of STZ is also associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001). Acute STZ injection has also been used to study cellular or tissue oxidative damage because it generates ROS and reduces antioxidant enzyme activities especially in pancreatic tissues (Coskum et al., 2005). In fact, STZ can stimulate H₂O₂ generation in islet cells (Friesen et al., 2004) where the activity of antioxidant enzymes such as SOD, CAT and GPX is relatively low when compared to other tissues (Tiedge et al., 1997). On exposure to STZ, most islets are impacted to
death and remaining islet cells almost exhibit a significant decrease in the activity of these enzymes (Srinivasan et al., 2003).

Generally, an acute intraperitoneal dose of 40–60 mg/kg b.w is employed to induce significant hyperglycemia in rats (Szkudelski, 2001). In our earlier studies (Singh et al., 2005a), we have induced significant hyperglycemia in adult rats with an i.p dosage of 52 mg/kg b.w. However for the present study, we employed a lower dose of 25 mg/kg b.w ('sub-diabetogenic dose') in order to examine if pretreatment with DDVP renders these rats more susceptible to hyperglycemia.

In the present study, STZ treatment at a dosage well below that needed to induce diabetes in rats was found to elevate blood glucose levels by 36% above control after 7 days of treatment. This is in contrast to the report of Mythili et al., (2004) who did not observe increased blood glucose after 7 days of treatment of rats with STZ at 30 mg/kg b.w. DDVP treated rats injected with STZ showed 30% more of blood glucose levels than that of STZ treated rats. Concomitantly, the liver glycogen was also further decreased in DDVP+STZ rats, suggesting a severely impaired carbohydrate metabolism in these rats.

Diabetes manifested by experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system (Baynes and Thorpe, 1999). Increased oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of diabetic complications (Baynes, 1991). Oxidative stress thus plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation (Kakkar et al., 1995).

We observed that LPO induced by STZ was associated with a decreased non-enzymatic (GSH) antioxidant content. GSH protects tissue from damage caused by diabetes, which impairs the antioxidant system. Depletion of tissue
GSH is one of the primary factors that permit lipid peroxidation (Konukoglu et al., 1998). It has been proposed that antioxidants that maintain the concentration of GSH may restore the cellular defense mechanisms, block lipid peroxidation and thus protect the tissue against oxidative damage (Chugh et al., 1999). GSH is an important substrate for GPX and GR. The reduction in GSH due to STZ was to a similar extent to that induced by DDVP treatment. Further depletion in pancreatic GSH content in DDVP+STZ rats clearly suggests an increased oxidative stress.

We did not observe any alteration in the levels of pancreatic marker enzymes due to STZ treatment. This is justified due to the fact that STZ does not affect the acinar cells of the pancreas (Richter et al., 1971). However, 'DDVP+STZ' rats showed altered pancreatic marker enzymes due to the impact of DDVP treatment alone.

Impaired glucose tolerance is a category that permits classification of individuals whose glucose tolerance is above the conventional normal range but lower than the level considered diagnostic of diabetes. Such persons do have a high risk of developing diabetes mellitus. Our earlier results (Section A, Chapter II) with DDVP had clearly demonstrated impaired glucose tolerance in rats treated at the dosage employed for the present study. Hence, based on the results of the present study, it could be hypothesized that the rats pre-treated with DDVP could be at a risk of developing diabetes if exposed to even a sub-diabetogenic dosage of a diabetogen.
7.0 SUMMARY

1. Blood glucose levels in rats treated with DDVP or STZ alone were only 22% and 37% respectively higher compared to control rats. Interestingly, blood glucose levels were further elevated (66%) among rats administered STZ pretreated with DDVP.

2. Liver glycogen levels were significantly lower in rats administered with DDVP (18%) or STZ (19%) alone while rats pretreated with DDVP followed by STZ injection had further lower levels of glycogen (46%) compared to that of the control rats.

3. Markedly higher ROS and lipid peroxide were evident in pancreas of 'DDVP+STZ' rats.

4. Reduced glutathione levels in pancreas were significantly lower among STZ+DDVP rats compared to those administered either DDVP or STZ alone.

5. Rats administered with DDVP+STZ showed significant increases in lipase and amylase activities in serum (30% and 83 % respectively), while pancreatic tissue lipase activity was decreased significantly.

6. These studies provide new evidence for the potential of OPI to damage rat pancreas and thereby exacerbate condition leading to diabetes.