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In vitro Study of Propylene Glycol on Bovine Blood Biochemical Parameters

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Abstract: The present work was undertaken to study the effect of different concentrations of propylene glycol (PG) on some biochemical parameters in blood of cattle. For this purpose, blood was aseptically collected by jugular vein puncture over acid citrate dextrose anticoagulant from six different cattle under pathology shed of IVRI. The RBCs pellets were used for analysis of glutathione, lipid peroxidation and total lipid extraction. Total protein, their electrophoretic analysis and alkaline phosphate activity were seen in blood plasma. The total protein content is not altered significantly. In electrophoretic analysis of plasma protein certain bands of molecular weight 81, 92 and 105 kDa observed in the control plasma sample were not seen in treated sample from 5-25 μM PG concentration. The alkaline phosphatase activity of plasma increased significantly, lipid peroxidation was found declined and no marked alteration in phospholipids of erythrocytes was obtained by TLC at higher concentration, it did not cause apoptotic DNA fragmentation in the peripheral blood mononuclear cells. It was concluded that, a certain dose of PG can be used for the treatment of ketosis in dairy cows. However, its higher dose may influence the blood constituents and cause mild oxidative stress on erythrocytes as well as RBC lipid peroxidation.

Key words: Propylene Glycols · Ketoses · Biochemical markers · Serum proteins · Reduced Glutathione

INTRODUCTION

Propane 1, 2 diol (PG, propylene glycol) is a clear colourless, slightly syrupy liquid at room temperature [1]. It is practically odorless and tasteless, considered so for as innocuous and harmless substance, emerged as one of the most unsuspected, subtle and hazardous inversion pollutant. Its hidden and latent dangers could not have been anticipated because its metabolism has been found linked to normal glycolytic pathway. PG is used as a solublizer, a stabilizer, an emulsifier, an additive, an additive and a preservative [2]. For instance, it became quite important as a solvent and co solvent for pharmaceuticals and antioxidants, a stabilizer for vitamin, an additive for foods, beverages and syrups and in animal feeds as energy source, as humectants improves the shelf life of packaged foods, as a preservative for retarding the growth of certain molds and fungi [1]. PG is also used in the cattle feed which help in the metabolic functions. It is metabolized by normal metabolic pathways and it is known to produce gluconeogenesis and used extensively for treatment of bovine ketosis [3-5]. However, recent studies showed that chronic ingestion of this solvent at a sub lethal dose level brings undesired metabolic changes at tissue level. The subclinical and ultramicroscopical changes observed at low level exposure are the genesis of the present investigation. Thus it was proposed to study the effect of these xenobiotics, namely PG on erythrocytes circulating in the peripheral blood obviously for the following reasons. The erythrocytes are subjected to the insults of the xenobiotics during their biodynamic processes for which maintenance of the normal erythrocyte constituents, e.g. enzymes, hemoglobin, glutathione, cations etc. is of crucial importance for the internal functions within the red cells [6]. In general, the erythrocyte membrane quite vulnerable to many physiochemical influences, structural and functional defects of circulating erythrocytes do arise

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as a result of *in vitro* treatment with a vast array of chemical and physical agents. These foreign agents may interfere with various oxidoreductive system of the erythrocytes –GSH, GSSG < NADPH/NADP, Ferro/ferric, Hb and SH/SS system of globular enzymes which ultimately leads to lipid peroxidation and hemolysis of the red cells. In the present study, it was proposed to investigate the biochemical changes in RBCs and plasma of cattle blood after short exposure to different concentrations of Propane diols. The biochemical parameters under investigation included RBC glutathione (GSH), RBC lipid peroxidation (LPO), alkaline phosphatase in plasma, total plasma protein and their electrophoretic profiles, total erythrocyte lipids and apoptosis in peripheral blood mononuclear cells [6].

MATERIALS AND METHODS

Blood was aseptically collected by jugular vein puncture from 6 cattle in test tubes by 18 gauge needle and 25 ml syringe, containing acid citrate dextrose solution (ACD) as an anticoagulant @ 15 ml/100 ml of blood. Collection was done under pathology shed of IVRI, India.

Blood was taken in six test tubes containing 5 ml each. Propylene glycol added in each test tube with different concentration i.e. 0, 5, 10, 15, 25 and 50 µ/ml, respectively, 0 µg/ml served as control and incubated at 37°C for 24 h. After incubation the blood was centrifuged at 3000 rpm for 10 min. Plasma was aspirated using a pasture pipette and stored at -20°C for analysis and removed the buffy coat. The RBCs pellets were washed three times with 0.9% normal saline (NaCl) at 4°C temperature.

Plasma Analysis: Total plasma protein was estimated by Biuret method of Robinson and Hogden [7]. Serum protein concentration was calculated using bovine serum albumin (BSA) (mg/ml) as standard, total serum protein values were expressed in g/100 ml serum. Standard protein solution was prepared containing 100 mg of BSA/ml in distilled water and diluted it 1:10 to prepare working standard solution just before use. The enzyme alkaline phosphatase was estimated by method of King and Armstrong [8] using p-nitrophenyl phosphate (PNPP) as substrate and p-nitrophenyl formed min^{-1} liter and it was converted to international unit /l.

RBCs Analysis: Glutathione was estimated spectro-photometrically by using DTNB reagent by method of Prins and Loos [9]. The lipid peroxidation was measured by thiobarbituric acid reacting substances (TBARS) by method of Jain [10] in the presence of EDTA, the interference of metal ions which could promote lipid peroxidation.

Electrophoretic Profile of Plasma Protein by SDS-PAGE: Molecular weight of protein from PG treated blood was estimated by SDS-PAGE according to method of Sambrook and Russell [11] using 10% gradient polyacrylamide phast gel. Samples were prepared in 0.312 M Tris HCl buffer (pH 6.8) containing 0.125% bromophenol blue, 10% SDS and 50% glycerol. A 205 kDa protein marker was used to estimate molecular weight (medium range, Genie, Cat. No. PMW-B). Protein bands were visualized by 0.25% coomassie brilliant blue dye.

Extraction of Total Lipid from RBCs: The total lipids were extracted from RBCs of PG treated cattle blood by using chloroform-methanol mixture (2:1) by method of Folch *et al.* [12]. One ml of RBCs suspension were taken in a tube and added 19 volume of chloroform methanol mixture and shaked vigorously and leave for 6-8 h at 4°C, then filtered it by Whatman filter paper no.1 in measuring cylinder. 0.2 volume of 0.9% normal saline was added of total volume of filtered solution and shaked invertally and leave it for 6-8 h. Two aqueous layers are formed, discarded the upper aqueous part by the pasture pipette. Some procedure was repeated at least three times. At least rest solution was evaporated at 56-60°C in dry incubator. Residues were dissolved in known volume of chloroform and tightly closed the neck and kept them at 4°C.

Separation of Lipids by Thin Layer Chromatography: Glass plates (20x20) were coated with silica gel G at 250 µm (0.25 mm) thickness by thin layer chromatography set according to method of Abramson and Blecher [13]. The plates were dried at room temperature to allow the thin layer to set. The dried plates were activated for 90 min by heating at 110°C and cooled in a desiccator chamber. Each activated plate was divided into 4 cm wide lane and a horizontal line 17 cm from one end of the plate was drawn. Lipid samples were applied in a streak, 2 cm from the unmarked and along with the authentic phospholipids standards.

Separation of Phospholipids: Alkaline solvent system for separation of phospholipids was used for better resolution, phospholipids were separated uni-dimentionally in that solvent system comprising of chloroform: methanol: ammonia (65:25:4 v/v/v) [14]. The plates were developed up to 15 cm length from the origin,

removed from the chromato tank and air dried. Authentic standards were chromatographed. The phospholipids spots on the plates were detected by exposing the plates to iodine vapors.

Statistical Analysis: Data obtained in each experiment were analyzed to calculate means and significant differences between treatments by using student't' test as described in standard statistical method [15].

RESULTS

The present study was done to investigate the effect of propane-1, 2-diol on blood constituents *in vitro* at different concentration and following results were observed. It is evident that the content of total serum proteins was not affected significantly by PG treatment. Although same amount of total protein (60 µg) was loaded per lane, some alterations were observed in electrophoretic profile in treated samples as compared to untreated control. Certain proteins of molecular weight approx. 81, 92 and 105 kDa observed in control sample were not seen in sample treated from 5-25 µM PG concentration. However, intense bands of low molecular weight proteins (6-14 kDa) were observed after PG treatment as compared to control (Fig. 1). This data revealed a statistically significant ($P<0.05$) increase in alkaline phosphatase activity of plasma after *in vitro* treatment of blood with different concentration of PG (Fig. 2). The level of reduced glutathione of RBC was found to be elevated significantly ($P<0.05$) *in vitro* treatment of blood with different concentration of PG. This increase was optimum at 15 mM PG concentration (Fig. 3). After 24 h incubation, some lipid peroxidation was observed in control erythrocytes as is evident from MDA levels. After different concentration of PG treatment, this levels of MDA were found to decrease which was found significant ($P<0.05$) (Fig. 4). No significant changes were observed in phospholipids pattern of RBC lipids, extracted after PG treatment as compared to control.

The PBMCs were separated from PG treated blood. The genomic DNA was extracted from the cells and was run in 1% agarose. Smearing of DNA in the control sample indicated fragmentation. In 15 µM and 50 µM PG treated sample similar results were observed. Although fragmentation was found in the DNA, yet no DNA ladder characteristic of apoptosis was observed.

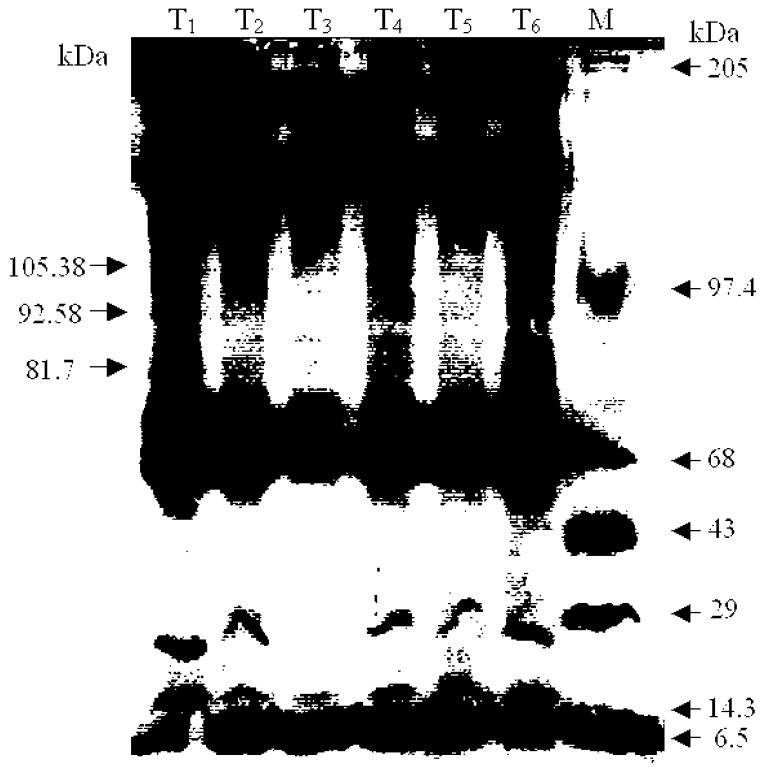

Fig. 1: SDS-PAGE of plasma proteins after *in vitro* treatment of blood with different conc. of PG. where T_1: Control; T_2 - T_6: Blood treated with 5-50 µM conc. of PG, M: Protein molecular weight marker

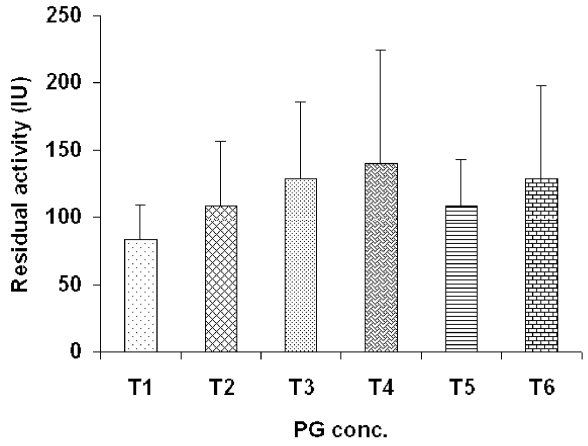

Fig. 2: Alkaline phosphatase activity (IU) of plasma after *in vitro* treatment of blood with different conc. of PG

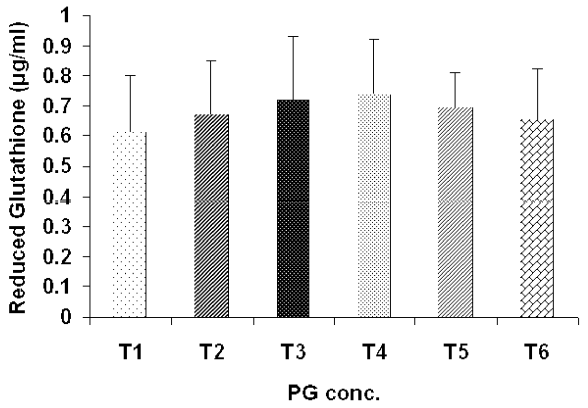

Fig. 3: Reduced glutathione (µ moles/ml packed RBC) after *in vitro* treatment of blood with different conc of PG

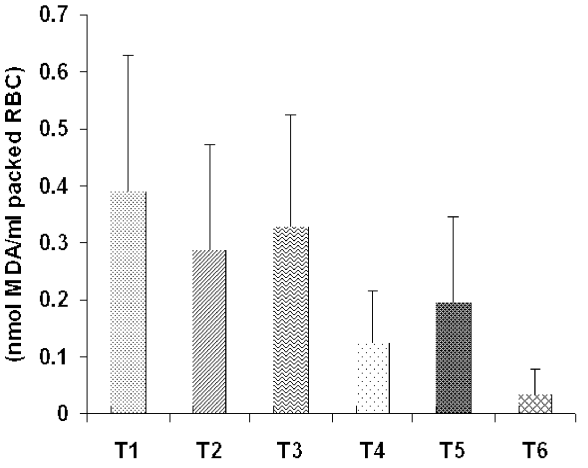

Fig. 4: Lipid peroxidation (µmoles MDA/ml packed RBC) after *in vitro* treatment of blood with different conc. of PG

DISCUSSION

PG has witnessed a versatile use in food and feed industry. It has been used as a suitable for carbohydrate in the diet of cattle [16] and in the treatment of bovine ketosis [17, 18]. It is known that in such circumstances it is metabolized via normal metabolic pathways and cause gluconeogenesis. After a single oral dose, high levels of PG persist in circulation for a few hours and within 24 h it gets cleared of the circulation [19]. High level of PG may persist in circulation of cattle because of continuous or regular use in feed containing it. In view of the adverse effects of PG reported so far in the review of literature. The present piece of work was planned to explore the effect of this relatively inert diol on the cattle blood biochemical parameters after *in vitro* treatment for 24 h.

The *in vitro* treatment of blood with PG did not change the total protein content of plasma. Yet minor alterations in the electrophoretic profile revealed increased presence of proteins of low molecular weight and disappearance of few bands of high molecular weight. These bands may be of some plasma enzymes influenced by presence of PG. The low molecular weight protein present in 5-25 µM PG treated samples could be hemoglobin released due to hemolysis observed in these samples. However, no hemolysis was observed in 50 µM PG treated blood plasma and it is clear from the electrophoretic profile.

The alkaline phosphatase activity was increased after PG treatment that could be due to either leakage of this enzyme from the cells or due to modified enzyme activity present in plasma. Similar results have been reported earlier during *in vitro* treatment of rat blood with 5-25 µM PG [20]. It has been observed earlier that PG dosing causes mild oxidative stress on erythrocytes and causes RBC lipid peroxidation in rats [21]. In view of its metabolism in liver via aldehydes, that is expected *in vivo*. In the present study, increased levels of reduced glutathione and decreased lipid peroxidaiton resulting from PG treatment indicate better redox state of the cell in the presence of the diol as such, since it is metabolized in the liver and during *in vitro* treatment it persist as such.

Lipid peroxidation was observed in the control cells due to surrounding oxidizing environment. PG is lipophylic molecule and can enter the cells. Inside the RBCs it may act as a substrate for dehydrogenases and alter the redox balance. No significant changes were observed in phospholipid profile of erythrocytes after PG treatment indicating lack of exchange of plasma lipids in the presence of PG.

PG has been reported to cause chromosomal abberrations and suppress the rat macrophage function *in vitro* [22]. In the present work, no marked effect of PG concentration in blood for 24 h was seen in DNA isolated from peripheral blood apoptosis due to PG treatment. Some DNA fragmentation observed in the samples could be due to *in vitro* condition and there may be stress on the cells during *in vitro* conditions.

It is inferred from the above discussion that when PG is present in the blood at 5-25 μM concentration for 24 h at 37°C some changes are observed in blood biochemical parameters. Better antioxidant state of red cells persists although, hemolysis is observed in the plasma. Alkaline phosphatase activity in plasma is increased. Non-significant changes were found in total plasma protein content, RBC PL profile and DNA fragment in peripheral blood mononuclear cells. At 50 μM concentration less severe changes were observed probably due to saturation of PG metabolism reversal phenomenon. Further work is needed to elaborate these findings.

From the above results it may be concluded that propylene glycol up to certain levels can be used as food supplements for the prevention of bovine ketosis. Severe cases of ketosis may require larger doses however; its high dose persisting in cattle blood may influence certain blood biochemical parameters.

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