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ORIGINAL ARTICLE

Histological alterations in Rabbit Liver after treatment with Dexamethasone

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ABSTRACT

Background One of the most potent glucocorticoids is known as Dexamethasone. Many metabolic side effect shave been reported on almost every organ after dexamethasone treatment specially its effect on liver.
Aim: To investigate harmful side effects of dexamethasone sodium phosphate on rabbit's liver that serve as human liver model via using light microscope, by administration of two doses (extreme) and two durations in order to depict the duration as well as dosage dependency.
Methods: Liver samples were taken via rabbits who were administered dexamethasone sodium phosphate. Then two Stratas were made namely, 1 and 2. The fixations of liver samples were carried out and underwent into evaluation in order to observe any histochemical and histological alterations. Study duration is from February to May 2021 Rabbits were brought from Veterinary Research Institute, Lahore. These Rabbits were kept in cages in the animal house of PGMI, Bird wood road Lahore.
Results: The ballooning and vacuolation of hepatic cells were seen in the liver in case of Stratas that were treated along with the degenerative alterations of these cells, congestion and dilatation of central hepatic vein with sinusoidal capillaries, positive periodic acid schiff's stain (PAS) reactions. The severity of all these alterations was dependent upon duration and dosage.
Conclusion: Morphological variations induced in the liver by dexamethasone sodium phosphate could be accepted as side effects of these drugs.
Keywords: Liver, dexamethasone, histology, glycogen.

INTRODUCTION

The liver sever as an most important organ in human body that help in nutrients processing that were absorbed via gastrointestinal tract and then help in transformation of these nutrients into such materials that is demanded by body tissues and organs[1].

Liver is in influence of several hormonal activities like adrenal steroids[4] glucagon[3] and insulin[2]. The glucocorticoids in synthetic form are given for many diseases but its effects clearly visible on almost all organs of body. The severity of complications are dependent on various factors, such as treatment duration, administration time, selected glucocorticoid preparation, administration route, dosage administered and intervals[5] between doses. Dexamethasone is regarded as strong glucocorticoid that is administered in case of many health conditions such as non-endocrine and endocrine diseases like osteoarthritis, rheumatoid arthritis and many other pathologies related to connective tissue. Moreover is given in case of inflammatory diseases like dermatological diseases, respiratory disease etc. However, dexamethasone has potent harmful effects of all systems of body[6].

A transcortin a serum corticosteroid-binding protein, has been synthesized as well as secreted by liver cells[7]. The role of transcortin is to act as selective glucocorticoid transporter through the plasma membrane and impacts the entry intracellularly and aid in transportation from the nucleus of steroid[8]. Glucocorticoids block uptake of amino acid and glucose in many scenarios and aid in lipolysis in case of adipose tissue). For gluconeogenesis, a wide hepatic capacity is present which along with substrate via catabolism (elsewhere) cause enhanced production of glucose. The net result of glucocorticoids in body cause hyperglycemia, fat loss and negative nitrogen balance[9]. On liver, the generalized glucocorticoid stimulating effect results in hepatocytes hypertrophy, since in the liver cells, the total protein content is enhanced[10].

The main aim of this case research is to investigate the dexamethasone sodium phosphate effects on the liver of rabbit that serve as human liver model via using light microscope, by administration of two doses and two using durations in order to make certain its dependency with respect to duration and dosage.

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METHODS

Separate plastic cages were arranged for white healthy rabbits that varied in weight from 2.kg-2.5kg, administered ad-libitum and then these were utilized for purpose of scientific research in 2019 from January to march in College animal house & Laboratory. Six Stratas of animals were made and each Strata contained seven rabbits in each. For consecutive 14 days, the first Strata was fed with (0.5mg/kg of body weight (b.w.) equal to 0.5 ml/kg b.w.) dexamethasone sodium phosphate intramuscularly via aid of single dose injection in thigh on every 24 hours. In case of second Strata of rabbits dosage of dexamethasone sodium phosphate was given (1.4mg/kg b.w. equal to 0.5 ml/kg b.w.) for 14 days on thigh as single dosage. The third Strata was administered dexamethasone sodium phosphate (0.5 mg/kg b.w.) for duration of 2 weeks whereas the fourth Strata was administered dexamethasone sodium phosphate (1.5mg/kg b.w.) for again duration of 2 weeks. The fifth Strata served as control (1) animals, who were administered equal quantity of 0.9% saline Strata 2weekssolution for 14 days intramuscularly The sixth Strata was administered same 0.9% saline solution for duration of 2weeks and served as the 2 control Strata[11]. By using chloroform, animals were anesthetized after twenty four hours of the latest administered injection. The liver of experimental rabbits were taken out via aid of dissection and then fixation was done by using 10% formalin solution for duration of 24 then afterwards samples were dehydrated, cleared, and then embedded via aid of paraffin and then blocks were sectioned following by staining using:

1. Haematoxyllin and Eosin stain (H&E): stains used in routine investigation of generalized liver structures[12].
2. Alcoholic periodic acid- Schiff's stain (PAS): for investigating carbohydrates that included mucin and basement membranes[13] and glycogen. Staining techniques and methods were executed depending upon Humason and Luna[12,13].

RESULTS

After staining with H&E, treated sections depict vacuolation and ballooning of cells of liver, which started to make its appearance more vividly in case of second and fourth Stratas (with the

increment of duration as well as dosage of treatment) as depicted in fig. 1. Liver cells were discovered to undergo degenerative changes such as destruction of nuclei with cytoplasm of hepartic cells began to make their appearances from second Strata uptill the fourth Strata that depicted pyknotic nuclei (Fig. 2). In case of first Strata , some sections depicted hepatic cells owing some nuclei showing prominent nucleoli (Fig. 3). In case of all treated Stratas, congestion as well as dilatation of central hepatic vein was clearly visible (Fig. 3). Moreover in case of fourth and second Stratas, Sinusoidal congestion and dilation (Fig. 3). Fifth Strata that was considered as controlled, rabbits were administered [(1)] , same quantities of 0.9% saline solution intramuscularly for duration of about 14 days. In case of sixth Strata, rabbits were administered 0.9% saline solution for duration of 2weeks and marked control[2] Strata[11]. By using chloroform, animals were anesthetized after twenty- four hours of the latest administered injection. The liver of experimental rabbits were taken out via aid of dissection and then fixation was done by using 10% formaline solution for duration of 24 then afterwards samples were dehydrated, cleared, and then embedded via aid of paraffin and then blocks were sectioned following by staining using :

1. Haematoxyllin and Eosin stain (H&E): routine stains for investigation of generalized liver structures[12].
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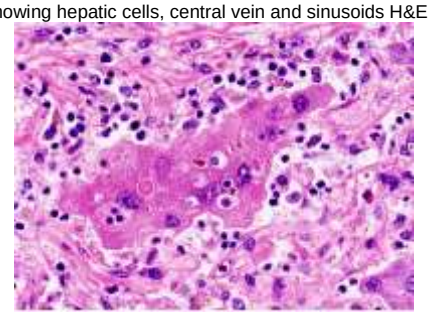
RESULTS

After staining with H&E , treated sections depicted ballooning as well as vacuolation of liver cells, which begin to give its appearance more vividly in case of fourth and second Strata of animals since the duration as well as administered dosage was raised up as depicted in fig. 1. The cytoplasm of liver cells begin to show up from second Strata and further seen till fourth Strata which depicted pyknotic nuclei (Fig. 2). In case of first Strata, some treated sections showed liver cells owing nuclei that contained noteworthy nucleoli (Fig. 3).

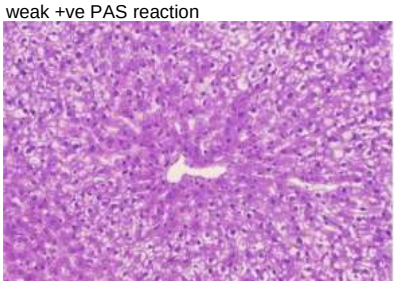
Fig1: Liver cells showing dilated and congested central vein

Fig 2 liver showing hepatic cells, central vein and sinusoids H&E



In case of all treated Stratas congestion as well as Dilatation of central hepatic vein was noted clearly (Fig. 3). The congestion and Sinusoidal dilatation were also seen significantly in case of fourth as well as second Strata (Fig. 3). All these alterations seen above were dosage and time dependent when comparison was made with control Strata animals (Fig.3). In case of PAS staining, PAS strength reaction was dependent upon distribution pattern of stain inside tissue. We observed that there were positive (+ve) PAS reactions in case of Stratas that underwent into treatment but showing difference with respect to degree of positivity. Moreover, this staining firstly visible clearly then gradually turned into a heterogenic pattern and then finally showed up a as clumped masses.

Fig 3 liver with weak +ve PAS reaction



DISCUSSION

One of the most leading cause of liver injury is drugs. Many cases have been seen where liver is being damaged because of administration of drugs. Physicians should be careful while prescribing drugs with respect to injury related to liver because detection in early stages can lower degree of liver toxicity if discontinuation of the medicine is made early[6]. In current case scenario, the histological examination of liver of rabbits was carried out, who were treated with dexamethasone. The histological sections depicted vacuolation and ballooning of liver cells, severity of which was directly proportional to the dose and duration of treatment planned. Hepatocyte that contained glycogen within cytoplasm, gave positive (+ve) PAS reactions in case of all rabbits who underwent into treatment, but showed dissimilarity with respect to degree of positivity, along with dispread staining presence that depicted PAS +ve reaction. With time, this staining appeared as heterogenic and later on showed as clumped bodies that showed Glycogen strong reaction to PAS, that alter in the different duration and dosage administered in this case research. With respect to some scholars[14] deposition of glycogen was dependentupon dose and time and moreover administration repeatedly of dexamethasone cause spikeup the net weight of liver as well as glycogen content and these alterations were lessened after treatment being ceased. In case of the liver, glucocorticoids activate gluconeogenesis inside liver and speedup hepatic synthesis and glycogen storage. Glucocorticoids also play an important role in declining uptake of glucose in peripheral tissues along with adipose tissue, further causing blood glucose levels to spikes up. In reaction to spiked up blood glucose levels, insulin secretion also increased as a compensatory mechanism[6]. However, glucocorticoids stops the gluconeogenesis suppression via insulin and result in insulin resistance typically in peripheral tissues that again exaggerates hyperglycemia[9]. Administration of dexamethasone results in time dependent alterations in levels of insulin and glucose and results increased insulin secretion, which contributes to deposition of glycogen, so that as with the passage of time, more deposition of glycogen happened in hepatocytes cytoplasm[15]. Moreover, dexamethasone cause up regulation of receptors of insulin depending upon time because of activation insulin receptors synthesis[16]. Many biochemical reactions are known to be effected via treatment with cortisone like glycogenesis, certain functions related to mitochondria, lipogenesis, hydrolytic enzymes release and protein synthesis. Many above mentioned processes can be linked to particular ultrastructural elements with respect to cytoplasm[17]. According to

many case researches, many scholars[18] suggested that dexamethasone cause smooth endoplasmic reticulum (SER) enhancement, which is linked with glycogen increased levels. Increment in quantity of SER has been observed in hepatocytes preceding to deposition of glycogen. Moreover dexamethasone increases glycogen synthase activity which ultimately leads to enhancement in accumulation of the glycogen[19] and prevents glycogen phosphorylase[20] activation. Hepatocytes degenerative changes like ballooning are not just because of deposition of glycogen, but also because of piling up of lipids. The main reason for that phenomenon is due to glucocorticoids that increases in hepatic synthesis and due to VLDL secretion[21]. Glucocorticoids are culprit in trapping time dependent triglyceride within hepatocytes cytoplasm due to decline in its release or because of speedup synthesis or fatty acids esterification [(22)] . The evidence suggested that glucocorticoids cause increase in fragility progressively with respect to intracellular organelles, like lysosomes with changes in the properties of plasma membrane[23] and decline in the number along with significant alterations in the mitochondria[17] ultra-structure. It is also well acknowledged fact that dexamethasone is involved in reducing mitochondria total number in case of hepatocytes that were treated and lowers the oxidative phosphorylation and their active respiration[24].These all sequences of events results in alteration of electrolyte balance via the "Sodium-Potassium pump". Since this process is energy dependent, the potassium ions efflux may take place in relation with the sodium ions influx, resulting in osmotic pressure to be raised up inside cytoplasm beside the disturbances in function of plasma membrane; which attract water molecules. Due to all these alterations, cells started to swelled up and also hydrolytic enzymes leakage may results in crowding of macromolecules[25]. Dexamethasone, which is regarded as glucocorticoid synthetic form, cause inhibition in arachidonic acid synthesis and prostaglandin that served as antiaggregant agents normally[26] . This, all together polycythemia and hypertension[27], resulted in congestion and sinusoidal dilatation, which have been observed in case of Stratas that underwent into treatment in this research with the significant dissimilarities among them indicating dependency with respect to duration and dose. Keeping in view our case research, we came to conclusion that the morphological alterations visible in case of liver induction by dexamethasone sodium phosphate and significant harmful effects of these medicines have been observed.

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