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Hepatotoxic effects induced by a traditional oral Anti-hyperglycemic Drug in healthy Mice

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ABSTRACT

Metformin is an oral anti hyperglycemic drug used by the patients of diabetes all over the world. In present study, Metformin hydrochloride (Glucophage) was used to determine the histological changes in liver of mice. Twenty mice (4-5 weeks old) were given oral gavages of 0.1 ml of 0.00, 31.25, 125 and 500µg/g B.W (Complete). of Metformin prepared in distilled water, for 04 weeks daily. After 24 hours of last dose, the animals were anaesthetized torecoverliver tissues following fixation in Bouin's fluid and processing for histological preparation using different grades of alcohol and xylene for dehydration. These tissues were later on infiltrated with paraffin wax, sectioned to size of 4µm and stained with Hematoxylin and Eosin. The histological analysis revealed that metformin could cause adverse malformations like pyknosis, necrosis, and steatosis.
Keywords: Metformin, Glucophage, Anti-hyperglycemic**,** Pyknosis, Necrosis, Steatosis

INTRODUCTION

Metformin is first line anti-diabetic drug which decreases plasma glucose and lipid profile levels in diabetic rats (Subhasree *et al.*, 2015), and improves the insulin sensitivity index, ISI (Hivert *et al.*, 2016). It is known to promote healthy aging by preventing sedentariness damages (Senesi *et al.*, 2016)and weight loss (Malin & Kashyap, 2014). It is cardio protective (Argun *et al.*, 2015; Sheta *et al.*, 2016) and therapeutic agent for the treatment of lung, ovarian, colon and renal carcinoma (Abdelsatir *et al.*, 2015; Al-Wahab *et al.*, 2015; Guo *et al.*, 2016; Tseng, 2016;Zhong *et al.*, 2015). It reduces oxidative stress (Othman *et al.*, 2016), attenuates the blood urea nitrogen and creatinine (Rafieian-Kopaei *et al.*, 2013), and inflammation (Wang *et al.*, 2015). Despite the risk of lactic acidosis (Lovas *et al.*, 2000; Reeker *et al.*, 2000; Pilmore, 2010) coupled with vomiting, diarrhea, hypothermia, hypotension and transitory blindness (Chu *et al.*, 2003) metformin is a preferred forits positive results with dose management (DeFronzo *et al.*, 2016; Decker *et al.*, 2015;Garofalo *et al.*, 2015).

Metformin enhances the action of insulin in liver (Hostalek *et al.*, 2015) and causes reversal of hepatocellular carcinoma (Ling *et al.*, 2014). It is recommended for prevention of liver damage (Bergheim *et al.*, 2006). It may improve NAFLD (Linden *et al.*, 2015), but unfortunately not under the pre-existing hepatic malignancies (Bhat *et al.*, 2015). Mechanism of action of metformin is combined effect of increased antioxidation, reduced mitochondrial ROS synthesis and inflammation (Cahova *et al.*, 2015). It also acts by inhibiting gluconeogenic gene expression in liver (Cao *et al.*, 2014; Madiraju *et al.*, 2014) and ameliorating through regeneration (Ismail *et al.*, 2015). Besides advantages, adverse effects of metformin have been encountered in form of lactic acidosis leading to hypovolemia, acute cardiorespiratory illness, severe sepsis and acute renal or hepatic failure (Balogh & Matyus, 2012).

MATERIALS AND METHODS

Animal Rearing

Twenty, Swiss Webster Albino male mice (*Musmusculus)* at the age of 4-5 weeks were raised in four cages (14″x 10″x 7″). These mice were fed with commercially prepared Feed No.12, National feeds, Lahore with a constant supply of fresh drinking water throughout the experiment. Five mice were housed per cage in a room with controlled light (12hours light and dark cycle) and temperature (25±2˚c) maintained by heater with relative humidity of 40-55%.

Drug used

Metformin available in the tablet with the trade name "Glucophage" (500mg) (Merck

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Pharmaceuticals Pvt. Ltd. Pakistan) was used in the study.

Grouping and Dose Preparation

Twenty mice were divided equally into four groups. These groups were given 0.00, 31.25, 125 and 500µg/g B.W. of metformin. Doses were prepared in distilled water in such a way that each 0.1 ml contained the desired concentration. These doses (0.1ml of each dose group) were given to mice once a day for 4 weeks, with the help of especially designed glass syringe by pumping into the gullet to minimize the wastage of dose.

Recovery of Organs

Animals of each dose group were sacrificed after 24 hours of the last dose. Intact liver tissues were recovered from each dose group and placed into normal saline. Subsequently these tissues were chopped into small pieces ($3mm^3$) with sharp cutter, followed by preservation in Bouin's fluid (Prepared by mixing 70 ml of picric acid, 25 ml of glacial acetate and 5 ml of formalin) for 48 hours.

Histological Preparations

All tissues were processed for preparation of serial sections (4µm) using rotary microtome. Well air dried sections were further stained with hematoxylin and eosin.

Microscopic Study, Digital Photography and Processing

The slides were observed for histological study using trinocular microscope SWIFT (M4000-D). Selected sections were microphotographed with the help of digital camera BESTSCOPE (BUC2-500C) attached with the trinocular.

RESULTS

Histological analysis of cross section of liver from control group appeared with intact and normal association of sinusoids and hepatic cords. The binuclear hepatocytes were found in functional stage. Kupffer cells were in normal alignment along the lining of sinusoids (Fig 1A). A cross section of liver of adult male mice treated with 31.25µg/g B.W. of Glucophage, showed shrunken nuclei with deposition of collagen fibers which leads to pyknosis. Fat droplets were also seen in some areas (Fig 1B). A cross section of liver of adult male mice treated with 125µg/g B.W. of Glucophage, showed various abnormalities. In most areas, uni-nuclear cells with shrunken nuclei appeared as pyknotic hepatocytes which ultimately reached to necrosis. Sinusoids were highly diffused due to extra collagen and fat globules and became the cause of hepatic fibrosis (Fig 1C). A cross section of liver of adult male mice treated with 500µg/g B.W. of Glucophage, appeared with large volume of fat globules i.e. alcoholic liver. Extracellular matrix was increased due to deposition of collagen fibers which severely affected the nourishment of hepatocytes and resulted into necrosis (Fig 1D).

DISCUSSION

Metformin exposure has been found to induce malformations of varying intensities at different concentrations. In dose I (31.25µg/g B.W) and II (125µg/g B.W), smudge cells (cells lack identifiable cytoplasmic membranes and nuclear structures), balloon cells (hepatocytes that are injured but not yet dead), extra collagen fibers, karyorrhexis (nuclear fragmentation), fibrosis and necrosis was observed (Fig1B &C). These results are contrary to those of Conde *et al.,* (2015) where metformin is found protective against necrosis. It inhibits HCC (Qu & Yang, 2015; Yang *et al.*, 2015), however, at the same time, it has also been reported to induce hepatotoxicity in a 61-year-old obese man (Cone *et al.*, 2010), and hepatocellular and cholestatic hepatic injury (Kutoh, 2005; Saadi *et al.*, 2013).

In dose III (500µg/g B.W), macrovesicular steotosis, karyorrhexis and pyknosis was observed (Fig 1D). Such results were again found contrary to other findings where metformin is declared as therapeutic agent for hepatic steatosis through PRKA as well as SIRT1 and adenosine monophosphate-activated protein kinase pathways, and targeting the mammalian target of rapamycin signaling and anabolic processes (Song *et al*., 2015;Zheng *et al*., 2015; Sosnicki *et al.*, 2016). Meng*et al*. (2015) strengthened this concept with further elaboration that metformin even in low amount triggers the formation of AMPK complex. The involvement of AMPK as primary target was denied by Viollet *et al*. (2012) mentioning that it is actually mitochondrial respiratory chain complex and not AMPK responsible for protective action, however, this correlation of energy status and AMPK pathway has been explained recently. It is found that primary effect of metformin action is a decrease in cell energy status, which activates AMPK followed by a decrease in serum concentrations of insulin and insulin growth factor I (Sosnicki*et al.*, 2016). Metformin improves liver injury in PCOs patients without influencing serum nonalcoholic steatohepatitis (Tan *et al.*, 2015). In one study it is found to reverse the hepatic steatosis completely (Fu *et al.*, 2015).

CONCLUSIONS

Findings of this study suggest that use of metformin, considering the associated health conditions, may become harmful during therapy.

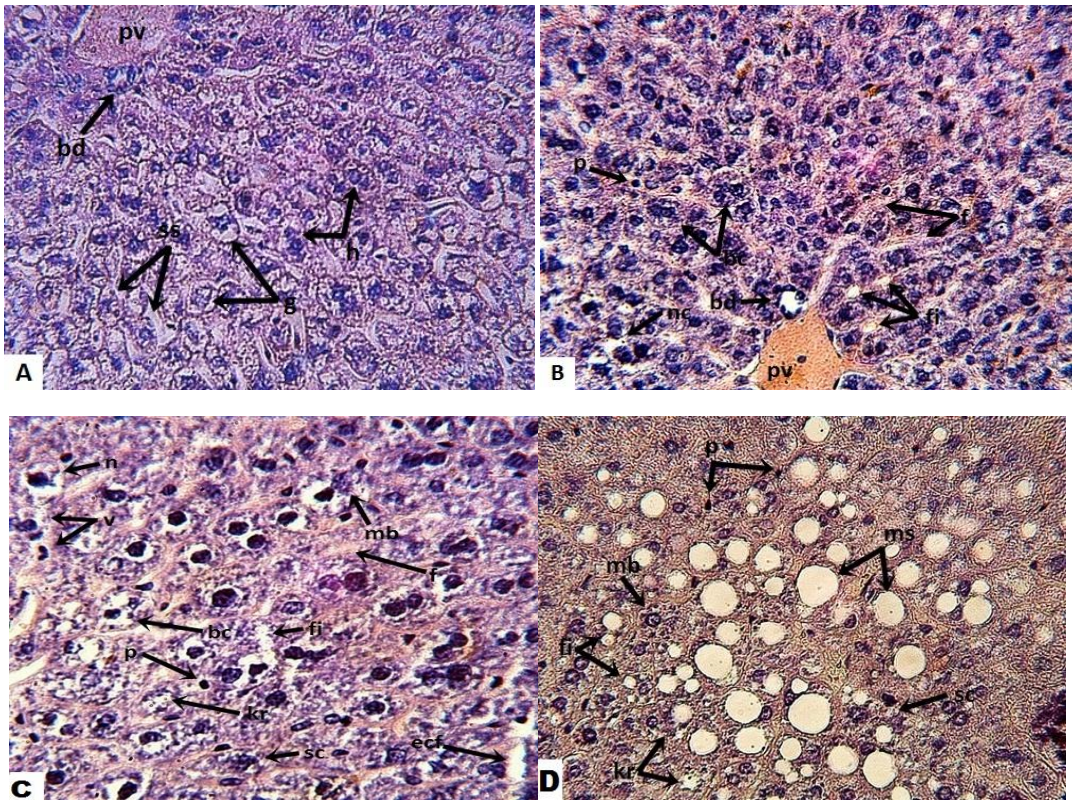

Fig 1: Selected sections (H&Ex400) of liver of adult male mice of (A) treatedwith 0.00µg/g B.W showing intact and normal association of sinusoids and hepatic cords, a portal vein and normal deposition of glycogen around the nucleus of hepatocytes., (B) 31.25µg/g B.W, (C) 125µg/g B.W, and (D) 500 µg/g B.W of metformin (Glucophage) showing various defects.

Note: h: hepatocytes; **ss:** sinusoids; **pv:** portal vein; **bd:** bile duct; **g:** glycogen; **n:** necrosis; **mb:** Mallory-denk body; **f:** fibrosis; **fi:** fatty infiltrate; **v:**vacuolation; **bc:** balloon cell; **p:**pyknosis; **ecf;** extra-collagen fibers; **sc:** smudge cells; **kr:** karyorrhexis**; ms:** macrovesiculartsteotosis; **nc:** necrotic nuclei

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