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Original Research Article

Potential effect of metformin and vildagliptin against isoniazid induced hepatotoxicity in Wistar albino rats

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ABSTRACT

Background: Metformin and vildagliptin both are anti-diabetic agent and they play an important role in diabetic patients as they reduce blood glucose levels. Studies revealed that both metformin and vildagliptin has the ability to promote beta cell neogenesis and regeneration. So, our study was planned to explore the hepatoprotective potential of metformin and vildagliptin in Wistar albino rats exposed to isoniazid (INH) induced hepatotoxicity.
Methods: Wistar albino rats weighing 150-180 g were obtained from Mass Biotech, Chengalpattu, Tamil Nadu. The animals were divided into 6 groups (n=6) and further treated orally against INH-induced hepatotoxicity except normal control group. group 1: normal control, group 2: INH, group 3: metformin+INH, group 4: vildagliptin+INH, group 5: metformin amd vildagliptin+INH, group 6: silymarin.
Results: In the present study, INH was administered for 21 days to induce liver damage to rats except normal group. Each group was treated with metformin, vildagliptin, (metformin+vildagliptin) combination and silymarin half an hour before INH challenge. On the 22nd day the blood samples were collected to estimate the AST and ALT levels. Immediately after blood collection the animals were sacrificed, the livers were removed and kept in 10% formalin for histopathological examination.
Conclusions: The study found that metformin, vildagliptin, and their combination showed hepatoprotective activity against INH-induced hepatotoxicity. The combination of metformin+vildagliptin was the most effective. Metformin reduces oxidative stress, while vildagliptin balances pro-oxidant and anti-oxidant levels, contributing to their hepatoprotective effects. This suggests their potential usefulness in drug-induced hepatotoxicity.

Keywords: Hepatoprotective, Metformin, Vildagliptin, INH, Liver enzymes

INTRODUCTION

The liver is one of the important organs, regulating homeostasis within the body. The various hepatic functions include protein synthesis, storage as well as metabolism of fats and carbohydrates. Apart from these, liver is essential for excretion of bilirubin, detoxification of toxins and metabolism of several endogenous and exogenous substances. Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences for the individual concerned.[1] Drug induced liver injury is an unresolved problem and often limits drug therapy in clinical practice. Liver injury may follow the inhalation, ingestion, or parenteral administration of a number of chemical and pharmacological agents.[2] The nature and extent of liver damage varies depending on the type of stage of its disease. Not all liver diseases produce the same patterns of change, at least while they are evolving, although many forms of chronic liver disease will ultimately lead to the typical clinical and histological picture of cirrhosis. The effects of liver disease on the hepatic metabolism of

drugs are complex and difficult to predict particularly when multiple drugs are administered simultaneously.[3] It is well known that drugs are structurally altered in the liver to form biologically inactive or active or toxic metabolites. Indiscriminate uses of analgesics, antimalarial, anti-tubercular drugs, oral contraceptives, antidepressants, and anticonvulsants are potential threats to the integrity of liver.[4-7] Quite often certain drugs even in therapeutic dose may cause hepatic damage in susceptible individual. Toxic effects of drugs on the liver or on its function may mimic any naturally occurring hepatic disease. The spectrum of drug induced liver injury ranges from asymptomatic increase in enzyme (markers of hepatic damage) levels to fulminant hepatic failure. It can occur in a number of different forms including acute drug-induced hepatitis, steatohepatitis, cholestasis, chronic hepatitis and may lead to liver failure. Many drugs may cause more than one type of hepatic injury patterns.[8] It appears that, the nature and extent of drug-induced hepatic damage is dependent on dose of causative agent and duration of exposure. Accordingly, single overdose of paracetamol and chronic consumption of alcohol certainly produce hepatotoxicity. Such hepatotoxicities though important clinically are preventable, while hepatic damage due to isoniazid (INH), an effective anti-tubercular drug that is administered chronically, often poses a problem in clinical practice. Measures to prevent and use of effective agents to treat the drug induced hepatotoxicity is essential to ensure the safety and efficacy of certain drugs like INH. Carbon tetrachloride and lindane has been used routinely to induce hepatotoxicity in Albino rats and mice which is not in clinical practice.[9,10] Despite tremendous strides in drug discovery and modern medical practice, there are hardly any drugs that stimulate liver functions or offer protection to the liver against injurious substances or help regeneration of hepatic cells. The presently used agents like folic acid, multivitamins and few polyherbal preparations provide only a supportive therapy and do not play an effective role in providing hepatic protection, so our search was towards a novel drug in treatment of liver injury.[11] Metformin is prescribed as the first-line medication for type 2 diabetes mellitus.[12] It has been reported as an anti-epileptic drug and, anti-aging drug and also used for treatment of PCOS, Parkinson's disease and adult neurogenesis, CVS disease and weight loss.[13-18] Vildagliptin significantly used as hypoglycemic agent by inhibiting DPP4 enzyme.[19] It has been also reported to have anti-inflammatory and reno-protective effect.[20,21] Metformin works by lowering glucose production in the liver, delaying glucose absorption from intestine, and increasing the body's sensitivity to insulin.[12] Vildagliptin works by increasing the release of insulin from the pancreas and decreasing the hormones that raise blood sugar levels.[19] This reduces both fasting and post-meal sugar levels. Metformin and vildagliptin together they provide better control of blood sugar levels.[22] Studies recorded that metformin has the potential to regenerate the liver cells against paracetamol-induced hepatotoxicity.[23] Earlier studies showed that vildagliptin promotes β cells neogenesis and regeneration and also prevents liver damage in cyclosporine-induced hepatotoxicity.[24,25] Therefore, the present study was planned to explore the hepatoprotective potential of metformin and vildagliptin in male Wistar albino rats exposed sub-acutely to INH-induced hepatotoxicity.

METHODS

In the present study albino Wistar rats served as an experimental animals.

Drugs

The drugs used were metformin (OKAMET-500, Cipla Ltd.); vildagliptin (Torglip-50, Torrent Pharmaceuticals Ltd.); metformin and vildagliptin combination (Vildapride M 1000/50 mg tablet, Micro Labs Ltd.); silymarin (Silybon suspension, Micro Labs Ltd.); isoniazid (Solonex-300, Micro Labs Ltd.).

The drugs (except silymarin) were freshly dissolved in the distilled water and were daily administered through oral gavage for 21 days.

Study type

The study was an *in vivo* study and sub-acute model.

Study period

The study duration was from October 2023 to November 2023.

Study place

The study was conducted in the animal house of Adiparasakthi College of Pharmacy.

Collection of animals

Healthy Wistar albino rats weighing 150-180 g were obtained from mass biotech, Chengalpattu, Tamil Nadu. Animals were placed in cages at room temperature (25°±2 °C) relative humidity of approximately (50±5%) and a 12 hours dark/light cycle with access to food and water ad libitum. Experimental protocols were performed in accordance with the institutional animal ethics committee approval.

Grouping of animals

The animals were divided into six groups (n=6) and further treated orally against INH induced hepatotoxicity at a dose of 100 mg/ kg through oral gavage for 21 days except normal control group.

Group 1: normal control; group 2: INH (100 mg/kg p.o.); group 3: metformin (200 mg/kg p.o.)+INH (100 mg/kg p.o.); group 4: vildagliptin (10 mg/kg p.o.)+INH (100 mg/kg p.o.); group 5: metformin and vildagliptin (100 mg+5 mg / kg p.o.)+INH (100 mg/kg p.o.); group 6: silymarin (100 mg /kg p.o.)+INH (100 mg /kg p.o.).[23-28]

All doses and the time interval between drug administration and the tests were selected based on pilot studies. At the end of the study, animals were fasted overnight and the blood was withdrawn by cardiac puncture under ether anesthesia and was analyzed for standard liver function tests (AST, ALT).

Liver function test

Serum AST and ALT were estimated by IFCC method and were expressed in IU/l.

Histopathological studies

Immediately after blood collection, animals were sacrificed under anesthesia, livers were removed and preserved in 10% formalin. Tissues were processed and embedded in paraffin wax, consecutive sections were stained with hematoxylin and eosin.

Statistical analysis

The results were analyzed by ANOVA followed by Dunnet's test and $p \leq 0.05$ was considered as significant.

Inclusion criteria

Healthy, weighs 150-180 g, Wistar albino rat species, free from pathogens and infections checked by veterinarian were included in the study.

Exclusion criteria

Food refusal, underweight, overweight, impaired mobility, showing unexplained clinical signs, Any abnormalities in previous 7 days were excluded.

Total samples

Total number of samples were 36.

RESULTS

Liver test findings

The liver test findings of INH group showed a significance increase in the serum AST and ALT. The metformin, vildagliptin, metformin and vildagliptin, silymarin group showed a significance protection against INH induced hepatotoxicity (Figure 7 and 8). The values are tabulated in Table 1.

Table 1: ALT and AST levels of the normal, INH, and drug treated groups.

Groups	AST	ALT
Normal	121****	27****
INH	191	62
Silymarin+INH	139****	34****
Metformin+INH	129****	31****
Vildagliptin+INH	160****	41****
(Metformin+vildagliptin)+INH	117****	25****

****P<0.0001 compared with INH treated group.

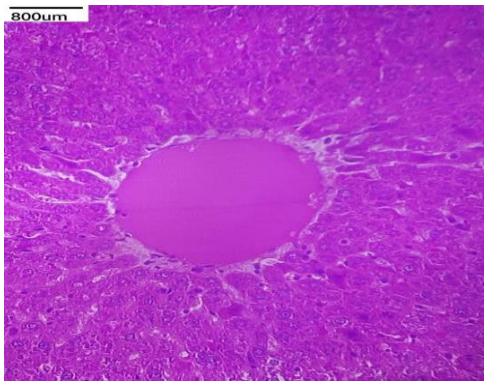

Figure 1: Control group (×800 H&E).

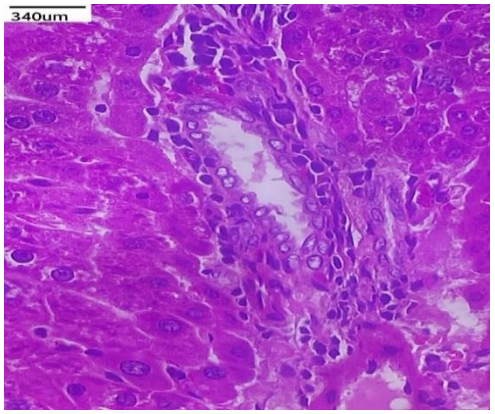

Figure 2: INH group (×340 H&E).

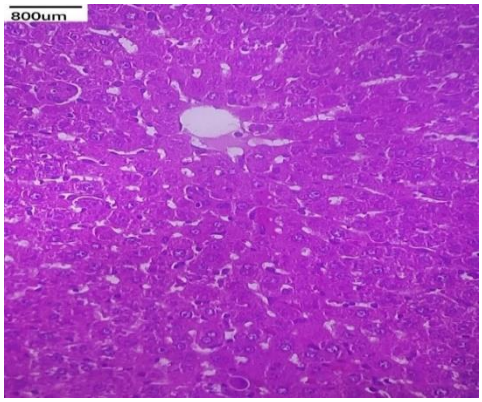

Figure 3: Metformin group (×800 H&E).

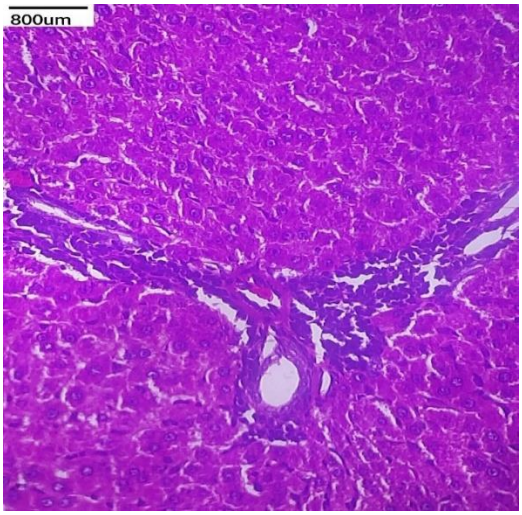

Figure 4: Vildagliptin group (×800 H&E).

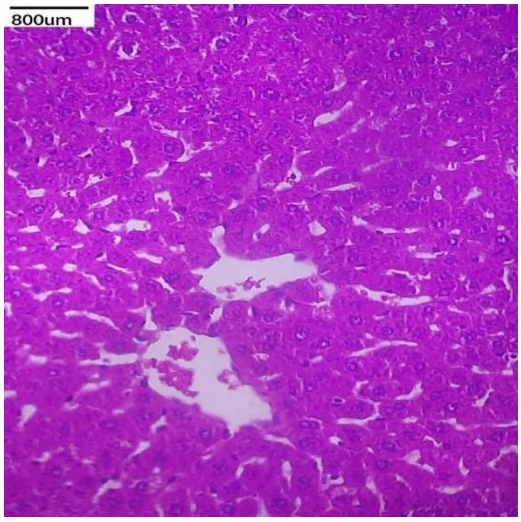

Figure 5: Metformin+vildagliptin) combination group (×800 H&E).

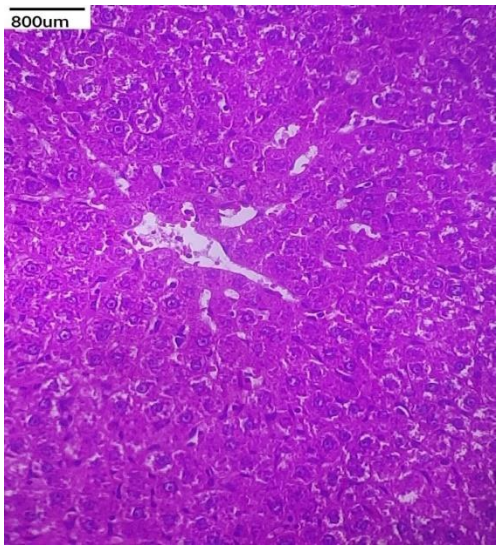

Figure 6: Standard silymarin group (×800 H&E).

Histopathological findings

Microscopical observations of control group show normal hepatocytes, normal sinusoids, and central vein (Figure 1). The INH-treated group shows periportal inflammation and fibrosis (Figure 2). The metformin group shows normal hepatocytes, normal sinusoids, and central veins (Figure 3). The vildagliptin group shows normal hepatocytes, normal sinusoids, periportal inflammation, and fibrosis (Figure 4). The metformin and vildagliptin group shows normal hepatocytes, normal sinusoids, and central vein (Figure 5). The silymarin group shows normal hepatocytes, normal sinusoids, and central vein (Figure 6).

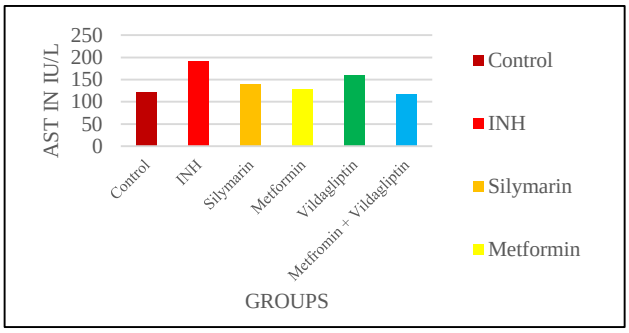

Figure 7: Effect of test drugs on INH induced changes in serum AST.

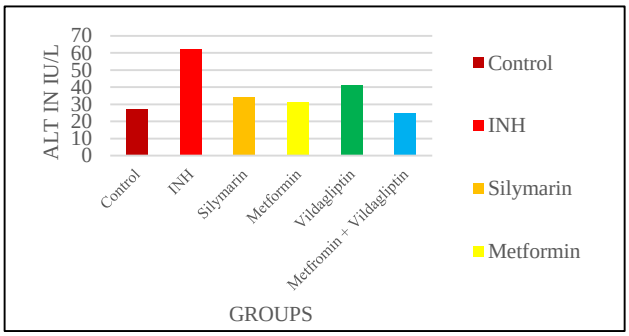

Figure 8: Effect of test drugs on INH induced changes in serum ALT.

DISCUSSION

In the present study, INH in the dose of 100 mg/kg p.o. was administrated for 21 days to induce hepatoxicity in groups, except group 1 normal control group. Group 2 only INH treated, group III treated with metformin+INH, group 4 treated with vildagliptin+INH, group 5 treated with combination (metformin+vildagliptin)+INH and group 6 treated with silymarin+INH. On the 22nd day the blood samples were collected to estimate the AST and ALT levels by cardiac puncture under ether anaesthesia. Immediately after blood collection the animals were sacrificed, and the livers were removed and preserved in 10% formalin for histopathological examination. The serum AST and ALT levels in the normal group was estimated as 121±2.57 and 27±1.55 respectively. AST

and ALT levels of only INH administered group was estimated to be 191±2.80 and 62±2.81 respectively. AST and ALT levels in standard (silymarin) treated group was 137±1.62 and 34±1.52 respectively. In metformin-treated group the levels of AST and ALT were 129±1.54 and 31±1.84 respectively. The levels of AST and ALT in vildagliptin treated group were estimated to be 160±2.59 and 41±2.09 respectively. Serum AST and ALT levels in combination (metformin+vildagliptin) group was estimated to be 117±1.59 and 25±1.66 respectively. AST and ALT levels in group 3, 4, 5 and 6 showed a significant decrease ($p<0.0001$) when challenged against group II (only INH-treated groups). AST and ALT levels of (group 1) normal control vs metformin (group 3), vildagliptin (group 4), combination (metformin+ vildagliptin) (group 5) and standard silymarin (group 6) were compared. Normal control vs. combination (metformin+vildagliptin) did not show any significant difference. The liver histopathology of the normal control group shows normal hepatocytes, normal sinusoids, and central vein. The liver histopathology of INH group shows periportal inflammation and fibrosis. The liver histopathology of metformin-treated group shows normal hepatocytes, normal sinusoids and central vein. The liver histopathology of vildagliptin group shows normal hepatocytes, normal sinusoids along with periportal inflammation and fibrosis. The liver histopathology of combination (metformin+vildagliptin) shows normal hepatocytes, normal sinusoids and central vein. The liver histopathology of standard (silymarin) seems to be normal showing normal hepatocytes, normal sinusoids and central vein. The abnormal levels of liver enzymes were recorded in group treated with INH alone in the present study coincide with aforesaid study.[26] The effects of INH were reduced by metformin and vildagliptin in this study. Similar results have been demonstrated by various studies; however, the toxicity induction model didn't coincide with the aforesaid study.[23-27] The observation of liver sections of INH treated group coincide with aforesaid study.[26] The observation of liver section of metformin and vildagliptin groups shows protective effects against the INH as similar to aforementioned studies in different models.[23,25] Some potential limitations of a study investigating the hepatoprotective activity of metformin and vildagliptin against INH-induced hepatotoxicity in Wistar albino rats could include: species-specific differences: findings in rats may not directly translate to humans due to physiological and metabolic differences between species.

Limited generalizability

Results may not be applicable to other strains of rats or animal models, let alone humans.

Duration of study

The study may not capture long-term effects of the treatments or provide insight into chronic hepatotoxicity.

Dose-dependency

The study may not explore the effects of different doses of metformin and vildagliptin, which could affect the observed hepatoprotective activity.

Lack of mechanistic insights

The study may not elucidate the underlying mechanisms of hepatoprotection by metformin and vildagliptin, limiting understanding of their therapeutic potential.

Single-induction model

Focusing solely on INH-induced hepatotoxicity may overlook potential interactions or effects in other liver pathologies or drug-induced liver injuries.

Sample size

Small sample sizes could limit the statistical power and reliability of the findings.

Duration of treatment

The study may not investigate the optimal duration of treatment needed to observe hepatoprotective effects or potential adverse effects associated with prolonged treatment.

Potential for off-target effects

The study may not thoroughly assess potential off-target effects of metformin and vildagliptin on other organs or systems.

Lack of clinical data

While the study may demonstrate efficacy in animal models, clinical trials in humans are needed to confirm the hepatoprotective effects and safety profile of metformin and vildagliptin in patients with INH-induced hepatotoxicity.

CONCLUSION

In the present study metformin, vildagliptin, metformin + vildagliptin and silymarin group were found to have hepatoprotective activity against INH-induced hepatotoxicity based on liver function test (AST and ALT) and histological studies. Among the three-test group, the combination of metformin+vildagliptin was found to be more effective because there was no significant difference between the normal control and combination of metformin and vildagliptin. Metformin is reported that reduced the activities of plasma xanthine oxidase and erythrocyte glutathione peroxidase so that reduces the oxidative stress, vildagliptin is reported that reducing the oxidative stress and apoptosis by pro-oxidant and anti-oxidant balance by reducing levels SSof

stress markers. Therefore, observed hepatoprotective activity of metformin and vildagliptin may be due to this property. Drug induced hepatotoxicity is a challenge, for many such toxic substance and there is no satisfactory antidote. The results of the present study shows that metformin, vildagliptin and combination of metformin and vildagliptin could be useful against hepatotoxicity produced by different drugs. Therefore, further studies is to evaluate the pharmacodynamic parameter of metformin, vildagliptin and combination of metformin and vildagliptin in drug induced hepatotoxicity is desirable. Further investigation on pharmacodynamic parameter of metformin and vildagliptin to be carried out in future to find appropriate mechanism responsible for the hepatoprotective activity.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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