Esculapio - Journal of Services Institute of Medical Sciences

Original Article

Hepatoprotective & other hepatic histopathological effects of Cinnamon, Pyridoxine and Pitavastatin in Treating High-Fat Diet-Induced Murine Dyslipidemia

Maryam Mansoor,[1] Saadia Shahzad Alam,[2] Iram Imran,[3] Hassan Farooq,[4] Rabia Tariq[5]

Abstract

Objective: To look for hepatic histopathological & hepatoprotective effects of pyridoxine, pitavastatin & cinnamon in diet-induced dyslipidemic rats.

Methods: 10 groups (60 albino male rats, age: 6 weeks) were included (Group 1: control). Group 2 (dietary preventive) and Groups 3-10 (therapeutic) were induced using HFD for 30 days (HFD continued throughout). They were treated for 30 days, after induction, orally, once a day with pitavastatin (PIT), aqueous cinnamon extract (ACCE) and pyridoxine (PYR) in various combinations: Group 3 (Pitavastatin 0.3mg/kg); Group 4 (Pyridoxine 18mg/kg), Group 5 (ACCE 200mg/kg), Group 6 (Pitavastatin 0.3mg/kg + Pyridoxine 18mg/kg), Group 7 (Pyridoxine 18mg/kg + ACCE 200mg/kg), Group 8 (Pitavastatin 0.3mg/kg + ACCE 200mg/kg), Group 9 (Pitavastatin 0.3mg/kg + Pyridoxine 18mg/kg + ACCE 200mg/kg) and Group 10 (Pitavastatin 0.15 mg/kg + Pyridoxine 9mg/kg + ACCE 100mg/kg). Animals were sacrificed (Day 60); slides were prepared for histopathology from livers (architectural distortion, epithelial damage, inflammation, fatty change, cytoplasmic changes, Kupffer cell hyperplasia). SPSS 20.0 ($P \leq 0.05$) was used to analyze data.

Results: Livers of rats in groups treated with pyridoxine and cinnamon (Group 4, 5 & 7) remained unaffected. Rats in groups 3, 6 & 10 showed mild while combination groups (8, 9) showed mild to moderate fatty change (46.7% rats) and inflammation (46.7% rats). 8 (13.3%) rats showed distorted architecture.

Conclusion: Cinnamon and pyridoxine can be "safer" alternatives to standard dyslipidemia treatment, either alone or in different combinations with pitavastatin.

Keywords: Pitavastatin, Pyridoxine, Cinnamon, Dyslipidemia

How to cite: *Mansoor M., Alam S.S., Imran I., Farooq H., Tariq R. Hepatoprotective & other hepatic histopathological effects of Cinnamon, Pyridoxine and Pitavastatin in Treating High-Fat Diet-Induced Murine Dyslipidemia. Esculapio 2021;17(03):269-273.*

DOI: ***https://doi.org/10.51273/esc21.2517311***

Introduction

Dyslipidemia, a recent term coined for hyperlipidemia, refers to elevated levels of plasma lipids & disturbance in metabolism of various lipoproteins[1]. Dyslipidemia if persists, can prove to be quite harmful (with high risk of developing cardiovascular diseases) and can give rise to various complications[2]. If the control is not achieved, pharmacotherapy becomes necessary to reduce the risk of complications by 30% in 5-years.[3] Dyslipidemia can lead to changes in hepatic histo-architecture. It can lead to fatty inclusions causing fatty liver, inflammatory changes, fibrosis, distortion of hepatic architecture and development of hepatic tumors and cancers. Excessive fatty acids can result in lipid-induced toxicity (lipotoxicity)-induced steatosis and hepatic dysfunction. [4]

Because of their effectiveness, statins have been commonly used for the pharmacotherapy of dyslipidemia. Pitavastatin, like other statins, is HMG (3-hydroxy-3-methyglutaryl) CoA-Reductase inhibitor which inhibits hepatic cholesterol biosynthesis. It lowers the total cholesterol and LDL-C and also raise HDL slightly. Pitavastatin enhances the LDL receptor expression and assembly in liver, hence, increasing LDL-C extraction from the blood. Pitavastatin has a

1. Maryam Mansoor
2. Saadia Shahzad Alam
3. Iram Imran
4. Hassan Farooq
5. Rabia Tariq

1. Department of Pharmacology, Al-Aleem Medical College, Lahore
2. Department of Pharmacology, SKZMDC, Sheikh Zayed Hospital, Lahore
3. Department of Pharmacology Amna Inayat Medical College, Lahore
4,5. Department of Pharmacology, Ameeruddin Medical College, PGMI, Lahore.

Correspondence:
Dr. Maryam Mansoor, *Assistant Professor of Pharmacology & Therapeutics, Al-Aleem Medical College, Lahore.*
Email: maryammansoor2013@gmail.com

Submission Date: 01-09-2021
1st Revision Date: 09-09-2021
Acceptance Date: 15-09-2021

highly liver-selective distribution due to carrier-mediated uptake and an extensive first-pass hepatic extraction[5].
Pitavastatin and other statins are generally safe and not toxic.[6] However, an issue of poor compliance and discontinuation of therapy can be there because statins (in high-doses) may lead to some adverse effects. Adverse effects may include raised liver enzymes, hepatotoxicity, myopathy, teratogenicity, cognitive dysfunction on prolonged use, impaired glucose control and drug-drug interactions (DDIs) due to cytochrome enzyme inhibition.[7] Hepatotoxicity from statins can range elevated liver enzymes to hepatic failure but serious injury is very rare and is mostly seen in underlying liver disease or alcoholism.[7] Statins are generally safe and well-tolerated. Their wide-spread use has a positive effect on the global burden of cardiovascular diseases. They are believed to protect the liver from dyslipidemia-induced damage and actually play a hepatoprotective role there.[8] Others advocate absence of hepatic adverse effects in mice treated with pitavastatin and protect against lipid-induced hepatocellular tumors. Moreover, pitavastatin, out of all other statins is superior in terms of safety because it is less involved in drug interactions.[9]
Use of phyto-products is gaining importance, as people are reverting to more natural choices. Drugs can become difficult to consume due to non-compliance: fear of adverse effects or effects themselves, issues with affordability, etc. Many plants and herbs are making a comeback e.g. for dyslipidemia and cardio-protection, dates, ginger, fax seeds, lemon grass and cinnamon are notable.
About cinnamon, it has been postulated that it significantly reduces lipid levels by inhibiting HMG-CoA reductase activity like statins. It may also activate the insulin receptor and launch the insulin cascade system to lower the lipid burden.[10] Some experiments link its lipid-lowering effect to PPAR-γ and PPAR-α activation[11]. It protects against lipotoxic effects on liver. It has been found to be free of adverse hepatic effects especially when used alone.[12] It has anti-inflammatory properties and is found to be hepatoprotective.[13,14]
Several micronutrients have also been found useful for definitive treatment of dyslipidemia e.g. pyridoxine.[10] Pyridoxine (Vitamin-B6) is believed to act in the cholesterol biosynthesis. It may be involved in defense mechanisms against lipid peroxidation in tissues because its deficiency accelerates the process and produces mild dyslipidemia.[15,16] So, its deficiency affects lipid metabolism, modifies the fatty acid composition of some tissues and increases triglycerides and other lipids. As a vital micronutrient, it is generally safe: free of adverse hepatic histopathological effects.
Various combinations of pharmacological drugs e.g., statins and fibrates, fibrates and niacin, etc. are often required to achieve maximum possible control of dyslipidemia in clinical settings, but their combinations can exaggerate adverse muscular and hepatic histopathological effects. Here, in this study, we are experimenting with a safer statin i.e., pitavastatin, hepatoprotective cinnamon and a friendly vitamin, pyridoxine; on diet-induced dyslipidemic rats.

Methods

25mg Pyridoxine and 2mg Pitavastatin tablets were purchased from Clinix Pharmacy, Lahore. Cinnamomum cassia bark was purchased from Hamdard Dawakhana, Lahore. Cinnamomum cassia bark (aqueous) extract was made at UHS, Lahore.
Cinnamomum cassia bark (1kg) was cleaned and shade-dried, extracted, twice, with 4L of distilled water at 90°C for 16 hours. Extract was filtered and freeze-dried for storage at room temperature. It was to be used after being diluted in normal saline (0.9%) and orally administered at 200mg/kg. Dry yield was 8% (w/w).[17] 60 healthy young male albino rats of 6 weeks of age (weight: 150-170 g), were purchased from UHS, Lahore. Rats were kept under standard housing conditions (maintained throughout) in Animal House, UHS. Female, diseased, under-aged and already overweight rats were excluded from the study. Study duration was 60 days.
Groups distribution: Group 2 (dietary preventive group) had High-Fat Diet intake for only 30 days (later on reverted to standard diet till Day 60) and were studied for vital preventive role of "dietary modification" on dyslipidemia. Groups 3-10 were continued to be fed HFD throughout 60 days. University of Veterinary and Animal Sciences, Lahore provided the HFD for the study. 10 groups of 6 animals each (total 60 rats) were used in this study (Table 1).
HFD administered for dyslipidemia induction consisted of: Casein - 120g, Corn starch - 549.6g; Soybean oil - 250g; Cholesterol - 10g, Choline - 0.4g; Salt mixture - 50; Vitamin mixture – 10g and Cellulose – 10g. Total calories (Kcal)/1000g of diet were 5018.4 which was 1000Kcal/1000g greater than normal control diet.[18]
After consumption of HFD and treatment with drugs for 30 days, rats were sacrificed (Figure 1). The livers were removed and fixed in 10% formalin solution to be preserved for histo-architectural studies. From paraffinized tissue blocks, 4µm thick slices were prepared & stained with hematoxylin and eosin.
Slides were prepared at Histopathology Laboratory, Department of Histopathology, Postgraduate Medical Institute, Lahore. They were studied and reviewed at Histopathology Laboratory, Department of Histopathology, Shaikh Zayed Hospital, Lahore. Images were taken at Histopathology Department, Shalimar Hospital, Lahore. Pearson Chi-square test was applied on histopatholo-gical parameters (magnification: 40x).

Table 1: *Groups Details*

Groups	Group Details (Diet & Treatment)
Group 1	Control
Group 2	Preventive Model (HFD + standard diet)
Group 3	HFD + 0.3mg/kg Pitavastatin
Group 4	HFD + 18mg/kg Pyridoxine
Group 5	HFD + 200mg/kg Aqueous Cinnamon Extract – ACCE
Group 6	HFD + 0.3mg/kg Pitvastatin + 18mg/kg Pyridoxine
Group 7	HFD + 18mg/kg Pyridoxine + 200mg/kg ACCE
Group 8	HFD + 0.3mg/kg Pitavastatin + 200mg/kg ACCE
Group 9	HFD + 0.3mg/kg Pitavastatin + 18mg/kg Pyridoxine + 200mg/kg ACCE
Group 10	HFD + 0.15mg/kg Pitavastatin + 9mg/kg Pyridoxine + 100mg/kg ACCE

Results

Hepatic histopathological effects are shown for Group 9 (Figure 1) & Group 10 (Figure 2)

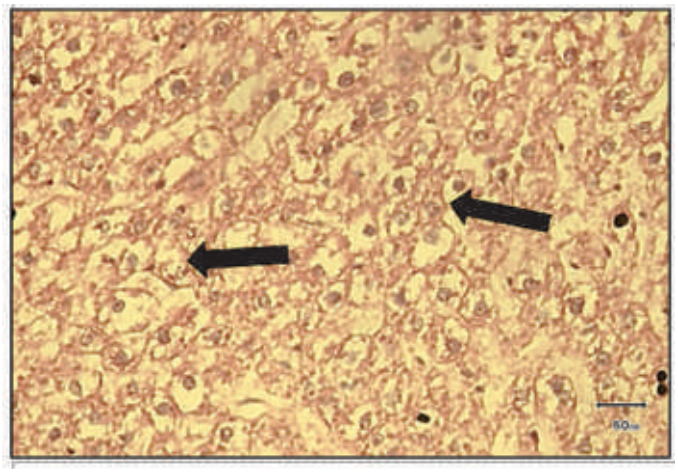

Figure 1: *Group 9 (Pitavastatin 0.3mg/kg + Pyridoxine 18mg/kg+ACCE 200mg/kg) Liver Cross-Section. Archi-tectural Distortion and Epithelial Disruption Seen. Fatty change & Inflammation can also be Seen.*

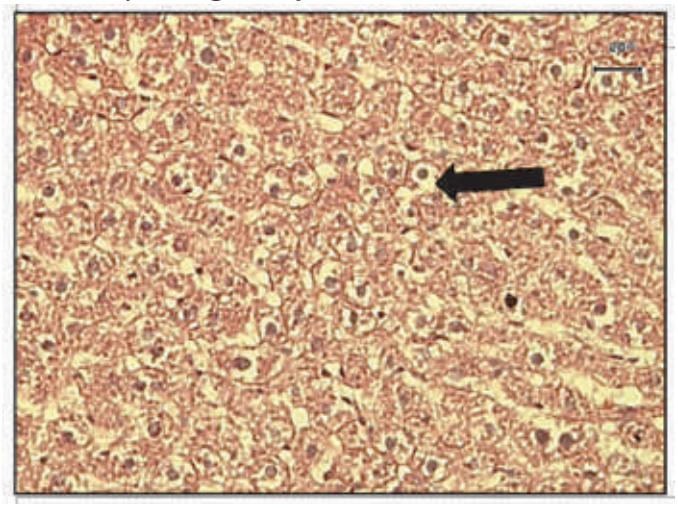

Figure 2: *Group 10 (Pitavastatin 0.15mg/kg + Pyridoxine 9mg/kg + ACCE 100mg/kg) liver cross-section. A: mild steatosis*

Legend:

Group 1: Control
Group 2: high fat diet + no treatment
Group 3: high fat diet + pitavastatin 0.3mg/kg
Group 4: high fat diet + pyridoxine18mg/kg
Group 5: high fat diet + cinnamon 200mg/kg
Group 6: high fat diet + pitavastatin 0.3mg/kg + pyridoxine 18mg/kg
Group 7: high fat diet + pyridoxine18mg/kg + cinnamon 200mg/kg
Group 8: high fat diet + pitavastatin 0.3mg/kg + cinnamon 200mg/kg
Group 9: high fat diet + pitavastatin + pyridoxine + cinnamon (full doses)
Group 10: high fat diet + pitavastatin + pyridoxine + cinnamon (in half doses)
None of the rats showed liver Kupffer cell hyperplasia in the treated or untreated group.

Discussion

The results of hepatic histopathology came out to be favorable (especially combination Group 10
Regarding lobular architecture, 86.7% (52/60) rats belonging to different group, exhibited normal hepatic lobular architecture. Rats in Groups 4 and 5 (pyridoxine and cinnamon, respectively) had normal architecture, highlighting their safety and hepatoprotection. Probable mechanism can be their antioxidant effects. Cinnamon possesses regenerative properties in the liver. Only 8 (13.3%), rats belonging to Groups 2 (preventive), Group 3, Group 8 and Group 9, showed distorted architecture. 13.3% rats with distorted architecture shows generally favorable results. Presence of architecture distortion in 4 out of 6 rats in Group 9 indicates pitavastatin's role in hepatotoxicity.[18,19] The association of treatment with full doses of all 3 treatment drugs with disturbance of hepatic architecture, was significant (P: 0.007).
In untreated Group 2, architecture might have got destroyed due to fatty-diet induced damage. Hepatic damage caused by high-fat diet is due to the deposition of fatty inclusions in form of lipid droplets in the cytoplasm, which join together to push nucleus to side, lead to disruption of architecture and disruption of bile ducts and portal triads: termed as "lipotoxicity"[4]. In other groups, few rats with damaged architecture could also be due to HFD as treatment was only given to all rats for 30 days.
53.33% rats (32/60) did not show any inflammation, including whole Group 4 Group 4 (PYR 18mg/kg) and Group 6 (PIT 0.3mg/kg + PYR 18mg/kg). Findings suggest that oyridoxine has dual actions in treating dyslipidemia: prevention of fat-induced liver inflammation as well as safety against toxic drug effects. The end result of fatty changes in the cytoplasm of the

hepatic cells can lead to inflammation and fibrosis while long-standing liver inflammation can also lead to carcinogenesis & tumorogenesis.

Out of 60, only 46.77% rats (28 rats) displayed mild to moderate inflammation distributed among groups 2, 3, 5, 7, 8, 9 and 10. All the rats of Group 2 and Group 8 showed inflammation in the liver. A strong, significant (P:0.000) association was observed. Hepatic inflammation in (untreated) Group 2 rats shows highlighted severe fatty change (caused by HFD), causing inflammation, fibrosis and hepatic damage. Livers of the rats in Group 8 also showed very mild inflammatory changes which may probably be statin-related.

Pyridoxine, cinnamon and statins, all seem to be hepatoprotective against HFD-induced inflammation & lipotoxicity. Cinnamon and pitavastatin, have significant general anti-inflammatory effects as well. The reported reason for statins' reduction of liver fibrosis in rats is through decrease in the turnover of stellate cells, thus, diminishing the inflammatory response 20. Cinnamon's phytochemical screening (bark) reveals flavonoids, alkaloids, glycosides, tannins, coumarins, anthraquinone, steroids and terpenoids most of which are known to possess hepatoprotection owing to anti-oxidative mechanisms[20,21].

53.33% rats showed exclusive protection from any intensity of hepatic fatty change. Notable among them are Group 4 (PYR 18mg/kg) and Group 6 (PIT 0.3mg/kg + PYR 18mg/kg). However, 46.77% rats exhibited some degree of mild to moderate fatty change. Group 2 (untreated) and Group 9 (all drugs in full doses) showed the most cases of fatty change, probably due to absence of drug treatment. The association was found significant (P: 0.000)

Fat deposition in the hepatocytes can lead to increased lipid peroxidation culminating in damage by free oxygen radicals & development of oxidative stress and mitochondrial dysfunction. All this can lead to hepatic steatosis and steatohepatitis development of which can be favorably retarded with statins 8. Group 9 showed fatty change and the findings were suggestive of 30-day long HFD plus the drug's toxicity (notably statin). But generally statins are safer for considerable periods of time in moderate doses & lead to serious hepatic injury very rarely. So, there should be no serious concerns in terms of prescribing statins.[22] Cinnamon and pyridoxine reduce total hepatic cholesterol content plus slow down and abolish the fatty change because of reduced fat oxidation. Cinnamon also augments fat utilization, upping the overall metabolism.[16]

88.33% rats showed liver specimens with intact epithelia signifying favorable results. 11.7% (7/60) rats distributed among only 3 groups had non-intact epithelia. Group 9 (PIT 0.3mg/kg + PYR 18mg/kg + ACCE) had the most cases, followed by Group 8 (PIT 0.3 mg/kg + ACCE 200mg/kg) and Group 2 (untreated) in order of decreasing frequency.

Aqueous cinnamon extract induces protein synthesis, one of many hepatoprotective mechanisms as it participates in liver cell regeneration[23]. Pitavastatin, as discussed earlier, and pyridoxine, both possess powerful anti-oxidant properties. Anti-oxidant mechanisms help in correcting lipotoxic damage to the cell. The association of the likely causative agent (pitavastatin) with epithelial damage was found significant (P: 0.000). The HFD-induced fatty change and lack of treatment thereof (Group 2) probably caused intracellular fat accumulation leading to disrupting epithelium.

91.7% (55/60) rats showed normal, healthy cytoplasm which signifies that all experimental groups (with exception of Groups 8 and 9) were protected from damage to nuclei and dispersion of nuclear material into cytoplasm. This finding advocates the "hepatoprotection" offered by all these three agents, to varying degrees. Pyridoxine, as suggested by the findings, has been found to be literally free of hepatic adverse effects. In addition, it also augments glutathione activity.

As discussed earlier, cinnamon, like pitavastatin and pyridoxine, retards fat-induced toxicity by anti-oxidant effects. Presence of normal cytoplasm also signifies the positive role of all these agents in providing hepatoprotection. Only a few rats i.e. 8.3% (5) rats belonging to two groups: Groups 8 and Group 9, exhibited acidophilic cytoplasm. The association of the presence of acidophilic cytoplasm with the causative agents (combination of all three drugs in full doses) was significant (P: 0.000).

Kupffer cell hyperplasia was not found to be a positive finding in any group. It vindicates immunological insult to the hepatic tissue, which vouches for the favorability of our three drugs in this case.

Conclusion

The hepatic histopathological effect of treatment of pitavastatin, pyridoxine and aqueous extract of Cinnamomum cassia bark on high-fat diet induced dyslipidemiahave been found fruitful as they have been found to halt the progression of the hepatic damage. It can be deduced from the results that the treatment agents may not only be free of deleterious hepatic effects but also possess "hepatoprotective" potential against high-fat diet-induced hepatic damage. Overall, pitavastatin showed very mild adverse hepatic histopathological effects devoid of any clinical significance. As liver histopathology of untreated dylipidemic rats showed a diverse spectrum of hepatic damage, it is evident that treatment is essential in dyslipidemia. The synergistic effects showed by the combination of all 3 agents (in

half doses) must be investigated further to design beneficial therapeutic combinations with good safety profile.

Conflict of Interest

None

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Author's Contribution

M.M.: Conceptualization of Project, Principal Researcher, writing of Manuscript.

S.S.A.: Supervision of manuscript writing, project

I.I.: Literature Search

H.F.: Statistical Analysis, drafting

R.T.: Drafting, Revision, Data Collection