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Short Communication

Evaluation of Hepatoprotective Effect of Vanillin in Isoniazid-Rifampicin Induced Hepatocellular Damage

Rashint Tiwari, Ayushi Chourasia, Aman Chaturvedi, Aditya Ganeshpurkar*, Nazneen Dubey

ABSTRACT

Objectives: Natural products are greatly acknowledged for antioxidant and hepatoprotective effects. Vanillin has been studied for radical scavenging effect. The aim of this study was to examine hepatoprotective effect of vanillin against isoniazid and rifampicin induced liver damage in rats. **Methods:** Wistar rats were used in present study. All the animals study protocols were duly approved by Institutional Animal Ethics Committee of the Institute. Hepatotoxicity was induced by administration of against isoniazid (50 mg/kg) and rifampicin (100 mg/kg) for 14 days. Vanillin was used in the dose of 50, 100 and 200 mg/kg body weight. At the end of study blood was collected and biochemical studies were performed to assess antioxidant status. **Results:** Oral administration of vanillin at test doses (50, 100 and 200 mg/kg body weight) resulted in restoration of AST, ALT and ALP. There was a notable decrease in production of SOD and catalase. **Conclusion:** In the present study, vanillin demonstrated a notable hepatoprotective effect. The protective efficacy of vanillin is possibly because of radical scavenging and antioxidant property.

Key words: Liver, Toxicity, Antitubercular, Peroxidation, Vanillin.

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INTRODUCTION

Drug induced liver injury are one of the leading cause of liver damage worldwide.[1] Incidence, progression, and outcomes of NAFLD and nonalcoholic steatohepatitis (NASH Long term use of some drugs adversely influences liver by inducing oxidative stress which arises due to imbalance between antioxidant and pro-oxidant system.[2] Such oxidative stress for the long period of time may be predispose to fatty liver which further leads to fibrosis, inflammation and cirrhosis.[3] Isoniazid and rifampicin are the two important classes of anti-tubercular drugs used widely for the management of tuberculosis.[4] However, these drugs suffer from a serious adverse effect of hepatocellular damage.[5] Phytochemicals are the chemicals that are produced by plants as a consequence of a regular metabolism. They are widely distributed in plant kingdom. About 8000 polyphenolic structures are known out of which nearly 4000 are identified.[6] Polyphenols are the secondary metabolites that are widely distributed in plant kingdom. They have antioxidant property which helps them to provide protection against oxidative crisis caused by reactive oxygen species. Evidences provide an insight towards their biological role to arbitrator the antioxidant activity.[7,8] Vanilla (*Vanilla planifolia* L.) is one of the most popular aromas worldwide, which has given a unique taste to bakery products and confectionary. Vanilla seems to be originated from Central America and Mexico.[9] Natural vanilla is a complex blend of flavors from the cured pods of *Vanilla planifolia* and *Vanilla tahitensis.*[10] Vanillin is the chief component which constitutes of 1-2 %% of vanilla extract.[11] It is a white powder crystalline in nature. It is a sweet substance with vanilla flavor. Chemically, vanillin is a phenol 'substituted' with an aldehyde and methoxy group. Vanillin has been studied for various biological effects which includes analgesic,[12] neuro-protective p-ERK[13] and antimutagenic[14,15] as well as mutagenesis, induced in Escherichia coli by N-methyl-N-nitrosourea (MNU effect. In a study, vanillin caused a significant inhibition on oxygen radical absorbance capacity assay and in the oxidative hemolysis inhibition assay.[16] Similarly, vanillin afforded a significant protection against lipid peroxidation and protein oxidation in mitochondria of hepatocytes sensitized by methylene blue and light.[17] The present study was aimed to determine the protective effect of vanillin on isoniazid-rifampicin induced hepato-cellular damage in rats.

Experimental

Animals

Laboratory-bred Wistar rats (180-200 g) of either sex were housed in polypropylene cages, maintained under standardized conditions (12 h light/dark cycles, 28±2°C) were used in the study. Animals were provided with standard pellet food and had

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free access to drinking water. All the animal study protocols were duly approved by Institutional Animal Ethics Committee.

Selection of dose

The aqueous solution of vanillin in doses of 50, 100 and 200 mg/kg body weight was freshly prepared and administered orally.[18]

Animal group and dosing

Animals were divided into six groups with six animals in each group.

Group, I Normal Saline 2 ml/kg

Group II Rifampicin (50mg/kg) and Isoniazid (100mg/kg)

Group III Rifampicin (50mg/kg) and Isoniazid (100mg/kg) + Silymarin

Group IV Rifampicin (50mg/kg) and Isoniazid (100mg/kg) + Vanillin 50mg/kg

Group V Rifampicin (50mg/kg) and Isoniazid (100mg/kg) + Vanillin 100mg/kg

Group VI Rifampicin (50mg/kg) and Isoniazid (100mg/kg) + Vanillin 200mg/kg

On the 14th day blood was withdrawn by retro orbital puncture for the estimation of biochemical parameters. After that, animals were sacrificed under ether anaesthesia. The liver was collected, washed and used for histopathological studies.

Biochemical analysis

Blood samples were collected into the epindrop tubes and centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic-pyruvic transaminase (SGPT/ALT) serum alkaline phosphatase (SALP) were estimated using commercial kits (Span Diagnostics, India).

Antioxidant enzymes

Superoxide dismutase assay

Superoxide dismutase (SOD) activity in liver homogenate was determined according to the method of Minami and Yoshikawa.[19] The method was based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan colour development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan colour development). The liver homogenate was centrifuged to 10000 rpm for 15 min at 4°C. To 0.25 ml of supernatant, 0.5 ml of tris cacodylic buffer, 0.1 ml of 16% triton x- 100 and 0.25 ml NBT were added. The reaction was started by the addition of 0.01 ml diluted pyrogallol. Incubation was maintained for 5 min at 37°C. The reaction was stopped by the addition of 0.3 ml of 2M formic acid. The formazan colour developed was determined spectrophotometrically at wavelength 430 nm. Enzymatic activity was expressed as µg/gm of tissue.

Catalase activity

The catalase activity was measured according to method of Sinha.[20] 0.1ml of liver homogenate was mixed with 1.0 ml of 0.01M phosphate buffer (pH 7.4) and incubated with 0.4 ml of 0.2M H_2O_2 at 37°C accurately for 1.0 min and reaction was stopped with 2.0 ml of 5% potassium dichromate (1:3 with glacial acetic acid). Further the samples were incubated in boiling water bath for 15 min. Tubes were centrifuged at 5000 rpm for 15 min and supernatant was used to quantify the amount of H_2O_2 to calculate catalase activity at 570 nm. One unit represents 1.0 µmole of H_2O_2 consumed/min/mg protein

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis was carried out by using One way ANOVA followed by Dennett's' test and $p<0.01$, $p<0.001$ was considered significant.

RESULTS

Effect on marker enzyme levels

Rats treated with isoniazid and rifampicin developed a significant hepatic damage observed as elevated serum levels of hepato-specific enzymes such as ALT, AST, ALP and decreased level of total protein when compared to normal control. In the present study, there was restoration of antioxidant enzyme level (AST, ALP and ALT) animals of the group IV to VI treated (50, 100 and 200 mg/ kg) with vanillin (Figure 1-3). Pre-treatment with silymarin also afforded a significant protection against isoniazid and rifampicin induced toxicity in liver.

Superoxide dismutase and Catalase

The administration of isoniazid and rifampicin to animals resulted in a decrement in levels of SOD. But, treatment with vanillin (50, 100 and 200 mg/ kg) resulted in a significant ($p<0.001$) increment in SOD levels as compared to toxic control group (Group II). Congruently, the administration of vanillin (50, 100 and 200 mg/ kg) in animals resulted in significant ($p<0.001$) increment in the levels of SOD and catalase. The administration of silymarin also resulted in decrement in the levels of SOD and catalase levels (Figure 4-5).

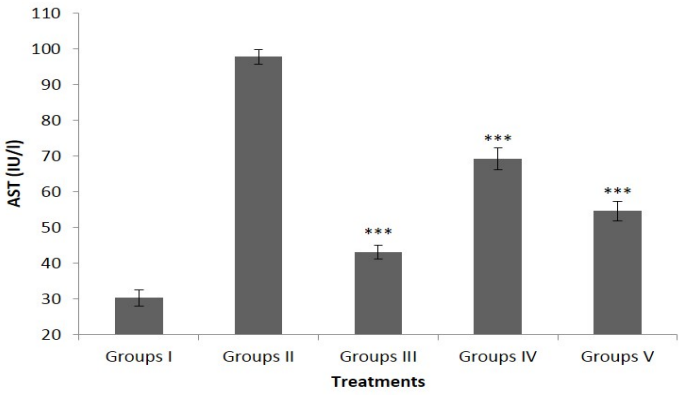

Figure 1: Effect of vanillin administration on AST levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

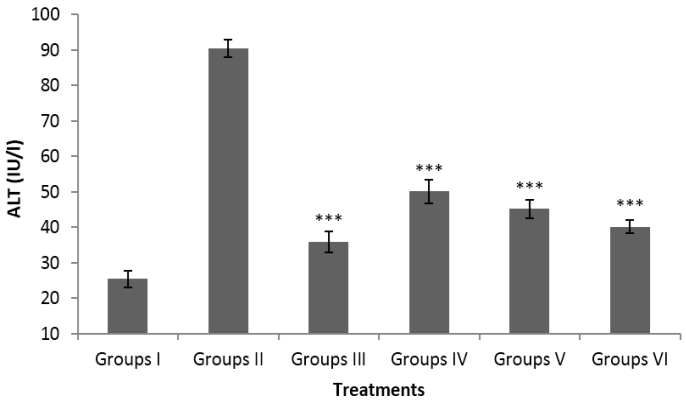

Figure 2: Effect of vanillin administration on ALT levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

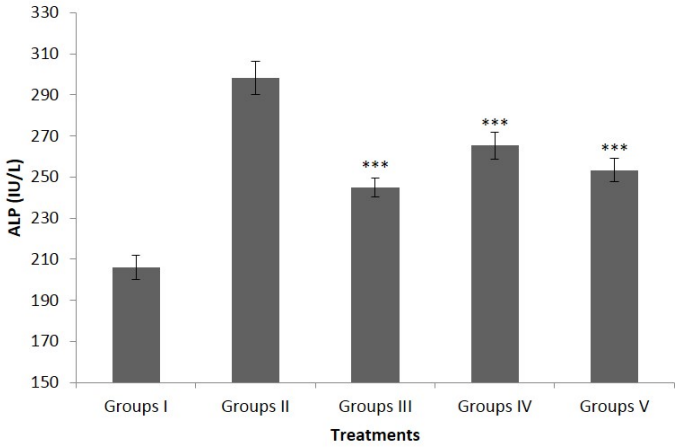

Figure 3: Effect of vanillin administration on ALP levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

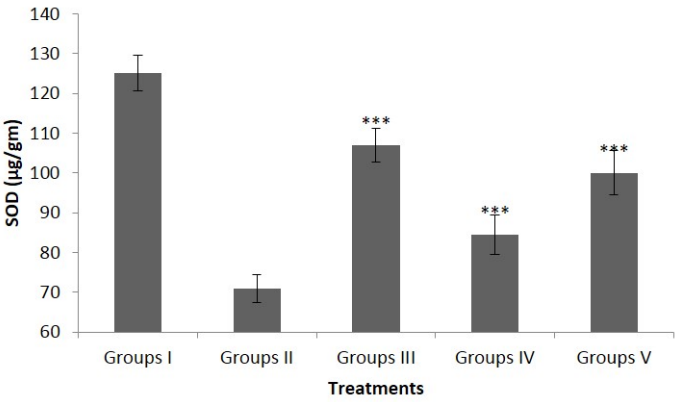

Figure 4: Effect of vanillin administration on SOD levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

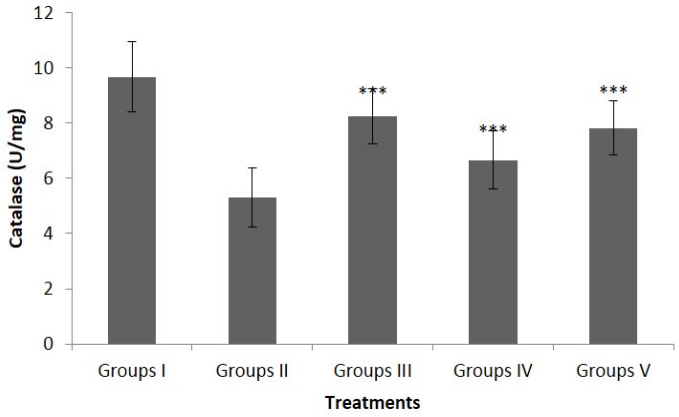

Figure 5: Effect of vanillin administration on catalase levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

DISCUSSION

Isoniazid is a gold standard anti-mycobacterial agent used in the management of tuberculosis world-wide. The metabolic products arising due to biotransformation of isoniazid (hydrazine, acetyl-hydrazine) are responsible to predispose liver toxicity. This is due to formation of covalent adducts with liver macromolecules. These adducts form covalent bonds with many lysine residues of hepatic proteins[5] Further auto oxidation of isoniazid is also responsible for production of free radicals.[21] Due to this type of biological stress, there is generation of anti-isoniazid antibodies.[22] Rifampicin is another anti-tubercular agent used in the management of tuberculosis. The experimental studies have showed that co-administration of rifampicin with isoniazid resulted in a more significant liver injury.[23] The remedies to control and reverse hepatotoxicity include use of radical-scavengers and antioxidants. Antioxidants are known to revert oxidative stress inside the cell. The various antioxidants from natural sources have been experimentally tested for their hepatoprotective potential. They include rutin,[24] hesperidin,[25] resveratrol,[26] silymarin,[27] naringenin[28] etc.

The aim of present study was to evaluate protective effect of vanillin on isoniazid-rifampicin induced hepatotoxicity in rats. Vanillin was administered to animals in the dose of 50,100 and 200 mg/ kg. The administration of vanillin resulted in restoration of antioxidant enzyme level (AST, ALP and ALT) animals of the group IV to VI. The administration of vanillin (50, 100 and 200 mg/ kg) in animals resulted in notable increment in the levels of SOD, catalase and bilirubin. There was a decrement in lipid peroxidation near to normal levels. The bilirubin and serum ALP are closely allied with the functioning of liver cells. An increased level of serum bilirubin and ALP point to biliary pressure.[29] In the present study, administration of vanillin to animals resulted in a notable decrement in bilirubin and ALP.

There is a well-developed defence system developed in multicellular eukaryotic organisms which aids in neutralization of damage induced by free radicals. The antioxidant enzymes viz. SOD and catalase work proficiently to prevent free radical induced damage.[30] However, isoniazid and rifampicin alter the activity of this system by producing reactive species, which probably interact with enzymes making them non-functional and highlight hepatic damage. The treatment with vanillin resulted in a significant increase in these enzyme levels which highlight their ability to scavenge reactive oxygen species. An increased production of lipid-peroxides represents the damage to cells. The increment in raised free radical production damages cell morphology and results in oxidative stress induced cell damage.[31] The present study revealed a reduction in free radical production.

Liver is a vital organ of the body. Various processes like calorie-genesis, enzyme production, detoxification, drug metabolism and many more processes take place inside the liver. The liver damage is associated with altered metabolism which can lead to progression cirrhosis. Therefore, the drugs/ chemicals which protect the hepatocellular damage are of extreme interest. The outcomes of the present studies testified the beneficial effect of vanillin administration on rat model.

Based on the above observations, it can be summarized that vanillin treatment resulted in restoration of liver antioxidant status and antioxidant enzymes. It caused a decrement in lipid peroxidation. The cellular integrity of hepatocytes was guarded by vanillin. Thus, vanillin can be regarded as a potential antioxidant and can be tested for molecular mechanism for hepatoprotective effects.

CONCLUSION

The outcomes of the present study highlight the role of vanillin in preventing the harmful effects of isoniazid-rifampicin on rats. These promising results can help in development of antioxidant and hepatoprotective nutraceuticals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ALP: Alkaline phosphatase; **ALT:** Alanine transaminase; **AST:** Aspartate transaminase; **CAT:** Catalase; **H_2O_2:** Hydrogen peroxide; **SOD:** Superoxide dismutase.

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GRAPHICAL ABSTRACT

SUMMARY

The present work establishes hepatoprotective effect of vanillin. More studies at molecular level might be helpful in estabilishing molecular mechanism of action.

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Mr. Rashint Tiwari holds Post Graduate degree in Pharmacy (M.Pharm) with specialization in Pharmacology. His research interest includes evaluation of bioactive- compounds for organ protective effects.

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