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Research Paper

Hepatoprotective Effects of the Adenosine Monophosphate-Activated Protein Kinase Signaling Pathway in the Fluoxetine-Induced Hepatotoxic Model in Rats

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ABSTRACT

Background: Drug-induced liver injuries (DILIs) can have various clinical manifestations ranging from asymptomatic abnormal liver tests to symptomatic acute liver disease. Several studies have indicated that fluoxetine can lead to hepatotoxicity and affect liver enzyme activity. Metformin suppresses Sterol regulatory element-binding transcription factor 1. It reduces lipogenesis and fat accumulation by activating the adenosine monophosphate-activated protein kinase (AMPK), which can have a protective effect on the liver.

Methods: The present study investigated the role of the AMPK signaling pathway induced by metformin in preventing fluoxetine-induced hepatotoxicity in rats. Rats were randomly divided into four groups of five, including a Control Group, a Fluoxetine-induced hepatotoxicity (Flux) group, a Flux + Metformin group, and a Flux + Metformin + Dorsomorphin group. Hepatotoxicity was induced by fluoxetine, and then the serum-specific liver marker, oxidative markers, and histopathology were measured.

Results: The findings demonstrated that the activation of AMPK by metformin increased antioxidative activity and decreased necrosis, edema, and inflammatory cells in the liver. However, the results of the fourth group indicated that dorsomorphin administration reduced the beneficial effects of metformin by inhibiting the AMPK signaling pathway.

Conclusion: The findings suggest that a decrease in inflammation and inhibition of oxidative stress in the liver may mediate the AMPK signaling pathway in fluoxetine-induced hepatotoxicity.

Keywords: AMP-Activated Protein Kinases, Dorsomorphin, Drug-Induced Liver Injury, Fluoxetine, Metformin

Introduction

Drug-induced liver injury (DILI) continues to account for a considerable proportion of acute liver failure cases [1]. DILIs present with a broad spectrum of clinical manifestations, ranging from asymptomatic abnormalities in liver tests to acute liver disease accompanied by symptoms, disability, prolonged jaundice, and even overt acute or subacute liver failure. The diagnosis of DILI can be difficult and is often delayed because other, more common causes of liver injury must first be ruled out [2]. The risk of DILI increases as a drug becomes more widely prescribed [3]. Evidence suggests that many antidepressants, even at therapeutic doses, may cause hepatotoxicity. Furthermore, DILI can present with hepatocellular, cholestatic, or mixed patterns of liver injury [4]. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is prescribed for the management of various psychiatric and behavioral disorders, including depression, obsessive–compulsive disorder, panic disorder, fibromyalgia, bulimia nervosa, premature ejaculation, and trichotillomania [5,7]. Fluoxetine is a fluorinated antidepressant with a long half-life, primarily metabolized in the liver and eliminated through the urine [8,9]. However, several studies have reported that fluoxetine use may cause hepatotoxicity and alter liver enzyme activity [10,12]. Long-term use of fluorinated drugs may lead to liver injury, recognized as one of their potential adverse effects. Studies have indicated that high doses of fluoxetine can elevate liver function biomarkers and cause liver damage through oxidative stress [13]. Fluoxetine metabolism may lead to the excessive production of free radicals, which can potentially cause liver damage.

Additionally, inflammation associated with hepatic injury has been linked to elevated levels of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and hydroxyl radicals ($OH^{\bullet}$), which can recruit phagocytes and exacerbate tissue damage [14]. Animal studies have demonstrated that fluoxetine can induce hepatic necrosis and ischemia at various doses, as well as cause steatosis, cholestasis, lobular and portal inflammation, and hepatomegaly [15,17]. Metformin, a frequently used treatment for type 2 diabetes [18], is a biguanide that inhibits complexes I and III of the mitochondrial electron transport chain during its metabolism. As a result, partial leakage in the electron transport chain increases cellular ROS production, leading to a slight reduction in ATP levels. In metformin's metabolic pathway, this decrease in cellular ATP typically activates the adenosine monophosphate-activated protein kinase (AMPK), which subsequently phosphorylates and activates the transcription factor FOXO3a, playing a central role in regulating cell growth and death, glucose metabolism, ROS detoxification, and lifespan [19, 20]. Elevated cellular levels of antioxidant enzymes, such as catalase (CAT), help reduce oxidative stress by detoxifying ROS. This process ultimately facilitates the removal of superoxide via manganese superoxide dismutase (MnSOD) and the breakdown of hydrogen peroxide [21,22]. It has been hypothesized that fluoxetine may promote fat accumulation in hepatocytes and induce hepatotoxicity by suppressing AMPK activation and inhibiting its signaling pathway [23]. In contrast to fluoxetine, metformin activates AMPK, which suppresses SREBP1, reduces lipogenesis, and decreases fat accumulation, thereby exerting a protective effect on the liver. The mammalian system has developed numerous enzymatic and non-enzymatic pathways to counteract the adverse effects of drugs. Several meta-analyses have demonstrated that metformin can improve liver enzyme profiles [24,25]. Several studies have shown that metformin exhibits hepatoprotective effects through the AMPK signaling pathway [26,27]. The present study investigated the role of metformin-induced AMPK activation in preventing fluoxetine-induced hepatotoxicity in rats.

Materials and Methods

Animals

A total of 20 adult male Wistar rats, weighing 240–260 g, were obtained from the Animal Laboratory of Lorestan University of Medical Sciences, Iran. The animals were housed under standard conditions at 21–23°C, with a 12-hour light/dark cycle, and had free access to food and water. To acclimate to the laboratory environment, the rats were individually housed for one week prior to the start of the experiment, with unrestricted access to food and water. We have added a statement regarding ethical considerations. All experimental procedures were approved by the Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1401.089) and conducted in accordance with international guidelines for the care and use of laboratory animals.

Experimental Design

Metformin hydrochloride (Sigma-Aldrich, St. Louis, MO, USA), Dorsomorphin (Sigma-Aldrich, St. Louis, MO, USA), and Fluoxetine hydrochloride (Arya Pharmaceutical, Iran) were purchased and prepared for administration. Metformin and fluoxetine (Flux) solutions were dissolved in distilled water. Prior to the experiment, the rats were fasted for 20–24 hours. The animals were then randomly assigned to four groups, each consisting of five rats. Group I served as the control and received 1.0 ml of tap water once daily, while Group II received fluoxetine at a dose of 10 mg/kg/day for seven days [28]. Animals in Group III received fluoxetine at 10 mg/kg/day via intraperitoneal (IP) injection and metformin at 300 mg/kg/day via oral gavage. Group IV animals received fluoxetine (10 mg/kg/day, IP), metformin (300 mg/kg/day, gavage), and dorsomorphin at 10 mg/kg/day via IP injection. In Groups III and IV, the rats received metformin and/or dorsomorphin starting three days before hepatotoxicity induction and continuing for seven days afterward. The 300 mg/kg dose of metformin, recognized as a low dose, was selected for this study due to its ability to stimulate the AMPK signaling pathway [29] and confer liver protection [30, 31]. The animals were randomly assigned to experimental groups, and the allocation was performed using a simple randomization method to ensure unbiased distribution (Schematic Diagram 1).

Serum-Specific Marker of the Liver

Blood was obtained through cardiac puncture and centrifuged at 1,500 × g for 10 min at 4°C. To evaluate liver function, the activities of key serum enzymes—alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—were subsequently measured [32].

Histopathology Analysis

Following sacrifice, liver fragments were fixed in 10% formalin, dehydrated through an alcohol series, embedded in paraffin, and subsequently stained with hematoxylin and eosin (H&E) to assess tissue histology [33].

Enzyme-Linked Immunosorbent Assays (ELISA)

Glutathione peroxidase (GPx) and CAT activities were measured using a commercial kit with an automated analyzer. Liver tissues were first sectioned into small pieces, and 3 mL of 1.12 M KCl was added per gram of tissue. The samples were homogenized at −4°C, and the resulting suspension was centrifuged at 9,000 rpm for 21 min using a refrigerated centrifuge. The supernatant was then analyzed with the automated analyzer to determine GPx and CAT enzyme activities [34].

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 9 software. Central tendency and dispersion indices were computed. Data with normal distribution were analyzed using one-way analysis of variance (ANOVA), while non-normally distributed data were assessed using the Kruskal–Wallis test. Significant

results were further evaluated with Tukey or Bonferroni post-hoc tests. A p-value less than 0.05 was considered statistically significant.

Results

Effects of AMPK on ALT, AST, and ALP

The results indicated that serum levels of ALT, AST, and ALP were significantly elevated on day 7 following fluoxetine administration in Group II, compared to the healthy control group ($P < 0.001$). Treatment with metformin at 300 mg/kg markedly reduced ALT, AST, and ALP levels compared to Group II. Conversely, co-administration of metformin and dorsomorphin resulted in increased levels of ALT, AST, and ALP ($P < 0.001$, Group IV vs. Group III; Figure 1).

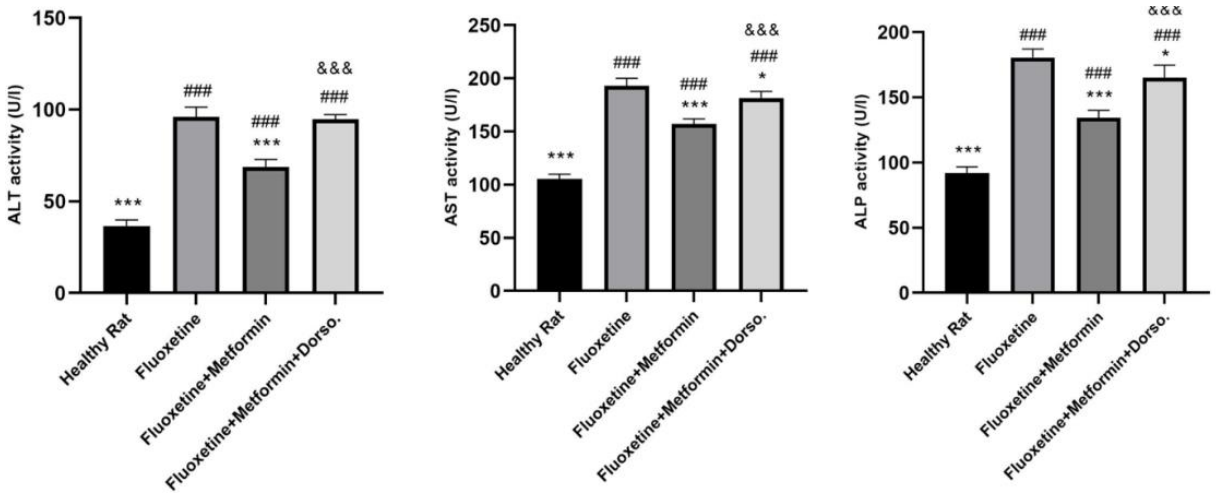

Figure 1. Comparison of the effect of drug administration on serum ALT, AST, and ALP activity. Group I: Healthy rats, Group II: Fluoxetine, Group III: Fluoxetine + Metformin, Group IV: Fluoxetine + Metformin + Dorsomorphin. * P <0.05 vs Group II, *** p< 0.001 vs Group II, ### p< 0.001 vs Group I, &&& p< 0.001 Group IV vs Group III.

Effects of AMPK on oxidative markers

Fluoxetine administration significantly increased GPx levels ($P < 0.001$, Figure 2) and decreased CAT levels ($P < 0.001$, Figure 3) compared to the healthy control group. In contrast, metformin treatment significantly elevated CAT levels ($P < 0.01$) and reduced GPx levels compared to Group II ($P < 0.001$). However, co-administration of dorsomorphin with metformin abolished the beneficial effects of metformin on these oxidative stress markers.

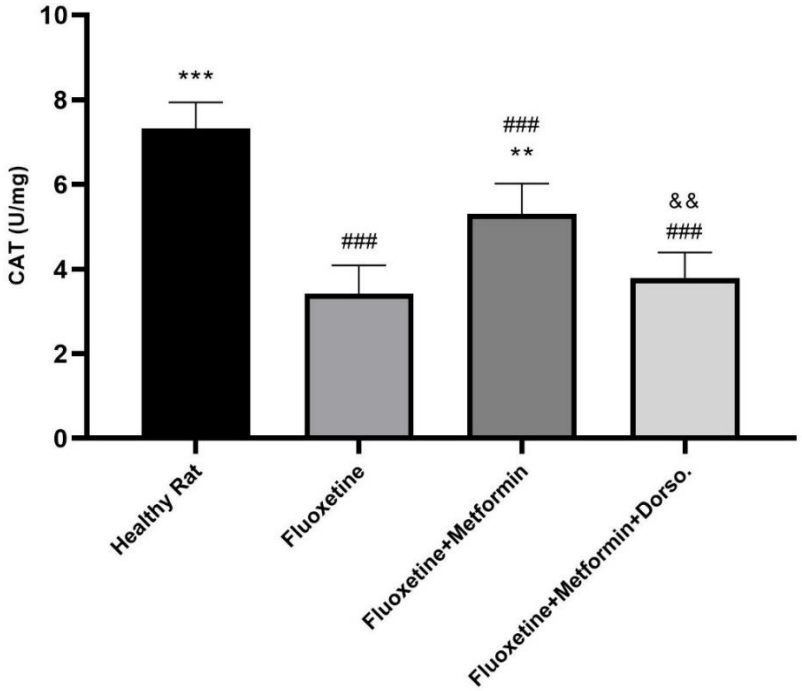

Figure 2. Comparison of the effect of drug administration on catalase (CAT) activity. Group I: Healthy rats, Group II: Fluoxetine, Group III: Fluoxetine + Metformin, Group IV: Fluoxetine + Metformin + Dorsomorphin. ** P <0.01 vs Group II, *** p< 0.001 vs Group II, ### p< 0.001 vs Group I, && p< 0.01 Group IV vs Group III.

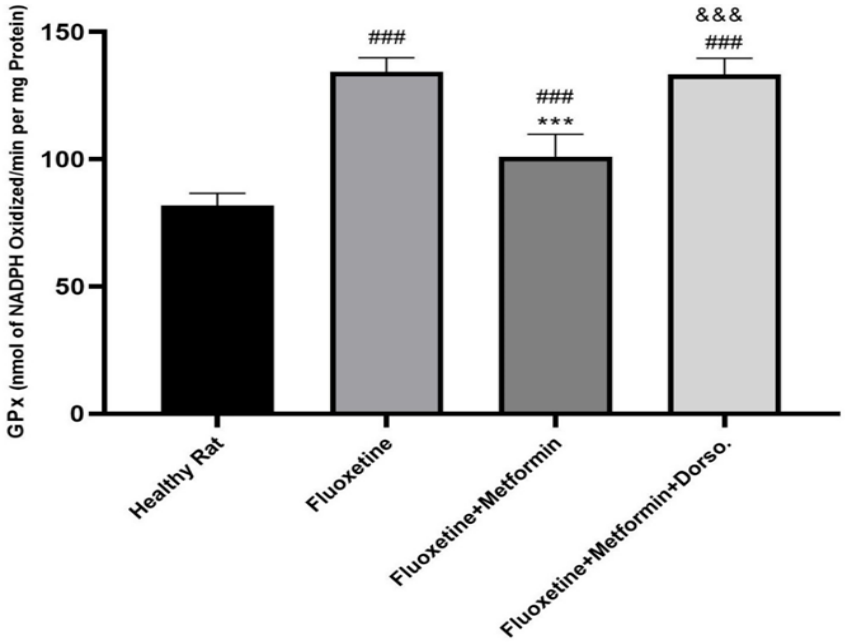

Figure 3. Comparison of the effect of drug administration on GPx activity. Group I: Healthy rats, Group II: Fluoxetine, Group III: Fluoxetine + Metformin, Group IV: Fluoxetine + Metformin + Dorsomorphin. *** $p< 0.001$ vs Group II, $^{###}$ $p< 0.001$ vs Group I, $^{&&&}$ $p< 0.001$ Group IV vs Group III.

Effects of AMPK on Histopathology

Figure 4 illustrates the grading of necrosis, edema, and inflammatory cell infiltration in the liver. Histological examination of Group II revealed a marked increase in bile duct proliferation, hepatic inflammation, and hepatocellular alterations. In contrast, Group III, treated with metformin, exhibited a significant reduction in bile duct proliferation, hepatic inflammation, and hepatocellular changes. However, in Group IV, which received combined metformin and dorsomorphin treatment, dorsomorphin antagonized the hepatoprotective effects of metformin, resulting in a significant increase in bile duct proliferation, hepatic inflammation, and alterations in hepatocytes.

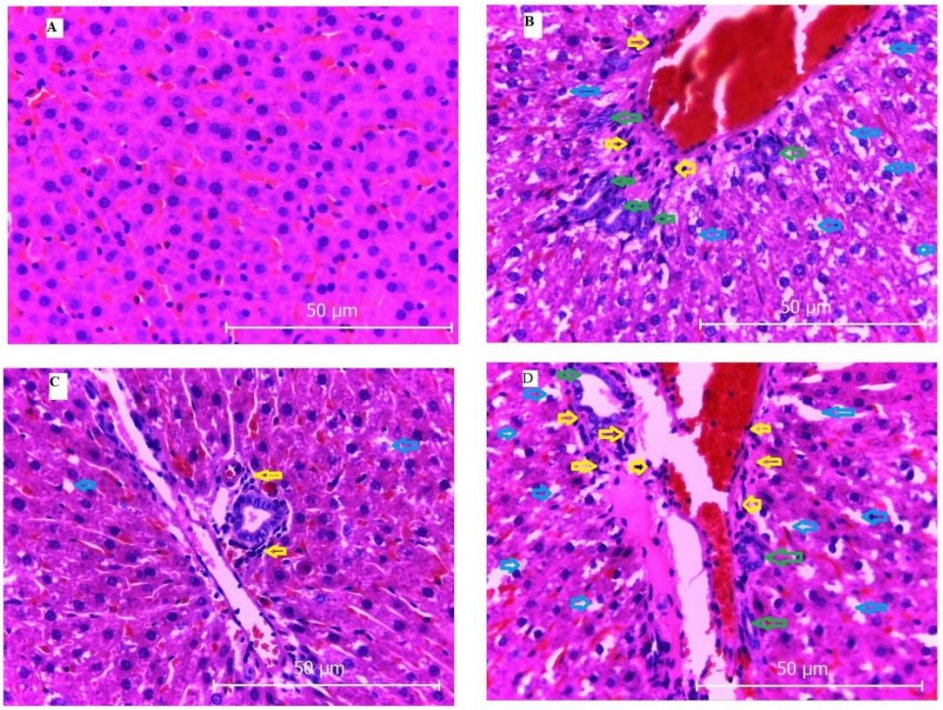

Figure 4. Effects of the AMPK signaling pathway on histopathological examination of Fluoxetine-induced hepatotoxicity. The yellow arrow indicates the location of inflammation, the blue arrow indicates the hepatocyte changes, and the green arrow indicates the proliferation of the bile ducts. **A**: Healthy rat (sham-operated), **B**: Fluoxetine (10 mg kg/day, IP), **C**: Fluoxetine (10 mg kg/day, IP) + Metformin (300 mg/kg, gavage), **D**: Fluoxetine (10 mg kg/day, IP) + Metformin (300 mg/kg, gavage) + Dorsomorphin (0.2 mg/ kg, IP).

Discussion

The results of this study demonstrated that treatment with metformin (300 mg/kg) for 10 days significantly improved serum markers of fluoxetine-induced hepatotoxicity, including ALT, AST, and ALP.

Additionally, metformin mitigated oxidative stress and reduced histopathological liver damage. However, co-administration of dorsomorphin, an AMPK inhibitor, markedly diminished the hepatoprotective effects of metformin. These findings suggest that activation of the AMPK signaling pathway plays a critical role in protecting against fluoxetine-induced hepatotoxicity.

The findings of the present work demonstrated a significant increase in serum liver function markers (ALT, AST, and ALP) in fluoxetine-treated rats, in agreement with earlier reports [9, 28]. Metformin treatment significantly reduced serum levels of ALT, AST, and ALP compared to Group II. However, co-administration of dorsomorphin attenuated the beneficial effects of metformin, resulting in elevated levels of these enzymes. Several clinical studies have demonstrated that metformin can reduce elevations in liver enzymes [35,37], findings consistent with the present study, which suggests liver injury resulting from damage to cytoplasmic and mitochondrial membranes. Furthermore, ALP serves as a biomarker of hepatobiliary injury and cholestasis [28]. Elevations in ALT activity are generally more pronounced than those of AST in hepatic diseases, reflecting the cellular localization of this enzyme [38]. The increased activity of the cytoplasmic enzyme ALT in fluoxetine-treated animals indicates hepatic dysfunction, likely associated with drug-induced cellular membrane damage [17]. Considering that mitochondrial dysfunction is linked to the adverse effects of several drugs, the observed increase in AST activity—a mitochondria-associated enzyme—in fluoxetine-treated rats may reflect compromised mitochondrial membrane integrity in hepatocytes induced by fluoxetine [39]. Another mechanism by which fluoxetine induces liver injury involves the generation of free radicals and ROS [40, 41]. Elevated levels of these species can disrupt cellular signaling and homeostasis, ultimately leading to cell damage. Studies have demonstrated that the AMPK signaling pathway exhibits antioxidant activity, reducing the activity of enzymes related to oxidative stress and ultimately providing hepatoprotective effects [42,44]. Oxidative stress can inhibit ATP synthesis and production [45]; however, the AMPK signaling pathway regulates ATP production and consumption, thereby increasing ATP levels and promoting cellular homeostasis [46,47]. Moreover, AMPK, a serine/threonine kinase that functions as an energy sensor, is activated when the AMP/ATP ratio increases due to a decline in cellular energy level [20]. In mammals, AMPK is activated through phosphorylation of its catalytic α-subunit by either Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) or liver kinase B1 (LKB1) [48]. Activation of AMPK inhibits anabolic pathways while promoting compensatory catabolic processes, including glucose uptake, glycolysis, and fatty acid oxidation [49]. Moreover, AMPK phosphorylation inhibits SREBP1, leading to reduced lipogenesis and lipid accumulation [50]. Fluoxetine is metabolized in the liver primarily via cytochrome P450, producing norfluoxetine and several other metabolites. Fluoxetine has been reported to affect hepatic lipid metabolism and may act as a hepatotoxic agent [8]. Furthermore, inhibition of AMPK activates SREBP1-mediated lipogenesis, resulting in the accumulation of chemically induced hepatic fat [51]. Studies have reported that fluoxetine's effects on lipid metabolism are mediated by changes in the expression of genes involved in lipogenesis and lipolysis, which are regulated by AMPK signaling inhibition [23,52].

Conclusions

In conclusion, the present study highlights the pivotal role of the AMPK signaling pathway in maintaining liver health by exerting hepatoprotective and antioxidant effects. Modulation of AMPK activity appears to be a key mechanism through which metformin confers protection against DILI. These findings suggest that targeting AMPK could represent a promising therapeutic approach not only for fluoxetine-induced hepatotoxicity but potentially for other forms of chemically induced liver damage as well. Future studies are warranted to elucidate further the molecular pathways involved in AMPK-mediated hepatoprotection and to explore its broader therapeutic applications in preventing or mitigating hepatotoxicity from various pharmacological agents.

Ethical Considerations

The Ethics Committee approved all animal protocols for this study (IR.LUMS.REC.1401.089). The authors affirm that they have fully complied with all ethical standards of research, and confirm that there was no duplicate publication, data fabrication, or plagiarism in this study.

Conflict of Interests

The authors declared that there is no conflict of interest.

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Authors' Contributions

Project idea: Amin Hasanvand and Nader Rahimi. Data analyses: Amin Hasanvand and Mehdi Birjandi. Literature search: Amin Hasanvand, Peyman Astaraki, Shakiba Shamsinia. Laboratory experiments: Amin Hasanvand, Peyman Astaraki, Shakiba Shamsinia, Mohammadjavad Nourmohammadi, Fatemeh Hatami, Zahra Haghighatian. Manuscript writing: Amin Hasanvand and Nader Rahimi, Manuscript revision: Amin Hasanvand, Nader Rahimi, and Peyman Amanolahi Baharvand, Final approval: All authors.

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