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Investigation of Effects of Apilarnil and Imatinib Use on Liver and Kidney Tissues in Rats via PI3K/AKT/mTOR and JAK2/STAT3 Signaling Pathways

Cüneyt ÇAĞLAYAN[1*], Aydın GENÇ[2], Sefa KÜÇÜKLER[3], Hakan İNCİ[4]

[1] Bilecik Şeyh Edebali University, Medicine Faculty, Medical Biochemistry Department, Bilecik, Türkiye
[2] Bingöl University, Veterinary Faculty, Biochemistry Department, Bingöl, Türkiye
3 Atatürk University, Veterinary Faculty, Biochemistry Department, Erzurum, Türkiye
[4] Bingöl University, Agriculture Faculty, Animal Science Department, Bingöl, Türkiye
Cüneyt ÇAĞLAYAN ORCID No: 0000-0001-5608-554X
Aydın GENÇ ORCID No: 0000-0001-5367-0743
Sefa KÜÇÜKLER ORCID No: 0000-0002-8222-5515
Hakan İNCİ ORCID No: 0000-0002-9791-0435

**Corresponding author: cuneyt.caglayan@bilecik.edu.tr*

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Keywords
Apilarnil, Imatinib, Liver, Kidney, Rat

Abstract: Imatinib, used in the field of molecular targeted therapy, has been reported to cause serious side effects, including liver and kidney failure. However, the mechanism of imatinib-induced liver and kidney toxicity remains unclear due to limited number of studies in this field. Apilarnil is a natural bee product produced from 3-7 day old drone larvae. The present study aimed to investigate the effects of apilarnil in rats with imatinib-induced liver and kidney toxicity using biochemical parameters. In the experiment, 35 wistar albino rats were divided into five groups (n=7): i) Control, ii) Apilarnil, iii) Imatinib, iv) Imatinib+Apilarnil-200, and v) Imatinib+Apilarnil-400. Rats were treated orally with imatinib (100 mg/kg) and apilarnil (200 and 400 mg/kg) for 14 days. Imatinib reduced PI3K, AKT and mTOR levels, while increasing JAK2 and STAT3 levels in liver and kidney tissues. Apilarnil given for treatment modulated these values and provided partial protection in liver and kidney tissue. In conclusion, it was determined that apilarnil has ameliorative effects against imatinib-induced liver and kidney damage.

Sıçanlarda Apilarnil ve İmatinib Kullanımının Karaciğer ve Böbrek Dokuları Üzerine Etkilerinin PI3K/AKT/mTOR ve JAK2/STAT3 Sinyal Yolakları Aracılığı ile Araştırılması

Anahtar Kelimeler
Apilarnil, İmatinib, Karaciğer, Böbrek, Rat

Öz: Moleküler hedefli tedavi alanında kullanılan imatinib'in karaciğer ve böbrek yetmezliği de dahil olmak üzere ciddi yan etkilere neden olduğu bildirilmiştir. Bununla birlikte, bu alanda sınırlı sayıda çalışma olduğu için imatinib kaynaklı karaciğer ve böbrek toksisite mekanizması belirsizliğini koruyor. Apilarnil, 3-7 günlük drone larvalarından üretilen doğal bir arı ürünüdür. Bu çalışmada, imatinib kaynaklı karaciğer ve böbrek toksisitesi olan sıçanlarda apilarnilin etkilerinin biyokimyasal parametreler kullanılarak araştırılması amaçlanmıştır. Deneyde, 35 wistar albino sıçan beş gruba ayrıldı (n=7): i) Kontrol, ii) Apilarnil, iii) İmatinib, iv) İmatinib+Apilarnil-200 ve v) İmatinib+Apilarnil-400. Sıçanlara 14 gün boyunca oral yoldan imatinib (100 mg/kg) ve apilarnil (200 ve 400 mg/kg) ile tedavi uygulandı. İmatinib, karaciğer ve böbrek dokularında PI3K, AKT ve mTOR düzeylerini düşürürken JAK2 ve STAT3 düzeylerini artırdı. Bununla birlikte, tedavi amacıyla verilen apilarnil ise bu değerleri modüle ederek karaciğer ve böbrek dokusunda kısmi koruma sağlamıştır. Sonuç olarak, imatinib kaynaklı karaciğer ve böbrek hasarına karşı apilarnilin iyileştirici etkilerinin olduğu tespit edilmiştir.

1. INTRODUCTION

Imatinib is one of the current anticancer drugs that is considered as a smart drug with antineoplastic effect used in the treatment of many types of cancer [1]. Imatinib is a selective inhibitor of BCR-ABL tyrosine kinase, mainly used in chronic myeloid leukemia, acute lymphoblastic leukemia, metastatic or unresectable gastrointestinal

stromal tumors [2]. Today, imatinib, which is called a smart drug and is frequently used in addition to existing chemotherapeutics is considered a crucial example in terms of reflecting the importance of molecular targeted therapy and perhaps the future of cancer treatment, considering its significantly positive side effect profile and reliability compared to conventional cytotoxic treatments. Although it is frequently preferred due to its beneficial effects in the fight against cancer, it can commonly cause unwanted side effects such as vomiting, diarrhea, muscle pain, headache and rash even in overdose or normal dose intake. Severe side effects include fluid retention, gastrointestinal bleeding, bone marrow suppression, liver problems and heart failure. It has been reported that it can cause damage to tissues and organs such as testicles, heart, liver and spinal cord, which can be considered serious health problems as a result of its use in cancer treatments [3-6].

Apilarnil (bee drone larvae) is a bee product obtained by lyophilizing 3-7-day-old male bee larvae and has strong antioxidant properties. Apilarnil, which is homogeneous, milky, has the consistency of boza, is yellowish gray in color and has a bitter taste, is easily adulterated, and is a bee product that must be stored in a cold chain in raw form or lyophilized [7,8]. It gets its antioxidant properties from the vitamins (A, D, C, E, B1, B6, choline etc.), minerals (Ca, P, Na, Zn, Mn, Fe, Cu and K) and polyphenols it contains. In addition to its rich vitamin and mineral content, it contains 66% water, 14.5% carbohydrates, 4.5% lipids, 13% amino acids and some biologically active substances [9].

In the present study, the effects of apilarnil and imatinib use in rats on some biochemical parameters in liver and kidney tissues were investigated.

2. MATERIAL AND METHOD

2.1. Drug and Chemicals

Imatinib (Glivec®, 400 mg/tablet) was obtained from Novartis Pharmaceuticals (İstanbul, Turkey). Apilarnil samples were taken from the honeycomb into falcon tubes and immediately stored at -20 °C. Then, for the lyophilization process, they were taken to a freezer between -20 °C and -80 °C and lyophilization was performed by taking them into lyophilized flasks. The lyophilized apilarnil was dissolved in water according to the experimental protocol and given orally to the rats. ELISA kits for mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), janus kinase-2 (JAK2) and signal transducer and activator of transcription-3 (STAT3) were obtained from Sunred Biological Technology Company (Shangai, China).

2.2. Animals

Thirty-five male Wistar albino rats, weighing 250-300 g and aged 12-13 weeks, was used in the experiment. The rats were obtained from Experimental Research Center, Bingol University (Bingol, Turkey). Animals were kept in cages in a controlled room, providing a constant temperature of 24-25 ºC and a twelve (12 h) hour light-dark cycle (07:00-19:00 light; 19:00-07:00 dark). They were provided with access to unlimited amounts of water and standard feed. The animal use protocol had been approved by the Animal Experimentation Ethics Committee of the Bingol University (Protocol No. 2022-02/01).

2.3. Experimental Procedure

Wistar albino male rats were randomly divided into 5 groups, with 7 rats in each group. Group I (Control): Physiological saline solution was given orally for 14 days. Group II (Apilarnil): Apilarnil at a dose of 400 mg/kg body weight was dissolved in physiological saline and given orally for 14 days [10]. Group III (Imatinib): Imatinib was dissolved in physiological saline and given orally at a dose of 100 mg/kg for 14 days [11]. Group IV (Imatinib + Apilarnil 200 mg/kg): Imatinib was dissolved in physiological saline and given orally at a dose of 100 mg/kg for 14 days. 30 minutes after imatinib application, apilarnil was given orally at a dose of 200 mg/kg. Group V (Imatinib + Apilarnil 400 mg/kg): Imatinib was dissolved in physiological saline and given orally at a dose of 100 mg/kg for 14 days. 30 minutes after imatinib application, apilarnil was given orally at a dose of 400 mg/kg. At the end of study period (15th day), the animals were sacrificed under mild sevoflurane anesthesia. The liver and kidney tissues from rats were evaluated for biochemical, analysis.

2.4. Determination of Liver and Kidney Tissues PI3K, mTOR, AKT, JAK2 and STAT3 by ELISA Method

Frozen liver and kidney tissues were ground with a tissue homogenizer machine (Tissue Lyser II, Qiagen, Netherlands) using liquid nitrogen. Then, 100 mg of ground liver and kidney tissues for each tissue were diluted 1:20 with phosphate buffer (0.1 M, pH 7.4) and homogenized with the homogenizer machine. Supernatants were prepared by centrifugation at 3500 rpm for 15 min. Measurements were performed using ELISA kits such as PI3K, mTOR, AKT, JAK2 and STAT3 in supernatants obtained from liver and kidney tissues according to the manufacturer's instructions and expressed as ng/g tissue.

2.5. Statistical Analysis

Biochemical data were analysed with ANOVA using SPSS (version 20.0; Chicago, IL). Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey test were used to determine the difference and significance levels between the groups. $p < 0.05$ were considered as statistically significant.

3. RESULTS

3.1. Liver and Kidney PI3K levels

When liver and kidney PI3K levels were analyzed (Figure 1A and B), it was found that PI3K levels decreased in the imatinib group compared to the control group ($p<0.05$), and apilarnil 200 and 400 mg/kg doses administered together with imatinib were effective in increasing PI3K levels ($p<0.05$).

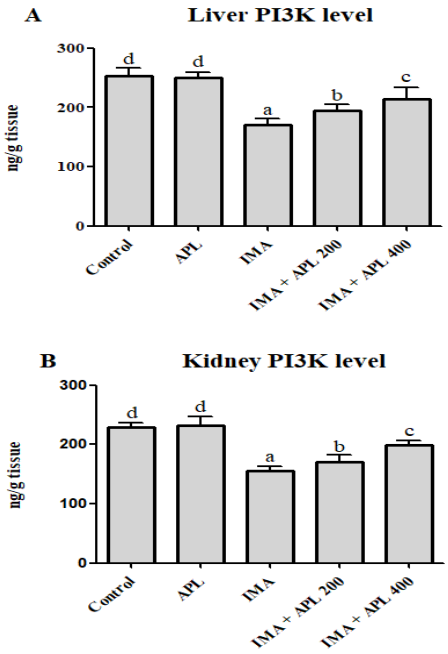

Figure 1. (A) Effect of oral administration of apilarnil (APL) on liver PI3K level in imatinib (IMA) treated rats. (B) Effect of oral administration of apilarnil (APL) on kidney PI3K level in imatinib (IMA) treated rats. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

3.2. Liver and Kidney mTOR levels

When liver and kidney mTOR levels were examined (Figure 2A and B), it was found that there was no difference between the control and apilarnil groups ($p>0.05$), mTOR levels in the imatinib group decreased compared to the control group ($p<0.05$), apilarnil 200 and 400 mg/kg doses increased mTOR levels ($p<0.05$).

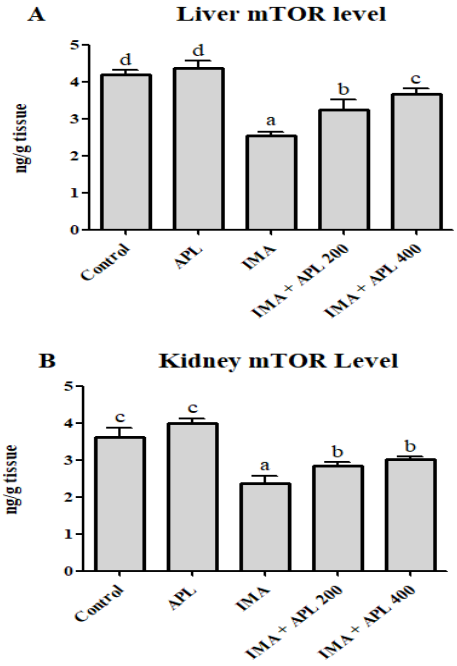

Figure 2. (A) Effect of oral administration of apilarnil (APL) on liver mTOR level in imatinib (IMA) treated rats. (B) Effect of oral administration of apilarnil (APL) on kidney mTOR level in imatinib (IMA) treated rats. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

3.3. Liver and Kidney AKT levels

It was found that AKT levels in the imatinib group decreased compared to the control group ($p<0.05$), there was no difference between the control and apilarnil groups ($p>0.05$), and both doses of apilarnil given together with imatinib were effective in increasing AKT levels ($p<0.05$) (Figure 3).

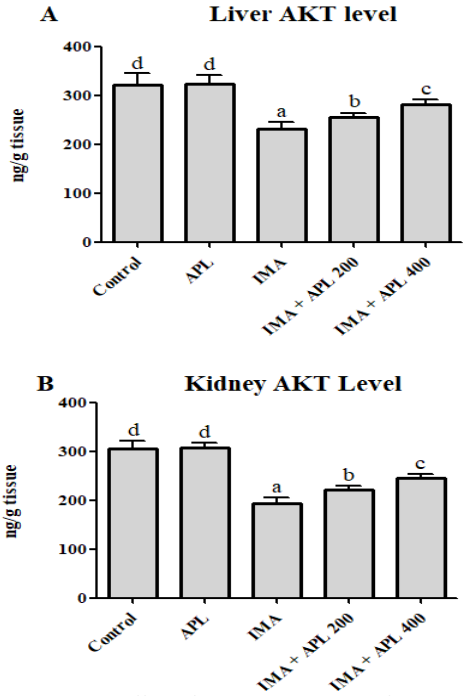

Figure 3. (A) Effect of oral administration of apilarnil (APL) on liver AKT level in imatinib (IMA) treated rats. (B) Effect of oral administration of apilarnil (APL) on kidney AKT level in imatinib (IMA) treated rats. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

3.4. Liver and Kidney JAK2 levels

While there was no difference between control and apilarnil group JAK2 levels ($p>0.05$), JAK2 levels in imatinib group incresed compared to control and apilarnil groups ($p<0.05$), JAK2 levels in IMA + APL 200 and IMA + APL 400 groups decreased compared to imatinib group ($p<0.04$) (Figure 3). There was no statistical difference between the two groups (IMA + APL 200 and IMA + APL 400).

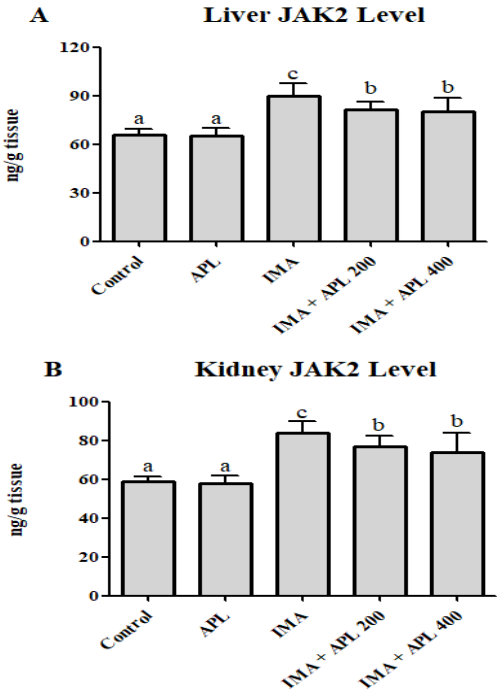

Figure 4. (A) Effect of oral administration of apilarnil (APL) on liver JAK2 level in imatinib (IMA) treated rats. (B) Effect of oral administration of apilarnil (APL) on kidney JAK2 level in imatinib (IMA) treated rats. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

3.5. Liver and Kidney STAT3 levels

When liver and kidney tissue STAT3 levels were examined, no difference was found between the control and apilarnil groups ($p>0.05$), STAT3 levels increased in the imatinib group compared to the control group ($p<0.05$), and 200 and 400 mg/kg doses of apilarnil given together with imatinib were found to be effective in reducing STAT3 levels ($p<0.05$).

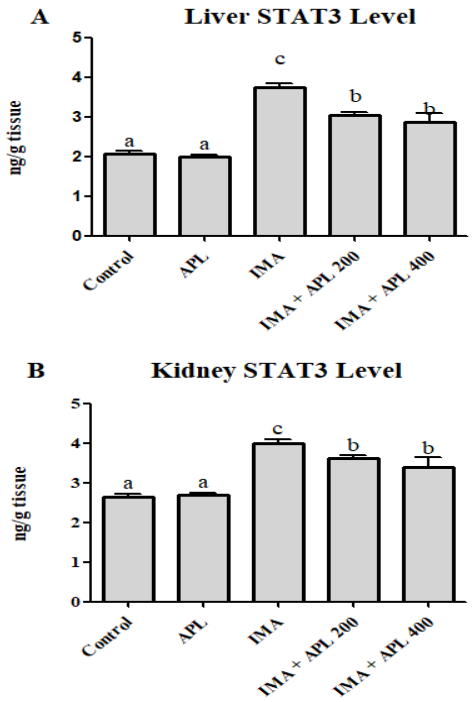

Figure 5. (A) Effect of oral administration of apilarnil (APL) on liver STAT3 level in imatinib (IMA) treated rats. (B) Effect of oral administration of apilarnil (APL) on kidney STAT3 level in imatinib (IMA) treated rats. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

4. DISCUSSION AND CONCLUSION

Imatinib is a drug used clinically against chronic myeloid leukemia and metastatic gastrointestinal stromal tumors. On the other hand, liver and kidney damage are associated with imatinib treatment [12, 13]. The current study investigated the possible effects of apilarnil against imatinib-induced liver and kidney toxicity in male rats from a biochemical perspective.

The PI3K/Akt/mTOR pathway is an important intracellular signal transduction pathway involved in regulating apoptosis, autophagy, cell proliferation, cell cycle, metabolism, and angiogenesis through communicating with its related downstream and upstream molecules [14]. PI3K is classified as type I, type II and type III. Among them, type I PI3K is a negative regulator of autophagy and its phosphorylation level plays an important role in regulating cell proliferation and death. AKT plays a role in regulating various signaling pathways including cell growth, proliferation, survival, chemoresistance and angiogenesis. It is a downstream effector of PI3K and an upstream regulatory molecule of mTORC1 [15]. The mTOR is a primary regulator with significant functions in autophagy and provides negative regulation of autophagy. mTOR is a serine/threonine kinase that is one of the major regulators of cellular functions such as growth, proliferation, and survival. While regulating cellular functions, mTOR; regulates cellular activities such as protein synthesis, energy metabolism, and stress response by bringing together different signaling pathways [16]. mTOR is considered an important downstream target of the PI3K/Akt pathway [15]. Many toxicants induce autophagy and apoptosis by downregulating the PI3K/AKT/mTOR pathway or

inhibiting mTOR [17]. Additionally, there is evidence from previous studies that imatinib inhibits the PI3K/AKT/mTOR pathway in various types of cancer [18,19]. However, there is no literature information on the PI3K/AKT/mTOR pathway regarding the effects of imatinib on liver and kidney damage. In this study, PI3K, AKT and mTOR levels in liver and kidney tissues were decreased by imatinib, while apilarnil given for treatment increased these parameters. In various toxicity studies conducted with experimental animals, it was reported that apilarnil protects liver and kidney tissues due to its antioxidant properties [10, 20].

The JAK2/STAT3 signaling pathway plays a role in regulating many important biological processes in the body, especially inflammation and oxidative stress. JAK2 can be activated by phosphorylation and then cause downstream STAT3 phosphorylation, which can be imported into the nucleus to initiate the expression of oxidative stress and inflammation-related genes and accentuate the oxidative stress and inflammation reaction in the tissue [21]. Activated STAT3 also stabilizes the mitochondrial membrane. Moreover, STAT3 is also recognized as an anti-apoptotic factor as it regulates several apoptosis-related genes such as Bcl-2 and Bcl-xL [22]. Previous studies have shown that the JAK2/STAT3 pathway plays a vital role in both liver and kidney injury [21, 23]. In another study, it was reported that low-dose imatinib (10 and 20 mg/kg) provided protection by reducing JAK2 and STAT3 protein levels in a model of ulcerative colitis experimentally induced with acetic acid in rats [24]. In this study, it was determined that imatinib increased JAK2 and STAT3 levels in liver and kidney tissues, while apilarnil given for treatment decreased JAK2 and STAT3 levels. There is no information in the literature about the relationship between apilarnil and the JAK2/STAT3 signaling pathway. However, it is thought that apilarnil has a therapeutic effect due to its rich content (vitamins, minerals and phenolic compounds).

As a result, it was determined that imatinib decreased PI3K, AKT and mTOR levels, increased JAK2 and STAT3 levels and triggered apoptosis and autophagy, while apilarnil application showed the opposite effect and tried to protect the tissues. In the light of the findings obtained, it is thought that more comprehensive studies are needed to clearly understand whether the use of apilarnil in imatinib-induced liver and kidney damage will be beneficial.

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