Al-Kitab Journal for Pure Sciences (2026); 10(2):87-103.
https://doi.org/10.32441/kjps.10.2.p7

Al-Kitab Journal for Pure Sciences
ISSN: 2617-1260 (print), 2617-8141(online)

https://isnra.net/index.php/kjps

The Effect of Tacrolimus and Triticum Aestivum Germ Oil on the Intrinsic Pathway of Apoptosis in the Liver of Male Albino Mice: An Immunohistochemical and Molecular Study

Thamer Ahmed Mohamed[1], Baidaa Abdulaziz Mohamed Salah[2]

[1,2] Department of Biology, College of Education for Pure Science, University of Mosul, Mosul, Iraq.

Corresponding author: thamer.20esp37@student.uomosul.edu.iq[1] baidaamohammed@uomosul.edu.iq[2]

Citation: *Thamer Ahmed Mohamed[1], Baidaa Abdulaziz Mohamed Salah[2]*. **The effect of tacrolimus and Triticum Aestivum Germ Oil on the intrinsic pathway of apoptosis in the liver of male albino mice: an immunohistochemical and molecular study**.
Al-Kitab J. Pure Sci. [Internet]. 2025 Sep. 14 ;10(2):87-103
DOI.
https://isnra.net/index.php/kjps/article/view/2026.
https://doi.org/10.32441/kjps.10.2.p7

©20--. THIS IS AN OPEN-ACCESS ARTICLE UNDER THE CC BY LICENSE
http://creativecommons.org/licenses/by/4.0/

Keywords: Tacrolimus, Wheat germ oil, Bax/Bcl-2 ratio, qPCR gene expression, Hepatotoxicity.

Article History
Received 09 Aug. 2025
Accepted 14 Sep. 2025
Available online 01 Nov. 2026

Abstract:

Tacrolimus is a commonly used as an immunosuppressant, while wheat germ oil is a recently discovered compound that is a potent antioxidant. The current study investigated the role of *Triticum Aestivum* Germ Oil in influencing the intrinsic pathway of apoptosis through its effect on the protein Bax. Forty male white mice, *Mus musculus* were used, divided into four sets, each containing 10 individuals: negative control (distilled water) set, positive control set (wheat germ oil, 1130 mg/kg), tacrolimus (0.4 mg/kg) set, and a set treated with tacrolimus and wheat germ oil together. The animals were treated for two periods, 14 and 28 days. The findings demonstrated that tacrolimus increases the activity of the Baxdelta2G9 (Bax protein) in hepatocytes; however, after 14 days of treatment with two certain chemicals, the group's immunohistochemistry activity of Bax declined. Over 28 days, the response and improvement grew. Additionally, the group that received tacrolimus revealed an increase in Bax transcription, whereas the group that received wheat germ oil showed a decrease in Bax gene expression. Additionally, the improvement was noted as the experiment's duration grew. We conclude from this that tacrolimus stimulates the activity of the intrinsic pathway of programmed cell death,

but the use of an antioxidant, such as wheat germ oil, improves and reduces the activity of this pathway in the liver.

Keywords: Tacrolimus, Wheat germ oil, Bax/Bcl-2 ratio, qPCR gene expression, Hepatotoxicity

تأثير التاكرولميس وزيت جنين القمح في المسار الداخلي للموت المبرمج في الكبد لذكور الفئران البيض: دراسة كيميائية مناعية نسجية وجزيئية

ثامر احمد محمد[١], بيداء عبد العزيز محمد صالح [٢]

[٢،١] قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل

الايميل الرسمي: thamer.20esp37@student.uomosul.edu.iq[1], baidaamohammed@uomosul.edu.iq[2]

الخلاصة:

يُعَدّ عقار التاكرولميس واحداً من أهم المثبطات المناعية المستعملة على نطاق واسع في عمليات زراعة الأعضاء، غير أنّ آثاره الجانبية على الكبد ما تزال مثار جدل علمي. أما زيت جنين القمح فهو غني بمركبات مضادة للأكسدة كالتوكوفيرولات والأحماض الدهنية غير المشبعة، وقد ثبُتت فعاليته في التقليل من الإجهاد التأكسدي وإبطاء مسارات الموت الخلوي. هدفت الدراسة الحالية إلى تقييم التأثير الوقائي لزيت جنين القمح ضد السمية الكبدية المستحثة بالتاكرولميس من خلال تتبّع التغيرات في بروتيني *Bax* و *Bcl-2*المرتبطين بالمسار الداخلي للموت المبرمج. استُخدم في التجربة أربعون فأراً أبيض ذكراً *(Mus musculus)*وزّعت إلى أربع مجموعات: مجموعة السيطرة السالبة (ماء مقطر)، مجموعة السيطرة الموجبة (زيت جنين القمح بجرعة ١١٣٠ ملغم/كغم)، مجموعة التاكرولميس (٠,٤ ملغم/كغم)، ومجموعة التاكرولميس مع زيت جنين القمح معاً. عُولجت الحيوانات لفترتين زمنيتين (١٤ و٢٨ يوماً). أظهرت نتائج الكيمياء المناعية النسيجية ارتفاعاً معنوياً في *Bax* في أكباد الفئران المعاملة بالتاكرولميس، يقابله انخفاض ملحوظ في مجموعة زيت جنين القمح. كما كشفت تحاليل *qPCR* عن زيادة في التعبير الجيني لبروتين *Bax* مع التاكرولميس، بينما أدّى الزيت إلى تعزيز التعبير عن *Bcl-2* وخفض نسبة *Bax/Bcl-2* مقارنة بالمجموعة المعاملة بالتاكرولميس فقط. تؤكد هذه النتائج أنّ التاكرولميس ينشّط المسار الداخلي للموت المبرمج عبر تعزيز*Bax* ، في حين يوفّر زيت جنين القمح حماية خلوية واضحة من خلال رفع *Bcl-2* وتقليل نسبة *Bax/Bcl-2*، مما يعكس دوره المضاد للأكسدة والواقي من السمية الكبدية.

الكلمات المفتاحية: التاكرولميس، زيت جنين القمح، نسبة، *Bax/Bcl-2*، التعبير الجيني بتقنية، *PCR*السمية الكبدية

Introduction:

Tacrolimus as an immunosuppressive agent is utilized for transplanted organs. It is a member of the calcineurin antagonist class, which blocks calcium-dependent processes. Since 1998, it has been applied to liver transplants [1]. Many studies are still investigating the adverse reactions of immune-suppressive agents, even though their use lowers the chance of organ

rejection. The immunophilin FK506-binding protein attaches to tacrolimus, forming an intricate structure in the process. T cells get blocked and their ability to damage cells decreases as an outcome of this complex's inhibition of calcium-dependent processes. Therefore, antigen presentation is prevented [2]. Numerous adverse effects, such as hepatotoxicity, compromised defenses, diabetes, kidney disease, and heightened vulnerability to severe acute respiratory syndrome coronavirus 2 and other immune-mediated illnesses as a result of immunosuppression, have been documented in organ transplant recipients [3]. When this medication is given to CD-1 mice, oxidative stress or cellular tissue degeneration is linked to elevated blood levels of urea nitrogen, renal creatinine, and free radical production [4]. The therapeutic index of the drug under study is narrow, and its administration, as mentioned, can cause a large number of side effects, extending to the appearance of toxic symptoms in the hepatocytes, cardiac muscle tissue, renal tissue, and cerebrum. However, the degree of damage depends on the dose and duration of administration. Unfortunately, the mechanism behind considering tacrolimus as a mediator of toxicity is still unclear and poorly understood, despite the drug's broad research and the many theories proposed to explain its mechanism of action, such as its metabolite-related toxicity through accelerated cell death and augmented inflammatory conditions [5–7]. Studies have shown that tacrolimus causes pulmonary toxicity, which is associated with morphological alterations and tissue changes such as cellular necrosis [8,9]. Because of its relationship to the digestive system, the liver is a significant organ that is susceptible to the harmful effects of medications, foreign substances, and other chemicals [10]. Cells can alter both biochemical and non-enzymatic responses to lessen the negative effects of foreign chemicals, and they can also digest nutrients high in phytochemicals via pathways akin to those of medications and insecticides [11].

Wheat germ oil enhances antioxidant protection by increasing lipid metabolism in the blood and liver. Rich in vitamin E, healthy fats, and antioxidants, this oil exhibits anti-oxidative activity and reduces DNA damage by neutralizing or balancing the increase in reactive oxygen species (ROS). Additionally, the oil from wheat germ inhibits the synthesis of fatty acids which promote the oxidation of lipids and aids the reactive oxygen species system through tocopherol [12]. Several energy-dependent chemical processes make up the intricate and sophisticated systems that regulate controlled cell death. The inherent, or mitochondrial, mechanism and the external, or killing receptor, pathway are the two primary routes for programmed cell death. Numerous pieces of data suggest that the two routes are interconnected and that molecules in one pathway can influence molecules in the other [13]. Numerous non-receptor stimuli are part

of the endogenous signaling processes that trigger cell suicide. These signals are produced intracellularly and have an immediate impact on targets inside the cell. These are the kinds of things that mitochondria trigger. Both positive and negative intracellular signals are produced by the stimuli that start the intrinsic route. The lack of specific growth regulators, hormones, and cytokines are examples of adverse signals that may end up in the initiation of suicide and the inability of death programs to suppress it. The process of apoptosis induced by mitochondrial dysfunction can be controlled and regulated with the help of the Bax-Bcl2 protein family [14]. Cellular tumor antigen p53 increases gene expression of the BAX protein. The protein in concern provides a role in death triggered by p53. Bcl2 is an apoptosis-inducing gene that is a member of the B-cell lymphoma 2 protein group. It is the first regulator of apoptosis found in living creatures. Bcl2-L-4 is found in the outer membrane of mitochondria, where it inhibits the activity of death proteins and promotes cell survival. Reactive oxygen species (ROS) and ferrocytochrome c, two crucial cell death signals, are released when these proteins, which include BAX and BCL2 antagonist/killer (BAK), engage on the mitochondrial outer surface to cause permeabilization. Bcl2-L-4 and its subordinate protein Bcl-XI are activated by Bcl2, which is activated by BH3-only proteins [15].

The current study aims to identify the effect of treating the livers of male white mice with tacrolimus at a concentration of 0.4 mg/kg for two periods, 14 and 28 days, on the BAX gene that is responsible for regulating the intrinsic pathway of programmed death. Furthermore, it aims to identify the role of Triticum aestivum germ oil in reducing the damage caused by tacrolimus, using immunohistochemistry and molecular techniques.

2. Material and methods:

Animals used in the research

The mice used in the current study were brought from Tikrit University / Tikrit / Iraq. Their number was 40 white male mice, *Mus Musculus* of suitable age. In the animal house of the College of Veterinary Medicine at the University of Mosul in Iraq, the creatures were housed in specialized nylon containers. They were fed the standard and prepared diet according to what was mentioned in [16]. The environmental conditions were suitable for the living conditions of laboratory animals and according to what was mentioned in [16]. The standard laboratory chow consisted of approximately 20% protein, 5% fat, and 55% carbohydrate, with an energy content of ~3.6 kcal/g. The work was carried out under the supervision of the Scientific Research Ethics Committee of the University of Mosul, and all relevant recommendations were implemented.

Preparation of tacrolimus and wheat germ oil for dosing:

The median lethal dose (LD50) was the primary criterion for selecting the tacrolimus (Pancea Biotech, India) (LD50, 138 mg/kg) concentration under study, which is 2 mg /kg of body weight. As for wheat germ oil (NOW company, USA), a 1130 mg/kg of germ wheat oil was used and prepared according to the weight of the experimental animals based on preliminary studies. The appropriate doses of both medications were given to the animals orally. The volume of dose given was (1.3-1.5 ml) depending on the animal's weight. The animals in the present research were split up into four distinct categories as indicated in tables (1 and 2) below:

Table 1: The experimental design for the first experiment is shown.

Groups	**Tacrolimus and Wheat Germ Oil Dose (mg/kg)**	**Experiment Duration (days)**	**Administration Route**	**Number of Animals**
Negative Control	Distilled Water	١٤	Orally	10
Positive Control	Wheat Germ Oil (1130 mg/kg)	١٤	Orally	10
Experimental (1)	Tacrolimus (0.4 mg/kg)	١٤	Orally	10
Experimental (2)	Tacrolimus (0.4 mg/kg) + Wheat Germ Oil (1130 mg/kg)	١٤	Orally	10

Table 2: The experimental design for the first experiment is shown

Groups	**Tacrolimus and Wheat Germ Oil Dose (mg/kg)**	**Experiment Duration (days)**	**Administration Route**	**Number of Animals**
Negative Control	Distilled Water	28	Orally	10
Positive Control	Wheat Germ Oil (1130 mg/kg)	28	Orally	10
Experimental (1)	Tacrolimus (0.4 mg/kg)	28	Orally	10
Experimental (2)	Tacrolimus (0.4 mg/kg) + Wheat Germ Oil (1130 mg/kg)	28	Orally	10

Detection of immunohistochemical reactivity to Bax and Bcl-2 proteins in liver tissue

BAX protein activity was detected in liver tissue and all treated groups in the current study using the BAX Monoclonal Antibody kit (Catalog Number: E-AB-22212) manufactured by Elabscience Bionovation Inc. The steps mentioned in the ready-made diagnostic kit were followed with high accuracy for the purpose of detecting the aforementioned protein in the liver

of male white mice and for all treated groups, and laboratory conditions were taken into account in terms of temperature, humidity, and sterilization.

Numerical Microscopic Evaluation

This Numerical Microscopic Evaluation process refers to determining a degree of importance, either low or high, or creating a numerical scale for the severity of cell reaction and the potential for tumour growth and spread. The process is often used to measure the effect of external toxins or physical factors on living tissues, as well as in cancer cases, based on what was approved by the National Cancer Institute (NCI). Table (3) below was used in the study of histological phenotypic variables [17, 18] in the liver tissue of male albino mice in the current study.

Table (3): Digital Microscopic Evaluation Adopted in the Current Study

Studied Protein	0+	1+	2+	3+	4+
Bax (Tsamandas et al., 2022)	Negative (-) 0 cell/ microscopic field	Weak (+) 1–5 cells/ microscopic field	Positive (+) 6–15 cells/ microscopic field	Strong Positive (++): 16–30 cells/ microscopic field	Very Strong Positive (+++): More than 30 cells/ microscopic field
Bcl2 (Qian et al., 2022)	Negative (-) 0 cell/ microscopic field	Weak (±) 1–7 cells/ microscopic field	Positive (+) 8–20 cells/ microscopic field	Strong Positive (++): 21–50 cells/ microscopic field	Very Strong Positive (+++): More than 50 cells/ microscopic field

Estimation of gene expression of genes encoding BAX and Bcl2 proteins in the liver of male albino mice for periods of 14 and 28 days

Quantitative real-time PCR (qPCR) was performed using a **Step-One-Plus™ Real-Time PCR System (Applied Biosystems, USA)** with the **TransStar® Top Green qPCR Super-Mix Kit**. The amplification reactions were carried out in a **20 µL volume** containing 10 µL of 2× Super-Mix, 1 µL of each forward and reverse primer, variable cDNA template, and nuclease-free water. Each sample was analyzed in **triplicate** to ensure reproducibility. The thermal cycling conditions included an initial denaturation at 94°C for 30 seconds, followed by 45 cycles of

denaturation at 94°C for 5 seconds, annealing at 60°C for 30 seconds, and extension. Melting curve analysis was performed to confirm amplification specificity. Relative gene expression levels of **Bax** and **Bcl-2** were calculated using the **2^–ΔΔCt method**, with β-actin serving as the internal housekeeping gene (Table 4).

Table 4: Primers used in the amplification of Bax and bcl2 genes by qPCR

Primer name	Primer sequence 5' – 3'	Reference
Bax-F	GGGACGAACTGGACAGTAACAT	Kandeil, M. A., Hassanin, K. M., Mohammed, E. T., Safwat, G. M., & Mohamed, D. S. (2018). Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin. Beni-Suef University journal of basic and applied sciences, 7(3), 257-262
Bax-R	GGAGTCTCACCCAACCACCCT	
Bact-F	ATGAGCCCCAGCCTTCTCCAT	
Bact-R	CCAGCCGAGCCACATCGCTC	

The components of the amplification mixture for each gene are described in Table (5) , and the qPCR conditions used to amplify the target genes are listed in

Table (5): Reaction components of qPCR

Components	**Volume (µl)**
Cdna	Variable
Forward primer	1
Reverse primer	1
2× *TransStar*® Top Green qPCR SuperMix	10
RNase-free Water	Up to 20

Table (6): Bax and bcl2 gene transcription is estimated using qPCR settings. Using the 2−ΔΔCT technique, the relative expression levels of bax and bcl2 were determined.

Step	**No. of repetitions**	**High Temperature ºC**	**Period of work**
initial strand separation".	1	94	0:30 sec
unwinding of DNA	٤٥	94	0:5 sec
primer hybridization and DNA synthesis		٦٠	0:٣٠ sec

Statistical Data Analysis

The statistical procedure to assess significant differences between the mean values of digital microscopic assessment across the experimental groups, immunohistochemistry analyses were conducted using SPSS software version 22.0. $P < 0.01$ was taken into consideration as the significance level. A post hoc analysis was performed after a single-factor ANOVA was used to assess the data. To find statistically significant differences between groups at the designated probability level, Duncan's Multiple Range Test was utilized. Regarding qPcr, GraphPad Prism version 10/USA was used for all statistical analysis.

3. Results:

Light microscopic examination of sections of the two groups of livers treated with distilled water (negative control group) and wheat germ oil (positive control group) for 14 days showed a negative reaction (-) in most of the tissue components of the liver (Figures 1, 2). Microscopic examination of liver sections of male albino mice treated with 0.4 mg/kg body weight of tacrolimus showed strong positive (+++) reaction to the antibodies of BAX protein in the cytoplasm of hepatocytes, which appeared as a dark golden color in the cytoplasm of the cells above, (Figure 3).The results also showed a more intense positive reaction (++) from the cytoplasm of hepatocytes from the livers treated the same concentration of tacrolimus (0.4) mg/kg and wheat germ oil towards antibodies to the BAX protein (Figure 4) .

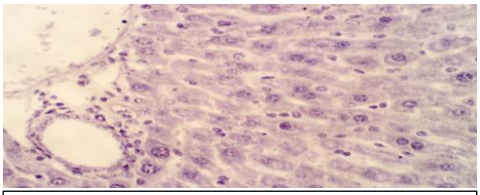


Figure 1: Liver of mice from the 14th day of the experiment, Distiled water (negative contol group) group. Negative reaction is shown in the liver tissue. BAX immunohistochemistry. Magnification 400×.

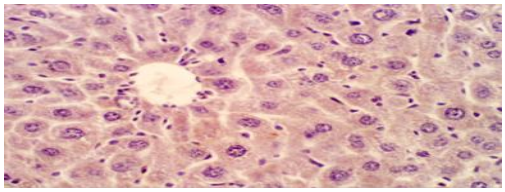


Figure 2: Liver of mice from the 14th day of the experiment, oil group (positive control group). Negative reaction is shown in the liver tissue. BAX immunohistochemistry. Magnification 400×.

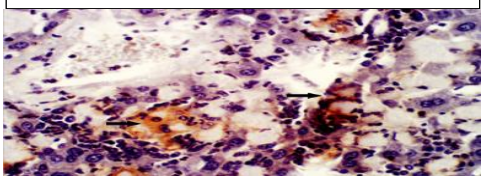


Figure 3: Transverse histological section of the liver of a male albino mouse from the second treatment group over 14 days. It shows a very strong positive reaction (+++) (black arrow) that appeared as a dark gold stain in the cytoplasm. BAX immunohistochemistry. Magnification ×400

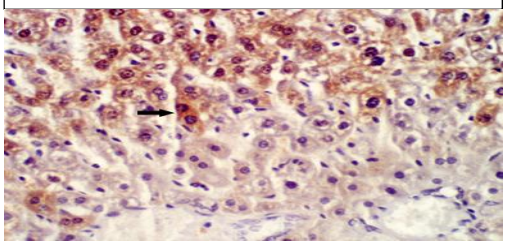


Figure 4: Transverse histological section of the liver of a male albino mouse from the second treatment group with wheat germ oil for 14 days. The positive (+) reaction (black arrow) appears as a dark golden color in the cytoplasm. BAX immunohistochemistry. Magnification ×400.

It was noted through histological reading using a light microscope of liver sections of mice from the negative and positive control groups for 28 days that there was no histological immune response (negative response -) of the components of the liver tissue towards antibodies of the BAX protein (Figures 5, 6).While the liver sections treated with tacrolimus at a concentration of (0.4) mg/kg of body weight showed a very strong positive reaction (+++) in the cytoplasm of the liver cells, which confirms the effect of the drug in increasing the gene expression of this protein through the excessive production of the protein, which was stained with a dark golden brown color in the cytoplasm of the liver cells (Figures ٧).The results also showed a more intense positive reaction (++) from the cytoplasm of hepatocytes from the livers treated with a concentration of tacrolimus (0.4) for 28 days mg/kg and wheat germ oil towards antibodies to the BAX protein (Figure 8).

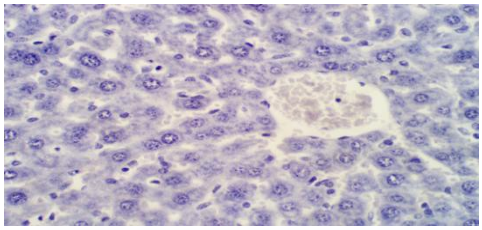


Figure 5: Liver of mice from the second period of the study, negative control set. Demonstrating a negative reaction in liver tissue. BAX

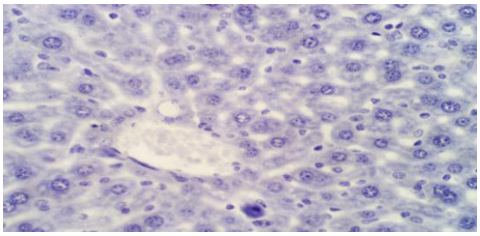


Figure 6: Liver of mice from the second set of the study, oil group. Demonstrating a negative reaction in liver tissue. BAX immunohistochemistry. Magnification 400×.

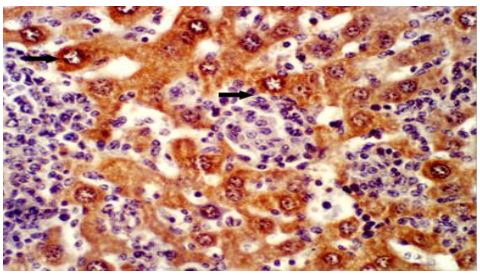


Figure 7: Transverse histological section of the liver of a male albino mouse from the second treatment group over 28 days. It shows a diffuse, strongly positive (+++) reaction (black arrow) that appears as a dark gold stain in the cytoplasm. BAX immunohistochemistry. Magnification ×400.

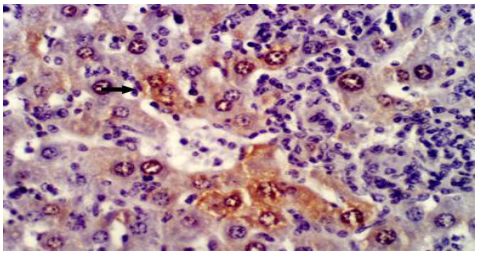


Figure 8: Transverse histological section of the liver of a male albino mouse from the second treatment group with whaeat germ oil for 28 days. The positive (+) reaction (black arrow) appears as a dark golden color in the cytoplasm BAXimmunohistochemistry. Magnification ×400.

The number of reactive hepatocyte cells in the group treated with a concentration of (0.4) mg/kg of tacrolimus for the periods of 14 and 28 days of dosing was considerably greater at the probability level of 0.01>P, according to the results of the digital microscopic evaluation, as indicated in Table (6).

Table (6): Digital Microscopic Evaluation of the Effect of Wheat Germ Oil on the Number of Hepatic Cells Reacting with BAX Protein Antibodies

Group	Treatment (mg/kg)	14 Days	28 Days
Control	Distilled water	0.10 ± 0.02 E	0.11 ± 0.01 E
Wheat Germ Oil	1130	0.11 ± 0.01 E	0.11 ± 0.04 E
Tacrolimus	0.4	3.51 ± 0.11 A	3.90 ± 0.36 A
Tacrolimus + Wheat Germ Oil	0.4 + 1130	2.47 ± 0.24 B	2.93 ± 0.27 B

Different vertical letters mean significant differences at a probability level of $P \leq 0.01$

Immunohistochemical staining for Bcl-2 protein revealed a weak or negative reaction in liver sections of mice treated with tacrolimus alone, indicating suppression of the anti-apoptotic pathway. In contrast, the wheat germ oil group showed stronger Bcl-2 reactivity, and the combined tacrolimus + wheat germ oil group exhibited moderate expression, suggesting a partial protective effect of the oil in restoring Bcl-2 activity.

Transcription and Translation of the Apoptosis Regulator Bax in the Liver Cells

The findings demonstrated that the livers of male albino rats had higher levels of the BAX gene, which in turn led to higher levels of BAX protein transcription in the group of animals that received wheat germ oil treatment (positive control). In comparison with the untreated and wheat germ oil-treated groups, the tacrolimus-treated group exhibited a greater gene production rate for this protein at the selected dose. Over the period of 14 days, the transcription and translation of the indicated gene were lower in the group treated with the researched drug alone, greater in the group treated with wheat germ oil and the selected medication than in the negative control group. In contrast to the negative control group, findings for the same treatments described above, but for 28 days, revealed a slight decrease in the liver's expression of the mentioned gene. Comparing the group treated with tacrolimus at the examined dose to the negative control group, findings similarly revealed a major rise in the translation and transcription rate of the studied gene (Figure 9). The rate of activity was comparable to that of the unaltered group (Figure 10).

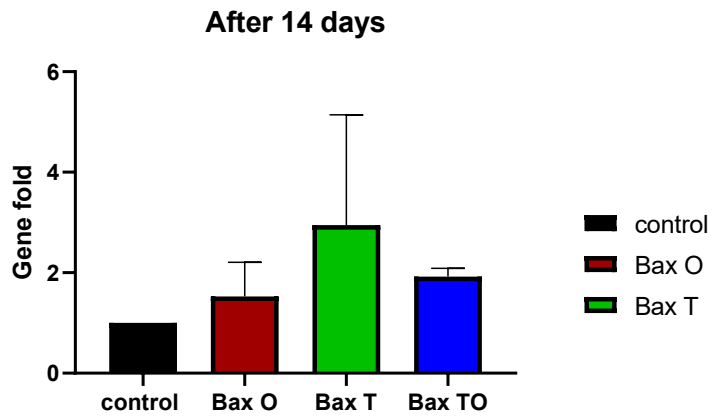


Figure 9: The gene expression of the Bax gene using q PCR technique is shown for all studied treatments. Control means the distilled water group, Bax O : wheat germ oil group, Bax T: tacrolimus group (0.4mg/kg), and Bax TO: Tacrolimus wheat germ oil group.Period of treatment: 14 days .

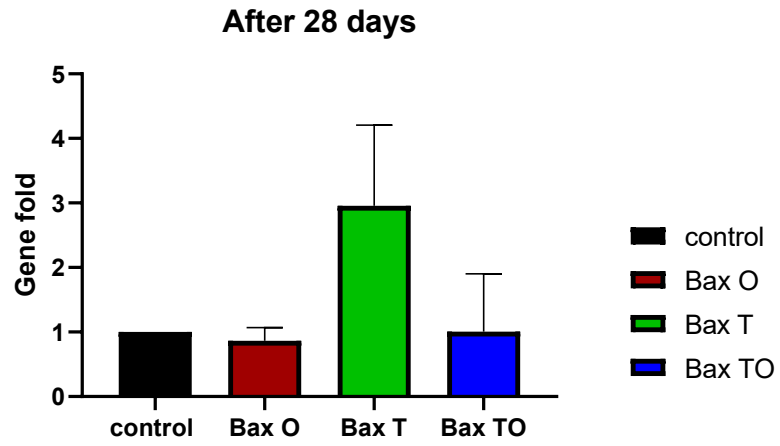


Figure 10: The gene expression of the Bax gene using q PCR technique is shown for all studied treatments. Control means the distilled water group, Bax O: wheat germ oil group, Bax T: tacrolimus group (0.4mg/kg), and Bax TO: Tacrolimus wheat germ oil group.Period of treatment: 28 days.

To better evaluate the balance between pro-apoptotic and anti-apoptotic signaling, the **Bax/Bcl-2 ratio** was calculated from qPCR data. Tacrolimus treatment significantly increased the Bax/Bcl-2 ratio compared with the control ($P \leq 0.01$), reflecting a shift towards apoptosis. Conversely, wheat germ oil markedly reduced this ratio, and in the tacrolimus + wheat germ

oil group, the ratio approached values closer to the control, indicating a protective modulation of apoptotic signaling by the antioxidant oil.

4. Discussion

Previous results showed that there is a positive response of the cytoplasm of hepatocytes to antibodies to the BAX protein in livers treated with tacrolimus (0.4 mg/kg) at the selected concentration and periods. The aforementioned results were consistent with what was stated by [19], who reported that livers treated with moderate concentrations of tacrolimus cause an increase in the production of the aforementioned protein. Current scientific findings are similar to what was mentioned by [20], that immunosuppressants, including the drug under study, when used for therapeutic purposes in living cells, including human liver cells, stimulate cell death and thus incite the occurrence of cell inflammation. Tacrolimus has side effects, including neurotoxicity, liver toxicity, and kidney toxicity, in addition to its induction of programmed cell death. The researchers identified this by using the TUNL test to detect this phenomenon.

These results are in line with recent research since the medication binds to molecules of protein phosphatase 2B (PP2B), which is involved in PIP catabolism, phosphotransferase activity, and cell protein synthesis [21]. This drug causes or can cause the production of types of free radicals, including OH radicals, O_2^-, and dihydrogen dioxide, and induces or stimulates programmed cell death, which in turn is due to the impairment of the functions of both mitochondrial and T cell functions [22]. The current results in terms of the drug's induction of programmed death are similar to what was mentioned by [23], and somewhat inconsistent with [24], who reported that immunosuppressants, when used in in vitro studies of human cells, inhibit the construction of hydrolyzed collagen without affecting the gene expression of collagen mRNAs, at a rate of 2 mg/kg. The present findings support [25], who demonstrated how the selected medication raises the production of the Caspase 3 protein in white rats. The data presented above showed that wheat germ oil had a beneficial effect on boosting Bax activity, the main suicide initiator. As the length of treatment against tacrolimus-induced damage grew, so did the severity of the response. The current results do not agree with [26], who showed that combining wheat germ oil with fluoxetine caused a negative cytoplasmic response to BAX protein in rat testicular tissue. This might be because of the oil's high vitamin E concentration, which strengthens its defenses against fluoxetine-induced apoptosis. Because wheat germ oil has anti-apoptotic characteristics, it plays a crucial part in the current investigation [27].

The scientific outcomes of the current study are similar to [28]. By regulating redox imbalance, apoptosis, swelling, and detrimental apoptotic pathways, the oil protected against kidney and liver failure at the physiological and cellular levels. Elainic, omega-6, and omega-3 fatty acids in wheat germ oil contribute to its beneficial impacts by promoting tocopherol-mediated antioxidant mechanisms and inhibiting eicosanoid synthesis, thereby reducing lipid peroxidation, inflammation, and apoptosis [29]. By decreasing the translation and transcription of genes that trigger cell death in rats exposed to toxins, one study demonstrated the anti-pro-apoptotic action of wheat germ oil [30].

The recent findings showed an increase in Bax transcription in livers treated with tacrolimus and a decrease in its activity in the tacrolimus + wheat germ oil group. This is consistent with [31], who showed that tacrolimus injections increased BAX expression in the hippocampus, and inflammation further enhanced this activity. Excessive ROS accumulation was highlighted by [32] as a key inducer of apoptosis via DNA damage, while [33] indicated that oxidative stress impairs cellular homeostasis.

The results of the current study, showing suppressed Bax expression with wheat germ oil, align with [34], who found that Nargencin A inhibited apoptosis proteins induced by tacrolimus. This is also consistent with [35], who reported that antioxidant phenolics such as those in green tea reduce tacrolimus toxicity. Similarly, [36] showed wheat germ oil has strong anti-apoptotic effects by regulating BAX and BCL2 gene expression in kidney cells. Finally, other authors emphasized wheat germ oil's richness in tocopherol and polyunsaturated fatty acids, reinforcing its protective roles [37,38].

5. Conclusions

Based on the latest study's achievements, we draw the conclusion that excessive levels of immunosuppressants harm hepatic cells' microcellular pathways and promote programmed death by raising the activity of the protein that triggers programmed death via the intrinsic pathway Bax. However, the recent study demonstrated that certain plant items with antioxidant activity, such as wheat germ oil, can reverse the detrimental effect of this medicine.

6. References

[1] Lin YC, Tsai CS, Li IH, Tsai YT, Huang TY, Lee KF, Kao LT. Transplant recipients using tacrolimus had higher utilization of healthcare services than those receiving

cyclosporine in Taiwan. *Front Pharmacol*. 2019;10:1074. doi:10.3389/fphar.2019.01074

[2] Akhtar T, Ali G, Sheikh N. Immunosuppressant-induced oxidative stress and iron: a paradigm shift from systemic to intrahepatic abnormalities. *Oxid Med Cell Longev*. 2020;2020:8675275. doi:10.1155/2020/8675275

[3] Ben-Fredj N, Hannachi I, Chadli Z, Ben-Romdhane H, Boughattas NA, Ben-Fadhel N, et al. Dosing algorithm for Tacrolimus in Tunisian kidney transplant patients: Effect of CYP3A4*1B and CYP3A4*22 polymorphisms. *Toxicol Appl Pharmacol*. 2020; 407:115245. doi: 10.1016/j.taap.2020.115245

[4] Oyouni AAA, Saggu S, Tousson E, Rehman H. Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by *Ocimum basilicum* L. in CD1 mice. *Toxicol Rep*. 2018; 5:687–694. doi: 10.1016/j.toxrep.2018.06.003

[5] Liu Z, Yuan X, Luo Y, He Y, Jiang Y, Chen ZK, Sun E. Evaluating the effects of immunosuppressants on human immunity using cytokine profiles of whole blood. *Cytokine*. 2009;45(2):141–147. doi: 10.1016/j.cyto.2008.12.003

[6] Kim HS, Lim SW, Jin L, Jin J, Chung BH, Yang CW. The protective effect of febuxostat on chronic tacrolimus-induced nephrotoxicity in rats. *Nephron*. 2017;135(1):61–71. doi:10.1159/000449289

[7] Tron C, Lemaitre F, Verstuyft C, Petitcollin A, Verdier MC, Bellissant E. Pharmacogenetics of membrane transporters of tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2019; 58:593–613. doi:10.1007/s40262-018-0717-7

[8] Goralczyk AD, Bari N, Abu-Ajaj W, Lorf T, Ramadori G, Friede T, et al. Calcineurin inhibitor sparing with mycophenolate mofetil in liver transplantation: a systematic review of randomized controlled trials. *Am J Transplant*. 2012;12(10):2601–2607. doi:10.1111/j.1600-6143.2012. 04157.x

[9] Akhtar T, Sheikh N, Shan T, Ghazanfar R. Tacrolimus induced nephrotoxicity and pulmonary toxicity in Wistar rats. *J Biol Regul Homeost Agents*. 2017;31(4):1061–1066. Available from: https://www.researchgate.net/publication/321946880

[10] Arafa UA, Fujiwara Y, Higuchi K, Shiba M, Uchida T, Watanabe T, et al. No additive effect between *Helicobacter pylori* infection and portal hypertensive gastropathy on inducible nitric oxide synthase expression in gastric mucosa of cirrhotic patients. *Dig Dis Sci*. 2003; 48:162–168. doi:10.1023/A:1021707103590

[11] Panemangalore M, Bebe FN. Short-and long-term exposure to low levels of pesticide and flavonoid mixtures modify endogenous antioxidants in tissues of rats. *J Environ Sci Health B*. 2009;44(4):357–364. doi:10.1080/03601230902801034

[12] Field R, Verghese M, Walker LT, Panala V, Shackelford L, Boateng J. Feeding wheat germ meal and wheat germ oil reduced azoxymethane-induced aberrant crypt foci. *Int J Cancer Res*. 2008;4(4):127–136. doi:10.3923/ijcr.2008.127.136

[13] Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer*. 2002;2(4):277–288. doi:10.1038/nrc776

[14] Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*. 2002;2(9):647–656. doi:10.1038/nrc883

[15] Hardwick JM, Soane L. Multiple functions of BCL-2 family proteins. *Cold Spring Harb Perspect Biol*. 2013;5(2): a008722. doi:10.1101/cshperspect. a008722

[16] Salah BA. Histopathological effect of fluoxetine drug on the brain of pregnant mice and their embryos. *Iraqi J Vet Sci*. 2020;34(1):71–76. Available from: https://web.archive.org/web/20201106084938id_/https://vetmedmosul.com/article_164198_acc17628f36302ee674334ab632ce9f0.pdf

[17] Tsamandas AC, Thomopoulos K, Zolota V, Kourelis T, Karatzas T, Ravazoula P, et al. Potential role of bcl-2 and bax mRNA and protein expression in chronic hepatitis type B and C: a clinicopathologic study. *Mod Pathol*. 2003;16(12):1273–1288. doi:10.1097/01.MP.0000097367.56816.5E. PMID:14681329

[18] Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Front Oncol*. 2022; 12:985363. doi:10.3389/fonc.2022.985363

[19] Love S, Mudasir MA, Bhardwaj SC, Singh G, Tasduq SA. Long-term administration of tacrolimus and everolimus prevents high cholesterol-high fructose-induced steatosis in C57BL/6J mice by inhibiting de-novo lipogenesis. *Oncotarget*. 2017;8(69):113403. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5768335/pdf/oncotarget-08-113403.pdf

[20] Lim EJ, Chin R, Nachbur U, Silke J, Jia Z, et al. Effect of immunosuppressive agents on hepatocyte apoptosis post-liver transplantation. *PLoS One*. 2015;10(9): e0138522. doi: 10.1371/journal.pone.0138522

[21] Naesens M, Kuypers DRJ, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol*. 2009;4(2):481–508. doi:10.2215/CJN.04800908

[22] Nash A, Samoylova M, Leuthner T, Zhu M, Lin L, Meyer JN, et al. Effects of immunosuppressive medications on mitochondrial function. *J Surg Res*. 2020; 249:50–57. doi: 10.1016/j.jss.2019.12.010

[23] Choi H, Baek K. Pro-apoptotic and anti-apoptotic regulation mediated by deubiquitinating enzymes. *Cell Mol Life Sci*. 2022; 79:117. doi:10.1007/s00018-022-04132-5

[24] Manojlovic Z, Blackmon J, Stefanovic B. Tacrolimus (FK506) prevents early stages of ethanol-induced hepatic fibrosis by targeting LARP6 dependent mechanism of collagen synthesis. *PLoS One*. 2013;8(6): e65897. doi: 10.1371/journal.pone.0065897

[25] Ibrahim S, Mousa A, Riawan W. Expression of AIF and Caspase-3 in New Zealand rabbit with cervical spondylosis myelopathy model. *Ann Med Surg*. 2021; 69:102604. doi: 10.1016/j.amsu.2021.102604

[26] Fawzy HM, Mahmoud FA. Histological protective effect of wheat germ oil versus zinc on fluoxetine–damaged testes in albino rats. *Ain Shams Med J*. 2023;74(3). Available from: https://asmj.journals.ekb.eg/article_321859

[27] Abdou HM, Mohamed NA, El Mekkawy AD, ElHengary SB. Vitamin E and/or wheat germ oil supplementation ameliorate oxidative stress induced by cadmium chloride in pregnant rats and their fetuses. *Jordan J Biol Sci*. 2017;10(1):39–48. Available from: https://jjbs.hu.edu.jo/files/v10n1/Paper%20number%208.pdf

[28] Elgendy SA, Baloza SH, Mohammed LA, Nasr HE, El-Shaer NO, Ghamry HI, et al. Ameliorative impacts of wheat germ oil against ethanol-induced hepatic and renal dysfunction in rats: involvement of anti-inflammatory, anti-apoptotic, and antioxidant signaling pathways. *Life*. 2022;12(10):1671. doi:10.3390/life12101671

[29] Alessandri C, Pignatelli P, Loffredo L, Lenti L, Del Ben M, Carnevale R, et al. Alpha-linolenic acid–rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2006;26(11):2577–2578. doi: 10.1161/01.ATV.0000242795. 08322.fb

[30] Mohamed MA, Ahmed MA. Apoptotic genes expression in γ-irradiated rats treated with wheat germ oil, zinc and/or bone marrow. *Int J Basic Appl Sci*. 2014;3(4):451–456. doi:10.14419/ijbas. v3i4.3657

[31] Badr R, Hashemi M, Javadi G, Movafagh A, Mahdian R. Assessment of global ischemic/reperfusion and tacrolimus administration on CA1 region of hippocampus:

gene expression profiles of BAX and BCL2 genes. *Bratisl Lek Listy*. 2016;117(6):358–362. doi:10.4149/bll_2016_071

[32] Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Squadrito F, et al. Oxidative stress: harms and benefits for human health. *Cell Prolif*. 2017;50(1): e12335. doi:10.1111/cpr.12335

[33] Sosa MS, Debnath J, Aguirre-Ghiso JA. Regulation of tumor cell dormancy by tissue microenvironments and autophagy. *Adv Exp Med Biol*. 2013; 734:73–89. doi:10.1007/978-1-4614-1445-2_5

[34] Park C, Kwon DH, Hwang SJ, Han MH, Jeong JW, Hong SH, et al. Protective effects of Nargenicin A1 against tacrolimus-induced oxidative stress in Hirame natural embryo cells. *Int J Environ Res Public Health*. 2019;16(6):1044. doi:10.3390/ijerph22050773

[35] Song J, Kim J, Jang H. Ferulic acid released by treatment with *Aspergillus oryzae* contributes to the cellular antioxidant capacity of wheat germ extract. *Food Sci Biotechnol*. 2014;23(4):1327–1333. doi:10.1007/s10068-014-0182-5

[36] Kandeil MAM, Hassanin KMA, Mohammed ET, Safwat GM, Mohamed DSh. Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin. *Beni-Suef Univ J Basic Appl Sci*. 2018;7(3):257–262. doi: 10.1016/j.bjbas.2018.02.001

[37] Rizzello CG, Coda R, De Angelis M, Gobbetti M. Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chem*. 2010; 119:1079–1089. doi: 10.1016/j.foodchem.2009.08.016

[38] Gelmez N, Kıncal SN, Yener ME. Antioxidant activities and phenolic contents of roasted wheat germ extracts. *J Supercrit Fluids*. 2009;48(3):217–224. doi: 10.1016//j.supflu.2008.11.002.