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Pharmata aims to contribute to the scientific literature by publishing manuscripts of the highest caliber. The journal accepts research articles, reviews, and short communications that adhere to ethical guidelines.
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17. Pharmaceutical chemistry
18. Synthesis and analysis of new drug molecules
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The target audience of the journal includes researchers and specialists who have an interest in or are working in any of the fields covered by the journal's scope.

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RESEARCH ARTICLES

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Ameliorative Protective Influence of Etoricoxib Against Chemo Convulsions Correlates with Blood Glucose Levels in Rats

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ABSTRACT

Objective: The effect of Etoricoxib (ETOR) on the link between blood glucose and seizure prevention in chemo-convulsive rats was investigated in this study.

Methods: Pentylenetetrazol (PTZ-105 mg/kg i.p.) at the CD97 dosage was administered to rats to cause seizures. ETOR (10 mg/kg p.o.) was administered for two weeks prior to seizure induction. On the 14th day, the animals were exposed to chemo convulsions, and the efficiency of ETOR in lowering clonus-type chemo convulsions (CC) as well as blood glucose levels were assessed. Morphometric analysis and chimney tests were performed to evaluate ETOR's neurotoxic profile. Actophotometer, rotarod, and hole board tests were employed for behavioral analysis.

Results: When compared to control mice, pretreatment with ETOR (10 mg/kg p.o) resulted in a substantial delay in the onset of CC (P<0.05) and duration of CC (P<0.01). Acute ETOR treatment also considerably lowered blood glucose levels. The chimney test findings show that it has no effect on motor control and is not neurotoxic to the animals.

Conclusion: Behavioral tests also demonstrated that, except for the diazepam-treated groups, no significant changes in muscle coordination, locomotion, or apprehensive behavior were detected in all experimental animals. Finally, the primary findings of this study showed that the PTZ group had considerably greater levels of hyperglycemia, which is reflected in the rat's early onset and longer duration of chemo-convulsions. However, ETOR medication reduced high blood glucose levels as well as the onset and duration of seizures, indicating ETOR's impact on blood glucose reduction and the relationship between blood glucose and seizure responses.

Keywords: Blood glucose, Chemo convulsions, Etoricoxib, Pentylenetetrazol, Seizure protection

INTRODUCTION

The significance of glucose proportion in the human body has been described in both *in vivo* and *in vitro* research, indicating that threshold glucose proportion is required to enable synaptic transmission.[1] However, circumstances such as hyperglycemia increase brain injury causing ischemia and hyper excitability in the neuronal environment. Hyperglycemia is a condition characterized by abnormally high blood sugar or glucose levels. It happens when the body doesn't make or use enough insulin, a hormone that converts glucose into energy in the cells.[2,3,4] Hyperglycemia is a common diabetes complication that can affect both diabetic and non-diabetics. Both Hyperglycemia and hypoglycemia can cause seizures if left untreated. According to known research, lowering extracellular glucose levels may reduce seizure activity by decreasing neuronal excitability.[5]

Seizures have been noticed in various central nervous system (CNS) disorders, and they have been linked to proinflammatory processes that cause CNS illness. Alternatively, metabolic abnormalities such as hyperglycemia and hypoglycemia can cause CNS damage, resulting in seizures; seizures in endocrine illnesses can be caused by autoimmune, metabolic, and neuroinflammation.[6,7] Inflammations are mostly caused by Cyclooxygenases. Cyclooxygenases are myeloperoxidases that mediates the conversion of arachidonic acid to PGH2, which is then converted to physiologically active end products like PGD2, PGE2, PGF2, PGI2, or thromboxane A2 (TXA2) via specific synthases.[8,9] COX-1 and COX-2 are two isoforms of cyclooxygenase. COX-1 is a housekeeping enzyme, whereas COX-2 is a fast-inducible

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inflammation-related enzyme. According to gene expression data, COX-2 was the dominant isoform in human pancreatic islets under baseline conditions.[10]

According to the data, the main and important endogenous prostanoid produced by COX-1 and COX-2 is PGE2. In a pancreatic cell line called HIT cells as well as in humans, PGE2 reduced glucose-induced insulin production.[11-13] As a result, inhibiting COX-1 or COX-2 lowers PGE2 levels, which improves insulin secretion. A recent *in vitro* study, found that using a selective COX-2 inhibitor called celecoxib, a pancreas-cell line called INS-1E cells released more glucose-stimulated insulin.[14] However, the *in vivo* effects of selective COX-1 or COX-2 inhibition on insulin secretion remain poorly understood and need to be clarified through additional research. Hence, this investigation was decided to establish the impact of ETOR an inhibitor of COX-2 on the correlation between blood glucose and seizure prevention in chemo convulsive rats, which was induced by toxic chemicals such as pentylenetetrazol (PTZ 105 mg/kg i.p) to the experimental animals.

METHODS

Drugs and Chemicals

The drugs etoricoxib, diazepam, and pentylenetetrazol were obtained from Yarrow chem. Pvt LTD, India, and dissolved in appropriate solvents. The dose of ETOR (10 mg/kg b.w) and diazepam has been used successfully in similar experimental models and the dose of ETC based on suggested human treatment regimes has been used successfully to closely replicate human scenarios of use.[15,16] Etoricoxib was suspended in 0.25% carboxymethyl cellulose, diazepam in 1% w/v gum acacia, and for PTZ normal saline is used. All chemicals that were purchased were analytical grade.

Animals and Ethical Approvals

Albino adult male rats with weight ranges between 160-175 g were chosen and housed in conventional laboratory-housing settings such as 23°C, humidity of 55%, and light-dark cycles. Many basic science studies of diabetic complications have concentrated on STZ-induced diabetes using exclusively male mice, despite the fact that diabetes is known to affect both sexes in patients and in some genetic animal models. Therefore, we have chosen exclusively male animals for our study.[17] All animals were given access to food and water ad libitum throughout the experiment. The experimental protocols were duly approved (SVCP/IAEC/II-10/19-20) by the IAEC (Institutional Animal Ethics Committee) in accordance with the recommendations of the Committee for Control and Supervision on Animal Experiments (CPCSEA), New Delhi, India. To avoid bias, randomized methods of allocation of animals were followed with respect to treatments as different experimental groups.[18]

Experimental Design

Induction of Chemo Convulsion

A total of 24 rats were randomly divided into four groups of six animals each (n=6) according to the following acute study design duration of 14 days. Group I was served as control and treated with normal saline (2ml/kg of NS 0.9%) whereas Non-Diabetic Hyperglycemia (Group II) received 20% glucose solution through intraperitoneally 30 minutes before administration of PTZ. Non-diabetic hyperglycemia is known as impaired glucose regulation, is defined as elevated blood glucose levels that are not in the diabetic range. Group III and Group IV animals received diazepam and standard ETOR, respectively, along with 20% glucose before the induction of chemo convulsions. To check the correlation between glucose levels and seizure protection, rats were given 20% glucose solution (to reduce drug-induced hypoglycemia mortality of animals) except control animals.[19-21]

PTZ was administered at a dose of 105 mg/kg i.p at its CD97 convulsive dose to induce chemo convulsions on 14th day. After administration, animals were kept separately in transparent cages (25 × 15 × 10 cm) to better observation of the occurrence of a clonus type of convulsion about the next 30 min.[22,23]

Group I - Control (NS-2ml/kg.p.o)

Group II- NDH (Glucose -20%) + PTZ (105 mg/kg i.p)

Group III - NDH (Glucose -20%) +DIAZ (2mg/kg.i.p) + PTZ (105 mg/kg i.p)

Group IV - NDH (Glucose -20%) +ETOR (10 mg/kg.p.o) + PTZ (105 mg/kg i.p)

Morphometric Analysis

The body weight of all experimental animals was carefully documented at regular intervals to examine the effect of acute ETOR administration on experimental animals.

Measurement of Blood Glucose Concentrations

Glucose measurement was performed in all experimental groups immediately before and after the seizure tests. To evaluate blood glucose concentration, a little drop of blood (10 µL) was collected from the tip of the rat's tail and placed on a test stripe (glucose oxidase/peroxidase reactive strips) of Dr. Morepen one touch glucometer. The

glucose-level reading appeared and was observed in 5 seconds. The glucose tolerance was measured hourly for three hours.

Acute Neurotoxicity Test

The neurotoxic profile of ETOR was evaluated using a chimney test. This test is considered as one of the gold standard tests to check the neurotoxic profile of ETOR. Rats were kept in a horizontal cylindrical tube with a diameter of 3cm and a length of 25cm. The capacity to exit the tube backward in a specified time limit (1 minute) has been calculated as a criterion for neurotoxicity.[24]

Behavioral Parameters Analysis

During the investigation, the animals were also observed for any behavioral changes. An activity cage was used to track the locomotor activity. Rats were kept one by one in the activity cage and the overall activity count was reflected as locomotion was noted until the cutoff period. The increased count was taken as stimulant activity in the central nervous system, whereas decreased count was regarded as depressing activity.[25]

The head dip test was used to assess the animals' exploratory behavior. Individually, they were kept on the hole-board apparatus till the cut-off period. During a three-minute period, the total number of head pocking was noted.[26-28]

The rotarod is one of the most extensively used experimental tools for evaluating the test drug's muscle grip strength and muscle coordination.[29-31] The rota-rod system supplied by Inco Instruments', Ambala, India, was used to automatically record the latency to fall animals from the revolving bar, which rotates at a speed of 25 rpm. To avoid experimental bias, all animals received sufficient trials.

Statistical Analysis

Results were expressed as mean ± S.E.M. The significance of the difference between the treatment group and control was determined by one-way ANOVA followed by Tukey's Multiple Comparison Test. Graph Pad Prism 5.0 Version (Graph Pad Software, Inc., San Diego, California, USA) was used to conduct the statistical analysis. The significance level was set at $P<.05$.

RESULTS

The Measurement of Blood Glucose

Blood glucose concentrations were assessed in all experimental groups hourly for three hours before and after the seizure induction by PTZ (Table 1). The results of the blood glucose levels before seizure induction show that all of the experimental animals' blood glucose levels were normal, with no deviations observed in any group. Except in the control groups, there was an increase in glucose levels after the induction of chemo convulsions preceded by glucose ingestion. However, the results indicated that a significant reduction of blood levels (at 1hr $^{*}P<.05$, at 2h** $P<.01$) was observed in ETOR-administered animals compared with NDH + PTZ groups. Similarly, blood glucose elevations were significantly reduced by the DIAZ groups also observed.

Table 1. Effect of ETOR on blood glucose level on chemo convulsive rats

Groups	Changes in blood glucose level (mg/dL)			
	Basal Values Before seizure induction	After seizure induction		
		1 h	2 h	3 h
CONTROL	97.70 ± 3.50	96.60 ± 1.24	94.40 ± 2.06	95.89 ± 1.74
NDH + PTZ	109.70 ± 2.40	135.50 ± 2.05	127.10 ± 5.71	131.00 ± 2.80
DIAZ + PTZ	111.50 ± 2.87	121.80 ±4.04^{***,a}	109.10 ± 1.18^{*,b}	107.20 ±1.75^{*,c}
NDH +ETOR + PTZ	107.80 ± 1.93	120.12 ±3.56^{***,a}	106.50 ± 1.60^{*,b}	113.140 ± 3.70^{*,b}

The number of animals (n=6): Values are mean ± SEM: One-way ANOVA was used to analyze the data, followed by Tukey's Multiple Comparison Test.$^{*}P<.05$, $^{**}P<.01$ & $^{***}P<.001$ when compared to control groups.$^{a}P<.05$,$^{b}P<.01$ & $^{c}P<.001$ compared to NDH+PTZ groups. ns: Non-Significant. The significance level was set at $P<.05$. **NDH:** Non-Diabetic Hyperglycemia; **DIAZ:** Diazepam; **ETOR:** Etoricoxib; **PTZ:** pentylenetetrazol

PTZ Induced Chemo Convulsions

In the PTZ model, the onset of clonus convulsion (Figure 1) and its duration (Figure 2) were observed and calculated in all experimental animals. Results of the study indicate that pretreatment with ETOR (10 mg/kg p.o) showed a marked delay in the onset of CC ($^{*}P<.05$) followed by a marked reduction of the duration of CC ($^{**}P<.01$) compared with control animals. From the results, it is evident that pretreatment with ETOR showed a protective effect ($P<.05$) upon the duration of the clonic seizure form in rats, along with the delay of onset of clonic seizures.

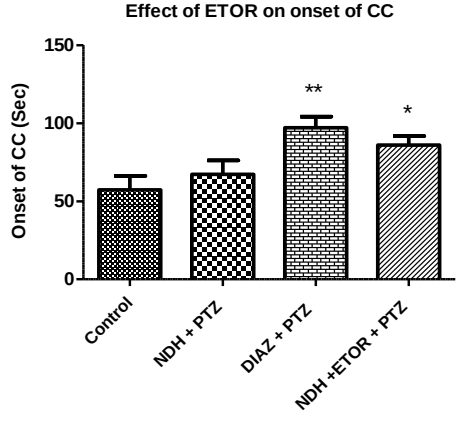

Figure 1. The effect of ETOR on the onset of CC

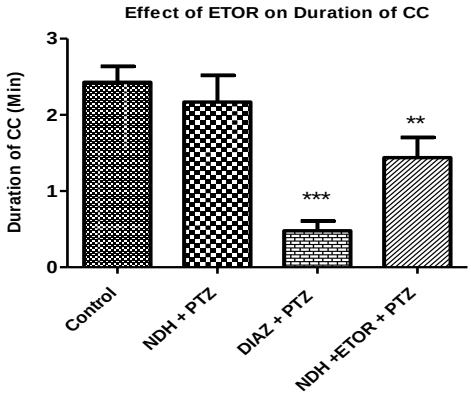

Figure 2. The effect of ETOR on duration of CC

The effect of ETOR on PTZ-induced chemo convulsions is depicted in Figures 1 and 2.According to the results, pretreatment with ETOR (10 mg/kg p.o) showed significant protection chemo convulsions caused by PTZ, which is evident by the delay of onset followed by a significant reduction of the duration of CC. Values are mean ± SEM; **P*<.05, ** *P*<.01 & *** *P*<.001 when compared to control groups; ns: Non-significant. The number of animals (n=6).

Morphometric Analysis

To demonstrate the effect of ETOR treatment, a morphometric examination of all experimental animals was performed, and the results are displayed in Table 2. Throughout the study period, the body weight of the experimental animals was monitored at weekly intervals. According to the findings, ETOR treatment resulted in significant changes in body weight, with a particularly noticeable increase in NDH groups treated with ETOR (164.30 ± 4.07, *P*<.05) on day 14 compared to control (145.70 ± 5.01) and NDH + PTZ NDH + PTZ (155.20 ± 5.47) treated groups. As a result, morphometric research revealed that ETOR has a favorable effect on body weight gain, which demonstrates the absence of harmful effects (Figure 3).

Table 2. Effect of ETOR on body weight of experimental animals

Groups	Changes in body weight(g)		
	Day 1	Day 7	Day 14
CONTROL	135.90 ± 3.16	142.40 ± 3.59	145.70 ± 5.01
NDH + PTZ	139.10 ± 3.47 ^{ns}	142.30 ± 2.73 ^{ns}	155.20 ± 5.47 ^{ns}
DIAZ + PTZ	136.70 ± 3.13 ^{ns}	141.50 ± 2.45 ^{ns}	151.50 ± 2.81 ^{ns}
NDH +ETOR + PTZ	140.43 ± 2.62 ^{ns}	145.20 ± 4.37^{ns}	164.30 ± 4.07*

NDH: Non-Diabetic Hyperglycemia; **DIAZ:** Diazepam; **ETOR:** Etoricoxib; **PTZ:** pentylenetetrazol **; Statistical comparison**: Values are mean ± SEM; **P*<.05,** *P*<.01, and *** *P*<.001 compared to the control group, NS: Non-significant. The number of animals (n=6).

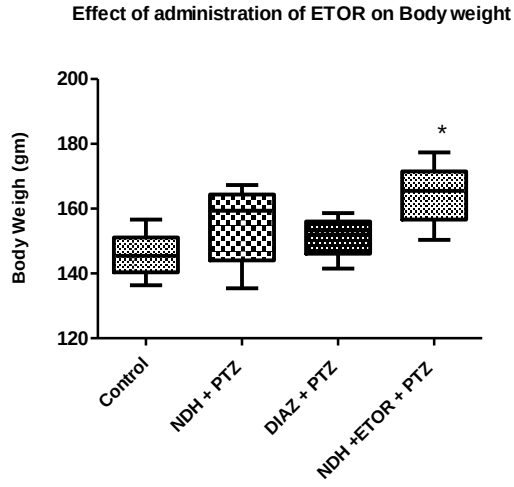

Figure 3. The effect of administration of ETOR on body weight

Figure 3. shows the effect of ETOR on experimental animals' body weight on day 14. Values are mean ± SEM; **P*<.05,** *P*<.01, and *** *P*<.001 compared to the control group, NS: Non-significant. The number of animals (n=6).

Acute Neurotoxicity Test

A conventional chimney test with rats was used to explore ETOR's neurotoxic characteristics. According to the findings, all experimental rats given ETOR at 10 mg/kg i.p. could evacuate the tube within the one-minute time limit by climbing back up as soon as they reached the other end, suggesting that ETOR has no effect on motor control and is not neurotoxic (Figure 4).

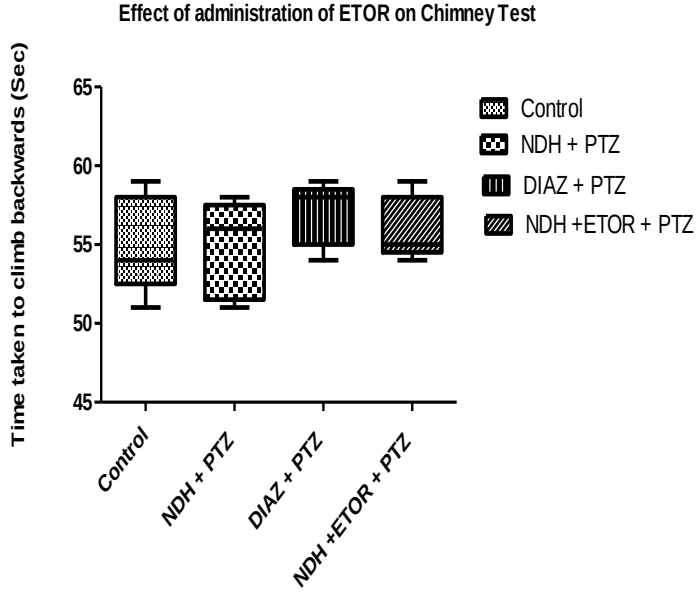

Figure 4. The effect of administration of ETOR on Chimney Test

Figure 4. shows the results of the chimney test, which demonstrated that all of the experimental animals could climb backwards out of the tube within a stipulated time (1 minute), indicating that ETOR has no effect on motor control and is not neurotoxic to the animals. Values are mean ± SEM; **P*<.05,** *P*<.01, and *** *P*<.001 compared to the control group, NS: Non-significant. The number of animals (n=6).

Behavioral Parameters Analysis

Figure 5(a) shows the effect of ETOR on the muscle grip strength of chemo convulsive rats in the rotarod test. The effects of ETOR on locomotion and the head tip test are depicted in Figures 5(b) and 5(c), respectively. In the rota rod test, animals treated with NDH+ PTZ and NDH + ETOR + PTZ showed no significant differences in muscle grip strength compared to control groups. However, diazepam-treated groups showed a steady decrease in muscular grip strength, confirming the conventional drug's relaxing characteristics. The locomotion results showed that, except the standard diazepam received ($^{*}P<.05$) group, the remaining experimental groups, such as NDH+ PTZ and NDH + ETOR + PTZ, showed no significant differences in locomotion compared to control groups. Similarly, no changes were observed in the head dip test except diazepam received groups (** $P<.01$). The results of behavioral tests revealed that all experimental animals, with the exception of diazepam-treated groups, showed no significant changes in muscular coordination, locomotion, or anxious behavior and the results were similar to those of control animals.

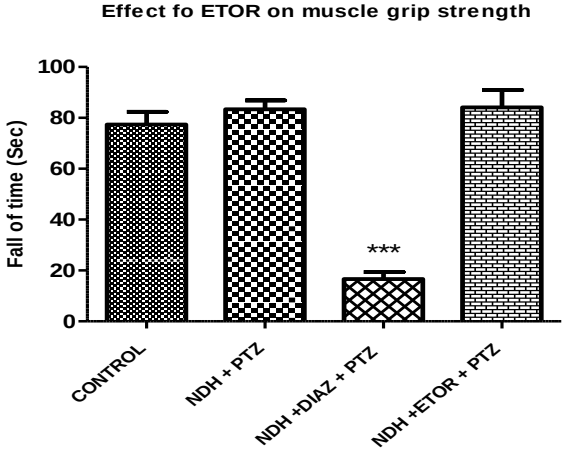

Figure 5a. The effect of ETOR on muscle grip strength

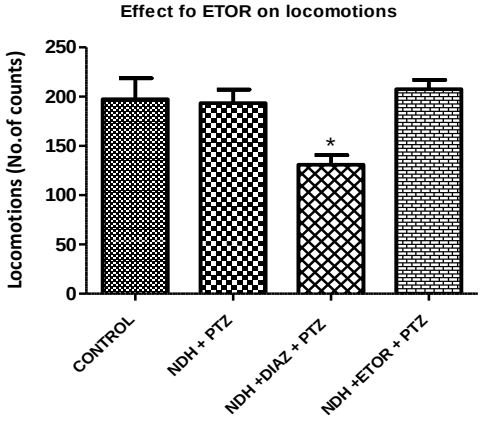

Figure 5b. The effect of ETOR on locomotion

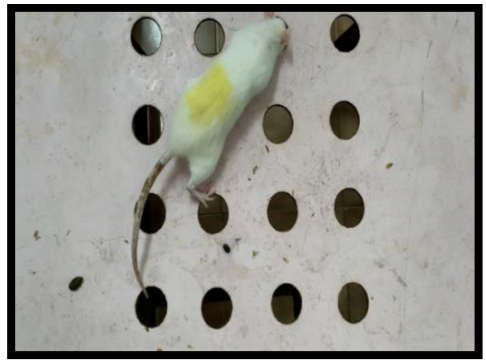

Figure 5c. Head tip test

Figure 5. Behavioral analysis: Muscle coordination, Locomotion and Head tip test. Values are mean ± SEM; *$P<.05$,** $P<.01$, and *** $P<.001$ compared to the control group, NS: Non-significant. The number of animals (n=6).

DISCUSSION

Metabolic diseases are predicted to cause widespread neurological impairments, including seizures. Experimental studies (both *in vivo* and *in vitro)* indicate that a certain level of glucose concentration is vital to enable synaptic transmission. But in rat models, elevated glucose is proconvulsant and has been linked to neuronal over activity followed by cerebral hypoxia-induced brain damage, whereas fasting-induced hypoglycemia protects against this neurotoxicity.[32,33] Hence, this investigation is designed to expound the correlation between blood glucose and seizure-provoking responses in experimental groups. The results of this study also reflect the results of earlier studies, which indicated that there was a massive rise of blood glucose in NDH groups when compared control groups. But the acute administration of ETOR showed significant reduction of blood levels at 1hr ($^{*}P<.05$), at 2h ($^{**}P<.01$) compared with NDH+ PTZ groups.

According to available reports, neuronal cell excitability owing to hyper stimulation may be affected by both hypoglycemia and hyperglycemia that leads to a short circuit of the brain that may cause seizures when left untreated.[34,35]

According to data about 25% of diabetics suffer from seizures.[36-41] In this investigation, to correlate the association between blood glucose and seizures, onset of clonus convulsion and its duration were observed and calculated in all experimental animals. From the results, it is evident that pretreatment with ETOR showed a protective effect ($P<.05$) upon the significant reduction in the duration of the clonic seizure (**$P<.01$), a delay of the onset of ($^{*}P<.05$) clonic seizures (Figure 3) along with the

significant reduction of blood glucose levels compared to control animals.

Alternatively, results from morphometric analysis show that ETOR administration caused a significant rise in body weight with a particularly notable increase were observed in NDH groups treated with ETOR (164.30 ± 4.07, $P<.05$) on day 14 compared to control (145.70 ± 5.01) and NDH + PTZ NDH + PTZ (155.20 ± 5.47) treated groups. Additionally, the results from conventional chimney test demonstrating that ETOR has no effect on motor control because all animals were all able to leave the tube backwards within a specified time limit, which indicates ETOR has no neurotoxic effects. Results of the behavioral test suggested that no significant changes were observed in muscle coordination, locomotion's and anxiety behavior of all experimental animals except diazepam-treated groups. However, a gradual decrease in muscle grip strength was observed in diazepam-treated groups, confirming the standard drug's relaxing properties. The results were comparable with control animals.

Overall, ETOR administration significantly lowered the glycemic control as well as the incidence and duration of seizures to a beneficial extent compared to control groups. As a result, the findings reveal that seizure duration is linked to blood glucose levels, implying that seizure duration is prolonged in NDH + PTZ animals. So, the findings of our study also reflect and confirm the findings of previous studies that found a link between hyperglycemia and prolonged seizure duration.[42-44]

CONCLUSION

The primary findings of this study revealed that the NDH + PTZ groups had significantly higher glycemic levels, which is reflected in the rat's early onset and longer duration of chemo-convulsions. As a result, the findings show that seizure duration is linked to blood glucose levels, implying that seizure duration is longer in the NDH + PTZ groups. Treatment with ETOR, on the other hand, significantly reduced elevated blood glucose levels as well as the onset and duration of seizures in rats, suggesting the link between blood glucose and seizure responses. In conclusion, the findings of this study suggest that ETOR has a beneficial effect on blood glucose-level reduction and seizure protection in PTZ-induced chemo convulsive rats.

Ethics Committee Approval: The experimental protocols were duly approved (SVCP/IAEC/II-10/19-20) by the IAEC-SVCP (Institutional Animal Ethics Committee of SVCP) in accordance with the recommendations of the Committee for Control and Supervision on Animal Experiments (CPCSEA), New Delhi, India. To avoid bias, randomized methods of allocation of animals were followed with respect to treatments as different experimental groups
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COVID-19 Fear and Uncertainty Intolerance in Medical Students

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ABSTRACT

Objective: To investigate the COVID-19 fear and uncertainty intolerance in first-year medical students in the first year of the pandemic.

Methods: In the academic year 2020-2021, a cross-sectional study was carried out among students in the first year of medical school. They were asked to complete an online survey which included demographic information and two five-item (1 to 5) Likert scales. The scales were the Fear of COVID-19 Scale (FCS) and the Intolerance of Uncertainty Scale-12 (IUS-12).

Results: Of the 237 students included (participation rate 81.7%), the median age was 19.0 [IQR=1.0] years and 60.3% were female (n=143). The median FCS score was 17.0 [IQR=7.0] and the mean IUS-12 score was 37.4±8.8. A significant positive correlation was found between FCS and IUS-12 scores (r=0.316, *P*<.001). Elevated FCS scores were significantly associated with female gender, never smokers, and those reporting a need for psychological support (*P*<.05). High IUS-12 scores were also significantly associated with the need for psychological support, but also with feeling lonely, home stress, and not watching online lectures (*P*<.05).

Conclusion: High levels of COVID-19 fear and uncertainty intolerance were found in first-year medical students. Students' need for psychological support tended to be significantly higher at both higher scaled.

Keywords: COVID-19, Medical student, Psychological support

INTRODUCTION

The coronavirus disease (COVID-19) that began in Wuhan, China, spread worldwide and was declared a pandemic on March 11, 2020,[1] has struck the whole world, not only in terms of health policies, lifestyles, and social distances, but also in terms of how COVID-19 affects people's psychological condition.[2-4]

The emergence of COVID-19 and its pandemic nature exacerbated global fears and, in some situations, led to stigmatization.[5,6] Fear is one of the characteristics of an infectious disease as compared to other diseases. This is directly related to the rate and nature of transmission (rapid and invisible), and to disease incidence and mortality rates.[7] Moreover, uncertainty is a powerful source of psychological and physical stress. Higher perceived levels of uncertainty were linked to higher levels of depression, anxiety, and lower quality of life.[8]

The current pandemic has led to the fear and associated anxiety of being infected with COVID-19.[9] One study found that fear of COVID-19 mediated a relationship between uncertainty intolerance and mental health.[10] In this context, an important community that is psychologically at risk are the students of the medical faculties, who are the health care workers of the future.[11]

First-year medical students who have just entered university and taken their first step on the road to becoming physicians were found to mostly adversely affected, since their lectures are given online by distance education methods, by lack of access to textbooks, no lecture hall environment, and no face-to-face communication with teachers.[12] Therefore, it is crucial to study the psychological changes that may occur in first-year medical students as a result of the pandemic.

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As psychological effects such as anxiety, fear, and stress may be observed in first-year medical students during the COVID-19 pandemic, potentially affecting their academic and later professional success, interventions should aim to detect such mood changes early to prevent the problem from becoming deep and chronic.[11,13]

In this regard, family physicians, the first health professional contact for all individuals and medical students in healthcare facilities, have a vital role to play in identifying and addressing this problem.14 Family physicians are expected to adopt a proactive approach to detect the problem with a biopsychosocial approach.15 For this purpose, screening individuals' fear and intolerance states with practical, fast and easy survey methods will enable the detection of possible problems and thus prevent future troubles. This study aims to assess COVID-19 fear and uncertainty intolerance in first-year medical students.

METHODS

Study Design

This study was designed as a cross-sectional study.

Ethical Approval

This study was carried out with the approval of the Ethics Committee of the Faculty of Medicine of the Atatürk University (approval number: 05.11.2020/¬09-14). In addition, necessary permission for the scientific research was obtained from the Turkish Ministry of Health General Directorate of Health Services (application no. T19.08.33, dated 27.10.2020). Informed consent of the participants was obtained online.

Study Period and Setting

The study was conducted at a university medical school during the 2020-2021 academic year between November 11, 2020 and November 16, 2020.

Sample Size

The study population consisted of first-year medical students at a medical school during the 2020-2021 academic year, with a total of 290 medical students who were eligible to participate in the study. The GPower 3.1 program calculated the sample size as 236 students, at 0.05 type 1 error and 90% confidence level.

Study Protocol

During the COVID-19 pandemic, it was deemed inappropriate to perform the research using face-to-face questionnaires in order to prevent the spread of infection, and due to the faculty's decision at the time of the research to continue the education through distance learning. For this reason, students were asked to complete an online survey using Google Forms. The survey was made available to be completed online over a period of five days. For informed consent, the first section of the form provided information about the study, and the first question afterward inquired about the student's willingness for participation. If the answer was "no," the questionnaire was immediately closed and the person was not included in the study.

Questionnaire

The questions in the first part of the survey examined the demographic characteristics of the participants and general effects related to COVID-19. Within the scope of the study, participants were asked to answer two different questionnaires to explore their fears of COVID-19 and their intolerance to uncertainty. The Fear of COVID-19 Scale (FCS) was used to determine fear of COVID-19 and the Intolerance of Uncertainty Scale-12 (IUS-12) was used to determine intolerance status.

The FCS was developed by Ahorsu et al. and then adapted to the Turkish language by Satıcı et al. who also confirmed the reliability and validity of the scale with a Cronbach's alpha value of 0.90.6,16 The items are scored on a five-point Likert-type scale ranging from 1 to 5. Possible scores range from 7 to 35. In the absence of a cut-off value, a high score indicates a high level of fear of a COVID-19 pandemic.

The short form of the IUS-12 was developed by Carleton et al. and adapted into Turkish by Sarıçam, and its reliability and validity were confirmed with a Cronbach alpha value of 0.85.17,18 The five-point Likert-type scale consists of 12 items, scored between 1 and 5. Possible scores range between 12 and 60. Higher scores indicate greater intolerance of uncertainty.

Statistical Analysis

The study data were analyzed by SPSS V23.0 (IBM, USA). Forms with missing data were excluded. Categorical data were expressed as frequencies and percentages, and continuous data were given as means and standard deviations if normally distributed, and medians and interquartile ranges (IQRs) if not. The Kolmogorov-Smirnov test was used for the assessment of the normal distribution of the data. Categorical data were analyzed using the Chi-square test. Student's t-test was used to analyze two independent continuous data, while one-way ANOVA test was used to analyze three or more groups

with normal distribution. For nonparametric analyses, Mann-Whitney U test was used to analyze two groups and Kruskal-Wallis test was used to analyze three or more groups. Spearman's correlation analysis was employed for correlations. The statistically significant parameters were further examined by multivariate regression analysis. For all analyses, *P*<.05 was considered significant.

RESULTS

A total of 237 first-year medical students (81.7% participation rate) who properly completed the survey were included in the study. The median age of the participants was 19.0 [IQR=1.0] years, and the majority were women (n=143, 60.3%). In terms of online lessons, only 24.1% (n=57) of students reported fully attending these lectures, while 18.1% (n=43) thought that studying at home was better. The sociodemographic and psychological characteristics and daily life behaviors of the participants are summarized in Table 1. One hundred thirty-three participants (56.1%) reported feeling lonely due to staying at home and social distancing during the COVID-19 pandemic, 135 (57%) stated that staying at home was more stressful, and 63 (26.6%) reported needing psychological support.

The participants' median FCS score was 17 [IQR=7] and the mean IUS-12 score was 37.4±8.8. FCS and IUS-12 questionnaire scores had a statistically significant positive correlation (r=0.316, *P*<.001).

The FCS and IUS-12 scores of the participants according to sociodemographic characteristics and psychological states are summarized in Table 2. Accordingly, participants who were female, never smoked, and needed psychological support had higher FCS scores, while participants who felt lonely, found it stressful to stay at home, needed psychological support, and did not watch online classes had higher IUS-12 scores. The only situation in which the score was statistically high in both scales was the need for psychological support.

Multivariable linear regression analysis was performed to predict the intolerance of uncertainty variable using the variables of fear of COVID-19, need for psychological support, and feeling alone. As a result of the analysis, a significant regression model of the variance in the dependent variables was found to be explained by the independent variables (Table 3).

Table 1. Sociodemographic and psychological characteristics and daily life behaviors of the participants

	N (%) *
Age (years) *median IQR*	19.0 [1.0]
Sex	
Female	143 (60.3)
Male	94 (39.7)
Chronic disease	
No	229 (96.6)
Yes	8 (3.4)
Smoking status	
Never smoked	192 (81.0)
Quit	21 (8.9)
Current smoker	24 (10.1)
Psychological support	
Not required	174 (73.4)
Required	63 (26.6)
Feeling alone	
No	104 (43.9)
Yes	133 (56.1)
Increased food intake	
No	68 (28.7)
Sometimes	64 (27.0)
Yes	105 (44.3)
Vacation plans for this year?	
No, I will not go until I am vaccinated	23 (9.7)
No, I will not go until the pandemic is over	109 (46.0)
No, I in any case had no intention of going	37 (15.6)
Yes, I have been	68 (28.7)
Perception of remaining at home	
It is less stressful	22 (9.3)
It is more stressful	135 (57.0)
Not changed	80 (33.8)
Able to follow classes online	
Yes	57 (24.1)
No	54 (22.8)
I follow them later	32 (13.5)
Not much	94 (39.7)
Contact with COVID-19	
Diagnosed	11 (4.6)
I came into contact and went into quarantine	40 (16.9)
Relatives were diagnosed	131 (55.3)
I did not come into contact and no relatives were diagnosed	55 (23.2)

* Total percentage may be more than 100% due to rounding up of decimal digits.

Table 2. FCS and IUS-12 scores of the participants according to sociodemographic characteristics and psychological states

	FCS SCORE (MEDIAN [IQR])	***P***	**IUS-12 SCORE** (MEAN ± STD. DEVIATION)	***P***
Age (Year)	19.0 [1.0]	.053*	19.0 ± 1.1	.568*
Sex		**<.001**†		.871†
Female	18.0 [6.0]		37.4 ± 8.3	
Male	14.0 [7.0]		37.3 ± 9.6	
Chronic Disease		.116†		.513†
No	17.0 [7.0]		37.4 ± 8.9	
Yes	13.0 [5.0]		35.9 ± 6.1	
Smoking		**.009**‡		.690‡
Never Smoked	17.0 [7.0]		37.1 ± 8.6	
Quit	16.0 [7.0]		39.2 ± 9.3	
Still Smoking	14.0 [6.0]		37.6 ± 10.6	
Psychological Support Requirement		**<.001**†		**<.001**†
No	16.0 [6.0]		35.9 ± 8.2	
Yes	19.0 [8.0]		41.1 ± 9.3	
Feeling Alone		.252†		**.003**†
No	16.0 [8.0]		35.2 ± 9.0	
Yes	17.0 [6.0]		39.1 ± 8.3	
Increased Eating		.739‡		.063‡
No	16.0 [8.0]		37.1 ± 8.7	
Sometimes	17.0 [5.0]		35.6 ± 8.3	
Yes	16.0 [6.0]		38.7 ± 9.1	
Vacation Plans for This Year		.556‡		.183‡
No, I will not go until a vaccine is found	19.0 [5.0]		34.6 ± 10.9	
No, I will not go until the pandemic is over	17.0 [6.0]		38.0 ± 8.6	
No, I in any case did not wish to go	15.0 [9.0]		34.6 ± 10.9	
Yes, I have been	17.0 [8.0]		38.4 ± 8.3	
Relationships When Staying at Home		.437‡		**.010**‡
Less Stressful	17.0 [7.0]		34.1 ± 9.1	
More Stressful	17.0 [7.0]		38.9 ± 8.9	
Not Changed	16.0 [5.0]		35.7 ± 8.0	
Able to Watch Classes Online		.057‡		**.022**‡
Yes	18.0 [7.0]		35.8 ± 8.6	
No	17.0 [6.0]		40.4 ± 9.7	
I Watch Them Later	15.0 [6.0]		38.3 ± 8.4	
Not Much	17.0 [6.0]		36.3 ± 8.2	
COVID-19 Contact		.671‡		.991‡
I have been diagnosed	16.0 [5.0]		37.7 ± 5.9	
I have been in contact and I was quarantined relatives have been diagnosed	16.5 [8.0]		37.0 ± 10.0	
	17.0 [5.0]		37.3 ± 9.0	
I have had no contact with COVID, and no relatives have been diagnosed with it	16.0 [7.0]		37.8 ± 8.2	

(Note: Bold *P* values indicate Significance below .05)
FCS: Fear of COVID-19 scale, IUS-12: Intolerance of uncertainty scale-12, IQR: Interquartile range.
Data is presented as median [interquartile range] and mean□standard deviation.
* Spearman's correlation test
† Mann–Whitney U test
‡ Kruskal Wallis test.

Table 3. The multiple linear regression model of intolerance of uncertainty scale

Variables	*Unstandardized Coefficients*		*Standardized Coefficients*		
	B	**Std.Error**	**Beta**	**t**	**Sig.**
Feeling alone	1.65	0.63	0.61	2.60	0.010
Psychological support requirement	3.21	1.28	0.65	2.50	0.013
Fear of COVID-19	0.46	0.18	0.26	4.25	0.000

R^2=0.162. *adjusted* R^2=0.151, *S E*=8.12, *F*=14.96, *P*<.001
Dependent variable = Intolerance of Uncertainty Scale
Independent variables = feeling alone, psychological support requirement, fear of COVID-19

DISCUSSION

People are afraid of catching COVID-19. The situation is still unclear, and people are still losing money because of the virus. Increased fear of Corona Virus was found to correlate positively with intolerance of uncertainty. Consistent with our study, Bakioğlu and Ercan observed positive correlation between fear of COVID-19 and intolerance of uncertainty.[20]

Mean FCS scores were higher among female students compared to male students. Women tend to adapt better to environmental stress, but may be prone to develop disease.19 More frequent illness may have contributed to fear of COVID-19 being at higher levels in women than in men. This finding is compatible with previous studies reporting higher fear levels in female students.[20, 21]

Women adapt better to stress, but may get sick more often. This may be why women are more afraid of COVID-19 than men. This finding is similar to previous studies that found women are more afraid than men. Some studies say women are more tolerant of uncertainty than men, while others say men are more tolerant. It is worth noting that both survey scores are positively correlated, and regression analysis revealed this association between them, such that fear of COVID-19 causes intolerance of uncertainty. In this study, the fear of COVID-19 disease and intolerance of uncertainty of first-year medical faculty students were examined by using the FCS and IUS-12 questionnaires. It is worth noting that both survey scores are positively correlated, and regression analysis revealed this association between them, such that fear of COVID-19 causes intolerance of uncertainty.

Some conditions capable of being caused by the disease may be regarded as negativities experienced by all, independently of sex. This may have led to no significant difference in levels of intolerance of uncertainty being observed between male and female students. Similar results have also been reported in previous studies.[22,23]

Education in Saudi Arabia also began being provided in the form of distance education as a precaution against the spread of COVID-19, and similar findings were observed to those of the present study.[24] Epidemics can lead to significant psychological difficulties, uncertainty, and fear of uncertainty, and this can result in undesirable effects.

High levels of fear and intolerance of uncertainty were determined among participants with family members who had been exposed to COVID-19. This may probably have derived from these students being anxious about their families' health, the infection being transmitted to other family members, an uncertainty about the course of the disease. Similar findings were obtained in a study of students in Saudi Arabia.[24]

People are afraid of catching the virus.[25] This is similar to other studies that found women are more afraid. Some studies have found different results. Parlapani says men are less uncertain than women.[26]

In conclusion, First-year medical faculty students in this study exhibited high levels of fear of COVID-19 and intolerance of uncertainty. The psychological problems of first-year medical students need to be investigated, and appropriate psychological counseling, support, and guidance needs to be provided.

Limitations: Our study has some limitations. Given that the survey was conducted as the pandemic began, results may change over time. In addition, pre- and post-pandemic comparisons were not possible due to the unknown scores of the participants before the pandemic. Thirdly, the study was conducted only among first-year medical students, and it is unknown whether COVID-19 fear and intolerance of uncertainty differs among medical students in other grades or other faculties. Furthermore, this was a single-center study, so the results of this study cannot be generalized.

Ethics Committee Approval: Ethics committee approval was obtained from Ethics Committee of the Faculty of Medicine of the Atatürk University (Date: 05.11.2020, Number: 09-14)
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ATATÜRK ÜNİVERSİTESİ YAYINLARI
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Determination of Hyaluronic Acid in Ophthalmic Solution by HPLC–UV and Stability Analysis

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ABSTRACT

Objective: Hyaluronic acid (HA) is a naturally occurring, biocompatible polysaccharide with unique viscoelastic and hygroscopic properties. Its role as a natural lubricant and its excellent water-retaining properties make it well-suited for use in ophthalmic products. The objective of this study is determination of sodium hyaluronate in ophthalmic solution liquid chromatography (HPLC) method with UV detection and, stability of the product.

Methods: The analysis was carried out using a polymer column (PolySep-GFC- P5000) with a mobile phase consisting of (dH2O: 20 mM Phosphate Buffer pH:6.5), (95:5, v:v) at a flow rate of 1.2 mL/min. UV detection was set 205 nm.

Results: Calibration curve was linear over the concentration range of 80%-120% concentration of the ophthalmic product. The back calculated concentrations of the calibration standards were within ±15% of the nominal value (±20% for LOQ). The stability of ophtalmic solution was found to be 92.5% at the end of the days at room tempereture.

Conclusion: The developed method is fast and reliable for HA analysis in ophthalmic products. Accordingt to stability study, these products must be stored at +4 C during usage and storage.

Keywords: Hyaluronic acid, ophthalmic product, HPLC

INTRODUCTION

Hyaluronic acid, also called hyaluronan or hyaluronate (HA), is an important kind of glycosaminoglycans due to its various physiological functions. This polysaccharide consists of a repeating of glucuronic acid and disaccharide bound by glycosidic bonds (Figure 1). HA is abundant in all organs and especially in connective tissue such as umbilicalcord, synovial fluid, skin and the vitreous body. HA sources can obtain from animal sources such as rooster combs, bovine's eyes, and microbial production.

The molecular weight of HA is dependents on its source, for example human synovial HA averages about 7 million Da per molecule.[1] It has high hygroscopicity, viscoelastic nature and good biocompatibility. It does not produce toxic products when broken. HA is used in cosmetics, surgery, drug delivery system, rheumatology, otolaryngology and urology. Especially Its role as a natural lubricant and its excellent water-retaining properties make it well-suited for use in ophthalmic products.[2,3]

Size-exclusion chromatography (SEC) is a kind of chromatographic method which substances are separated by their size or molecular weight; The advantages of this method are good separation of large molecules like quaternary structure of purified proteins, peptids, polysaccharides, biotechnological drugs by preserving the biological activity of the particles. Breakdown of polymer is not necessary part of method development. While sample preparation steps decreases, sensitivity and simplicity of method increases.[4]

There are several methos reported on sodium hyaluronate determination in literature such as HPLC UV-Vis[1-7], electrophoresis[8] ,LC-MS[9-13], turbidimetric[14-16], chemiluminescence[20]. Most of studies are based on analysing disaccarides or monomers of HA after enzymatic digestion, acid-based degradation or derivatization of HA.

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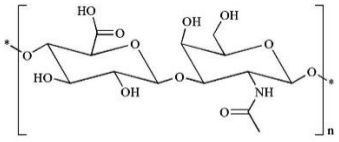

HA

Figure 1. Structures of hyaluronic acid

K. Ruckmani et al. developed a SEC chromatography method to analyse HA and applied in house pharmaceutical preparations.

High performance liquid chromatography (HPLC), is a widely used analysis technique to separate the components in mixtures and determine their quality and quantity. In this technique, analytes carried by the mobile phase pumped by pumps reach the chromatographic column. They interact with the column in different ways and reach the detector at different times. HPLC-UV absorption detection technique was used with a polymer column (PolySep-GFC- P5000) in this study. The developed method was used for determination of the HA, and evaluate stability of HA in a commercial ophthalmic formulation.

METHODS

Apparatus and Reagents

The HPLC system of Agilent Infinity 1260 Series with UV detector were used for analysis HA. The HPLC system consisted of 1260 Quat pump, 1260 ALS, 1290 Thermostat, 1260 TCC, 1260 DAD. Chemstation was used as instrument software.

Hyaluronic acid was obtained from Bloomage Freda Biopharm Co.,Ltd. Potassium dihydrogen phosphate and potassium hydroxide were purchased from Merck. Brand of opthalmic product is Artelac Splash® (0.24% (w/v)Hyaluronic acid) from Bausch + Lomb Health and Optic Products TIC. Inc.

Chromatographic Conditions

PolySep-GFC- P5000 (300×7.8mm) was chosed as column and (dH2O: 20 mM Phosphate Buffer pH:6.5), (95:5, v:v) was used as the mobile phase with a flow rate of 1.2 mL/min. The detector was set at 205 nm and the injection volume was 100 µL.

Preparation of Solutions

3% HA stock standard solution was prepared by weighing 3 g sodium hyaluronate into 100 mL of ultra pure water. The calibration standard solutions were prepared by dilution of stock solution with dH_2O. Calibration curves were constructed at five concentration levels ($1.920x10^{-1}$-$2.880x10^{-1}$ %, w/v **(**weight/volume percentage concentration)). Eye drop wasn't diluted. 20 mM potassium dihydrogen phosphate was prepared by weighing 1.3608 g into 500 mL of ultra pure water and pH are adjusted to 6.5 by 1M KOH solution. Opthalmic solution was analyzed directly without dilution or extraction procedure.

RESULTS

Chromatographic separation of compound and interference came from sample was developed succesfully. Any interference at retention times of analyte wasn't seen (Figure 2). It means that method is selective for HA in ophthalmic product.

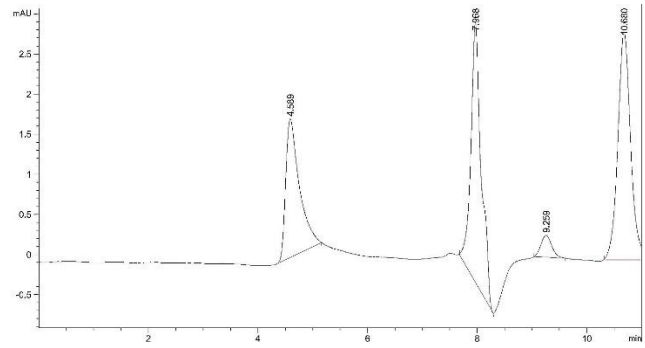

Figure 2. Chromatograms of ophthalmic solution, HA Rt: 4.589 min

System suitability parameters, tailing factor, theoritical plates, injection precision and capacity factor determined and summarized in Table 1. Values were found within acceptable limits .[23]

Table 1. System suitability parameters of hyaluronic acid

Parameter	Value (HA)	Limit (FDA guidline)
Tailing	0.735	$T \leq 2$
Theoretical Plates	5393	$N > 2000$
Injection Precision (RSD)	0.03	RSD% < 1% , $n \geq 5$
Resolution (R)	9.30	$R > 2$
Capacity Factor	4.62	$k' > 2$

HA: Hyaluronic acid, T: Tailing, N: Theoretical Plates, RSD: Relative Standart Deviation, R: Resolution, k': Capacity Factor

"The limit of detection (LOD) and lower limit of quantification (LOQ) were determined to evaluate the sensitivity of the analytical method. The calculated LOD and LOQ level based on signal to noise (S/N) ratio of 3:1 and 10:1, respectively. LOD and LOQ were found to be 0.012% and 0.039% (w/v), respectively. The method LOQ level is 0.192 % (w/v), the first point of calibration curve.

Calibration curve was plotted area of the peak versus HA concentration (1.920x10-1; 2.160x10-1; 2.400x10-1 ;2.640x10-1 ; 2.880x10-1 %, w/v). The linearity of the method was evaluated by correlation coefficient and equation of the standard curve.

They were found (r^2=0.9959) and (y =-127.29577.x-0.678689) respectively. Standart solutions were analyzed 2 times with an interwal 3 days. Accuracy of calibration curve was expressed as mean(%,w/v); precision was expressed as relative standard deviation (RSD %).The back calculated concentrations of the calibration standards were within ±15% of the nominal value (±20% for LOQ) (Table 2). The working range used to determine HA quantification was found acceptable accurate, precise, and linear (Figure 3).

Table 2. Accuracy and precision results of the calibration standard

Conc.(%,w/v)	Mean(%,w/v)	RSD%	CV%	n
$1.920x10^{-1}$	$1.852x10^{-1}$	3.550	1.079	6
$2.160x10^{-1}$	$2.051x10^{-1}$	5.051	1.219	6
$2.400x10^{-1}$	$2.388x10^{-1}$	0.483	2.070	6
$2.640x10^{-1}$	$2.738x10^{-1}$	3.698	0.724	6
$2.880x10^{-1}$	$3.014x10^{-1}$	4.638	1.396	6

Conc.: Concentration, w/v: weight/volume, RSD: Relative Standart Deviation, CV: Coefficient of Variation, n: number of experiment

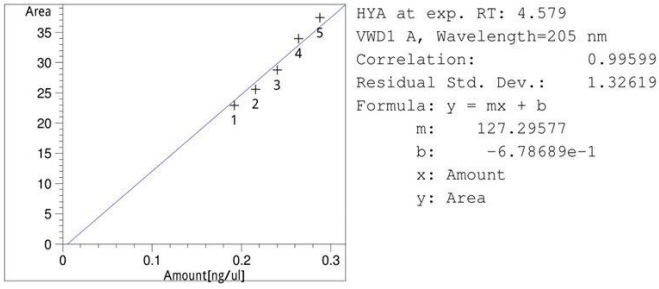

Figure 3. Calibration graph of HA

Stability of samples was performed. Samples containing HA at $2.400x10^{-1}$ % concentration in four replicate were kept at ambient temperature and, analyzed on days 1, 5 and 7. Recovery results of the samples are shown in Table 3.

Table 3. Recovery results for one week

Day	Conc.(%,w/v)	Mean(%,w/v)	RSD%	Accuracy	n
1.		0,239	0.417	99.58	4
5.	$2.400x10^{-1}$	0,227	5.417	94.58	4
7.		0,222	7.500	92.50	4

Conc.: Concentration, w/v: weight/volume, RSD: Relative Standart Deviation, n: number of experiment

DISCUSSION

Hyaluronic acid, due to its various physiological functions like high hygroscopicity, natural lubricant and good biocompatibility, is good candidate for ophthalmic products. Thus, routine analysis of HA in ophthalmic product is essential. HPLC- UV system was used for separation and detection. Polymeric column was chosen because of polymeric structure of HA .

This choice provides simplicity, repeatability and a fast analysis without sample preparation like derivative or digestion. In addition to this, while 100% aqua mobile phase system can't be used in C18 column because of phase collapse, it could be used in SEC coloumn to seperate and analyze HA which is stable in aqua phase at neutral pH. Literature survey shows us that pH effect the rate of the hydrolytic degradation of HA in aqueous solution extremely. HA is most stable at around neutral pH values than acidic or basic conditions.[21,22] Considering pH effect to HA stability, mobile phase composition was chosen at neutral pH.

Calibration range was suitable for quantitative of HA in commerical opthalmic product and, any sample preparation process also including dilution wasn't applied to product sample. Besides it wasn't used internal standard because of simple sample preparation procedure and without analyte loss. Weight/volume percentage concentration (%)" (w/v) was used as concentration unit because quantity of HA is defined on commercial product as % HA. The developed method was fast and reliable for HA analysis in ophthalmic products. The stability of ophtalmic solution was found to be %92.5 at the end of the days at room temperature. So, the ophtalmic products which is contain HA must be stored at +4 °C during usage and storage.

CONCLUSION

In literature, various degradation and derivatization process have been applied to determine hyaluronic acid content, but a few studies were about analysis of hyaluronic acid without these process. In this study, size exclusion chromatography technique were studied to quantify hyaluronic acid in ophthalmic product. The proposed method was simple, fast and reliable.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept -DÖÜ; Design- DÖÜ; Supervision-DÖÜ; Resources-DÖÜ; Data Collection and/or Processing-MA; Analysis and/or Interpretation-MA; Literature Search-MA; Writing Manuscript-MA; Critical Review-DÖÜ
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ATATÜRK ÜNİVERSİTESİ YAYINLARI
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Research Article DOI: 10.5281/zenodo.12626403

Antioxidant Activities of *Glycyrrhiza glabra L.* and *Momordica charantia L.* Collected From *Kahramanmaraş, Türkiye*

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ABSTRACT

Objective: Türkiye is one of the countries that are rich in plant biodiversity. This geographical structure and climatic conditions of Kahramanmaraş allow various plant species to coexist. The usefulness of bioactive molecules as a source of novel antioxidant chemicals may be revealed by studies aimed at determining the antioxidant activity of plant species. The antioxidant capacity of the crude products from two Kahramanmaraş plants, licorice (*Glycyrrhiza glabra*) and bitter melon (*Momordica charantia L.*), was examined.

Methods: This study systematically investigates, for the first time, various combinations of temperature, stirring rpm and time, and solvent to optimally extract the bioactive properties from these plants. Using ferric ion antioxidant potential (FRAP) reduction and DPPH (1,1-diphenyl-2-picrylhydrazyl free radical) scavenging tests, the antioxidant capabilities of plant extracts were investigated. Additionally, measurements were made of their total polyphenol levels (TPC), copper (II) ion reducing antioxidant capacity (CUPRAC), and iron (II) chelation activity (FIC).

Results: The results of the FRAP assay and the DPPH assay showed a good connection, suggesting that the extracts included chemicals that could reduce ferric ions and scavenge free radicals. A strong association between TPC and other findings indicated that the extracts' polyphenols contributed to some of the antioxidant activity.

Conclusion: The investigation indicates that consuming these plants would have a lot of advantageous effects due to the antioxidant properties they possess.

Keywords: Antioxidants, *Glycyrrhiza glabra L. Momordica charantia L.*, plant extracts

INTRODUCTION

Free radicals are atoms or molecules with one or more unpaired electrons in its outermost shell and are highly reactive. Radicals can be negatively, positively, or neutrally charged in terms of electrical charge. These reactive chemicals become stable by capturing electrons from other molecules around them. Thus, they convert the molecule from which they have taken its electron into a free radical, making it unstable or reactive. This situation leads to undesirable reactions in the human body. According to a study, it has been stated that a human cell is exposed to radical attacks approximately 10^5 times a day.[1,2] Also, studies have shown that free radicals can cause DNA damage, cell death, protein and lipid modifications, aging, cardiovascular diseases and even cancer stress.[3-6]

The human body employs its defense mechanism, the antioxidant defense system, to cope with the unwanted and damaging effects of such free radicals. Antioxidants present in the body neutralize free radicals. Factors such as improper and unhealthy diet, radiation, air pollution, infections and alcohol consumption contribute to the increase in free radicals solvents.[7]

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If an excess of free radicals is formed and the body's mechanism to deal with these radicals is disrupted, a phenomenon which is oxidative stress emerges. As a solution to this problem, consuming fruits and vegetables which are rich in polyphenols and flavonoids contributes positively to this defense mechanism, thereby inhibiting this effect.[8] The effect of antioxidants on free radicals stems from their ability to readily donate an H^+ atom and thereby convert radicals into low-density, less reactive forms, reducing oxidative stress in this manner.[9]

Glycyrrhiza glabra L., commonly known as licorice, is a flowering plant in the Fabaceae family that can reach up to one and a half meters in height. Due to the sweet aroma of its root, it is used to flavor food, beverages, toothpaste and tobacco products. Originally from the Mediterranean, Asia Minor to Iran, central to southern Russia, licorice species are perennial herbs that are now widely grown in Europe, the Middle East, and Asia. With the development of modern pharmacology, many important pharmacological activities of licorice root have been discovered, and with the help of these developments, natural compounds such as triterpenoids and flavonoids have been isolated from licorice root.[10]

Momordica charantia L. also known as bitter melon or bitter gourd is a tropical vine belonging to the gourd family and is grown as a one-year plant in warm climate conditions, particularly during the summer months. The origin of this plant is in China, although it is also cultivated in Africa and Asia[11] The fruit of this plant contains beneficial chemicals such as polypeptide-p, amino acids like threonine and alanine, and additionally, compounds like picolinic acid and luteolin, which make it highly nutritious. [12] It has been explained by scientists that the seeds contain rich antioxidant compounds such as phenolic acid, gallic acid, catechin, and chlorogenic acid.[13] Turkey holds an important position in terms of genetic diversity in the Cucurbitaceae family[14] Both of these plants are consumed in Turkey especially, Kahramanmaraş.

The active ingredients isolated from plants have been shown in numerous studies in recent years to have antitumor, antimicrobial, antiviral, anti-inflammatory, immunoregulatory, and several other properties that support the preservation and repair of the neurological, gastrointestinal, respiratory, endocrine, and cardiovascular systems.[15–18] In Kahramanmaraş, consumption of licorice is quite widespread, often consumed in the form of licorice sherbet. This sherbet is a refreshing beverage particularly favored during the summer months and the Ramadan period.

Licorice sherbet is frequently consumed due to its health benefits such as being beneficial for the kidneys, stomach, and strengthening the immune system. Bitter melon, on the other hand, is a medicinal plant consumed in various forms in Kahramanmaraş and generally throughout Turkey. Prevalent consumption methods include eating the fruit, consuming it as a paste, or mixing it with olive oil.

Antioxidant activity refers to the reaction occurring between a single antioxidant substance and free radicals. Antioxidant capacity signifies the reaction of a mixture of compounds possessing antioxidant properties with free radicals. Direct or indirect measurements can be used to determine a compound's antioxidant capability.[19] Antioxidant activity analyses, which measure the oxidative stress of antioxidants, are generally divided into two reaction mechanisms. First method based on hydrogen atom transfer (HAT) and the second one is based on singlet electron transfer (SET). In the first method, antioxidants interact with free radicals capturing the reactive hydrogen atoms they possess; it is monitored by color change. In the second method, antioxidants stabilize radicals by donating electrons to them. It can also be defined as a method based on the reduction of structures containing metals or carbonyl groups.[20]

In the first step of this article, extraction of dried bitter melon, and licorice plants will be compared using different solvent systems. To enhance the interaction between the bioactive compounds in the plant and the solvent, a magnetic stirrer was used at room temperature for specific minutes. More effective and efficient extraction of these components were facilitated in this way. After the filtration and drying process, the plant extracts were subjected to different instrumental analysis for the determination of their compositions and stored in the refrigerator at +4 °C to for further studies. After determining the extract yield, incubation time, and solvent polarity conditions with the highest efficiency, total antioxidant capacity determinations of these compositions were conducted by 5 different methods in the continuation of this study.

METHODS

Materials and Preparation of Extracts

An experiment was initiated to compare the extraction efficiency of dried bitter melon and licorice roots obtained from Kahramanmaraş, and to determine the most effective extraction conditions using different solvents of varying polarity and mixing times. First, 0.1 grams of the plant material was taken and 10 mL of pure water was added.

The mixture was then stirred with a magnetic stirrer for 5 minutes. Afterward, the resulting solution was filtered through ordinary filter paper. Subsequently, 2 mL of the filtrate was drawn using a micropipette and transferred to a pre-weighed watch glass. This process was repeated three times. The watch glasses were placed in an oven set to 120 °C for 2 hours. Each watch glass was weighed again, and the values were recorded. The same procedures were conducted for 0.1 grams of both power bitter melon and licorice roots, with 5, 10, 20, 30, 40, and 60 minutes of mixing times. During these processes, shown in Figure 1 different solvent systems such as pure water, ethanol, n-hexane, and boiling pure water were used.

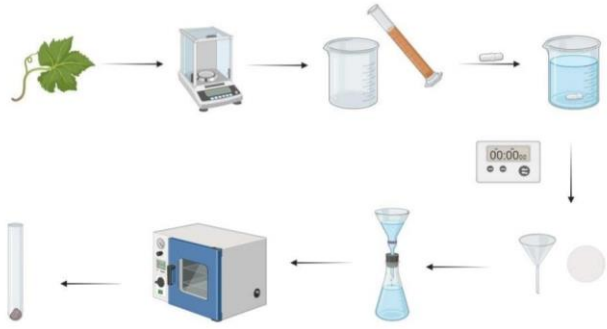

Figure 1: Plant extraction stages

Antioxidant Activity Methods

DPPH Radical Scavenging Activity Assay

DPPH, one of the most commonly used methods for scavenging free radicals, is a chemical compound with the chemical name 2,2-diphenyl-1-picrylhydrazyl. This structure, which is a nitrogen radical reacts with substances with high antioxidant content, causing the purple color of DPPH to disappear, and this process is examined spectrophotometrically at 515 nm.[21]

Firstly, a 10^{-4} M DPPH solution was prepared. Then, a 2000 µg/µL plant stock solution was prepared. From this prepared stock solution, 100, 200, and 1000 µL were taken and diluted with water to a total volume of 1000 µL. In contrast, a control group consisting of 100 µg/µL ascorbic acid stock solution was used to prepare ascorbic acid solutions at five different concentrations by taking 50, 100, 250, 500, and 1000 µL.1 mL of each prepared solution was transferred to Falcon tubes, 2 mL of DPPH was added to it. After vortexing each tube for 30 seconds, they were kept in a dark place for 30 minutes. 1 mL of solvent was used as a blank solution. Absorbance values were read at 517 nm using a UV-Vis spectrophotometer, and the values were recorded. These steps were repeated three times. Then, the percentage of free radical scavenging activity was determined using the formula:

$$\% \text{ Free Radical Scavenging Activity} = (A_c - A_s) \times 100/A_c$$

Where A_c is the absorbance of the blank and A_s is the absorbance of the sample. The antioxidant activity of the plant extract was expressed as IC_{50}, defined as the extract concentration (in µg/mL) required to inhibit the formation of DPPH radicals by 50%. The results were compared with ascorbic acid.

Total Phenolic Content (TPC) Determination Method

This method is based on the measurement of the absorbance of the colored product observed at 745 nm, resulting from the oxidation of phenolic compounds in the antioxidant extract with the Folin-Ciocalteu (FC) reagent. The obtained results are evaluated based on a reference standard of gallic acid to measure antioxidant activity [22].

Plant extracts with a concentration of 1 mg/mL were mixed with 5 mL of Folin reagent in a 1:10 ratio. A 7.5% (w/v) Na_2CO_3 solution was prepared with water. A stock solution of 1000 µg/mL gallic acid was prepared and diluted to concentrations of 20, 40, 60, 80, and 100 µg/mL. After preparing all solutions, 0.4 mL of plant extract and other standard solutions were transferred to Falcon tubes. Then, 2 mL of Folin reagent was added to it and vortexed. After 5 minutes, 1.6 mL of 7.5% Na_2CO_3 solution was added to the mixtures and vortexed again. Falcon tubes were left at room temperature for 1 hour, and absorbances were read at 765 nm against the blank containing 0.4 mL of water.

Iron Reducing Power Assay (FRAP) Method

This antioxidant determination method relies on the reduction of Fe (III) to Fe (II) chelate by the reaction of the Fe(III) tripyridyl triazine (TPTZ) complex with substances rich in antioxidants. This reduction reaction produces a dark blue color, and the measurement is determined spectrophotometrically at 595 nm.[23]

Firstly, 40 mM HCl aqueous solution is prepared. Then, a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution is prepared using a 40 mM HCl. Then, 20 mM $FeCl_3.6H_2O$ solution is prepared. Subsequently, a 0.3 M acetate buffer aqueous solution is prepared. After preparing all solutions, 30 mL of 0.3 M pH 3.6 acetate buffer, 3 mL of 20 mM $FeCl_3.6H_2O$, and 3 mL of 10 mM TPTZ solutions (in a ratio of 10:1:1) are mixed and incubated at 37°C for 10 minutes.1 mM $FeSO_4.7H_2O$ solution is prepared using ethanol as the solvent. The calibration curve is prepared by diluting this stock solution to concentrations of 100, 200, 300, 400, and 500 µM. Additionally, a 1000 µg/mL ascorbic acid solution is prepared. The plant extract solution is also diluted to a concentration of 1 mg/mL.

After preparing all solutions, 200 µL is taken from each and mixed with 1.8 mL of the prepared FRAP solution, followed by vortexing. The reaction mixtures are then incubated at 37°C in a water bath for 30 minutes. After reaching room temperature, absorbances are read at 593 nm against a blank solution. The difference between the sample absorbance and the blank absorbance is calculated to determine the FRAP value. The results are compared against the standard. All measurements are repeated three times.

Iron (II) Chelation Activity (FIC) Method

Ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine p,p'-disulfonic acid) is a compound that can form complexes with free ferrous ions (Fe(II)) and contains a chromophore that causes strong absorption. Antioxidant components capable of chelating Fe(II) reduce the concentration of free Fe(II) in the solution, thereby decreasing the concentration of the Ferrozine-Fe(II) complex, leading to absorption loss at 562 nm. Antioxidants that chelate with Fe(II) reduce the formation of the Ferrozine-Fe(II) chelate, resulting in less color formation.

Antioxidant activity is measured based on this process.[24] Different concentrations of plant extracts, ranging from 1 to 10 mg/mL, were prepared from the 1000 µg/mL plant extract solution. Then, 0.25 mM $FeSO_4.7H_2O$ solution was prepared using water.

After preparing all solutions, 0.2 mL of sample solutions at various dilutions and a solution were transferred to falcon tubes. Then, 0.2 mL of 0.1 mM $FeSO_4.7H_2O$ and 0.4 mL of 0.25 mM ferrozine solution were added to each tube. After vortexing the solutions, they were incubated in the dark place at room temperature for 10 minutes, and absorbances were measured at 562 nm. Na_2EDTA was used as a reference. The process was repeated three times. The chelation effect of a sample on ferrous ions was calculated as follows:

$$\text{Chelation effect (\%)} = (A_{Control} - A_{Sample}) \times 100 / A_{Control}$$

Copper(II) Ion Reducing Antioxidant Capacity (CUPRAC) Determination

This method bears similarities to the FRAP method mentioned in 2.2.3. The only difference is that it is based on the reduction of the Cu(II)-neocuproine (Nc) complex to Cu(I) chelate by the antioxidant substance instead of Fe metals.[25] Plant stock solutions with a concentration of 5 mg/mL were diluted to a final concentration of 1 mg/mL. Then, an ascorbic acid solution with a concentration of 1 mM was prepared using water.

From the stock solution, the required amounts were taken to prepare solutions with concentrations of 100, 200, 400, 600, 800, and 1000 µM, and the total volume was adjusted to 1 mL with water. 10 mM $CuCl_2$ solution was prepared. Ammonium acetate (NH_4Ac) was used as a buffer solution to maintain a pH of 7.

Finally, 7.5 mM Neocuproine solution was prepared using ethanol as the solvent. After preparing all solutions, a reaction mixture was prepared in a separate beaker by mixing 20 mL of $CuCl_2$, Neocuproine, and NH_4Ac in a 1:1:1 ratio. To prepare the blank solution, 0.5 mL of plant solution and 3 mL of reaction mixture without $CuCl_2$ were added. From the extract and standard solutions, 0.5 mL was transferred to Falcon tubes, and 3 mL of the reaction mixture was added to it.

After vortexing, the tubes were left for incubation at room temperature for 30 minutes. Absorbance values were read at 450 nm, and the values were recorded. All procedures were repeated three times, and the results were expressed as mM ascorbic acid equivalent per extract.

RESULTS

Impact of Extraction Method and Solvent on Extract Yields

The data presented in Table 1 and Table 2 show the results of extraction obtained using different solvents and durations for bitter melon and licorice plants.

As indicated in Table 1, it is observed that the highest yield of the bitter melon plant is achieved by treating 0.1g of dried plant powder with 10 mL of distilled water for 30 minutes using a magnetic stirrer. According to Table 2, soaking 0.1g of dried plant powder in 10 mL of distilled water for 10 minutes with a magnetic stirrer yields the maximum yield of the licorice plant.

Antioxidant Activity

The research of plant species' antioxidant activity has increased dramatically in recent years due to the fact that many of them are used as sources of phytotherapeutic substances.[26–29] Since phenols feature an aromatic ring that permits the stability and relocation of unpaired electrons within their structure, they are the primary chemicals in plants that exhibit antioxidant action. This property facilitates the transfer of hydrogen atoms and electrons from the hydroxyl groups of their structure.[30,31] The species, tissue, stage of development, and environmental elements—such as temperature, stresses caused by water, and light conditions—all affect the total phenol content of plants.[32,3]

Table 1. Extraction data of bitter melon

Solvents	5 min		10 min		20 min		30 min		40 min		60 min	
	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)
Water (10 mL)	9.30		7.50		9.70		10.20		10.20		10.70	
	11.30	**10.53**	6.60	**7.53**	9.10	**9.33**	10.80	**10.57**	10.30	**10.23**	10.60	**10.17**
	11.00		8.50		9.20		10.70		10.20		9.20	
Ethanol (10 mL)	0.70		0.50		0.60		1.10		0.70		1.20	
	0.60	**0.47**	1.50	**0.80**	0.30	**0.43**	1.00	**0.87**	1.40	**1.03**	1.10	**0.97**
	0.10		0.40		0.40		0.50		1.00		0.60	
N-hexane (10 mL)	0.30		0.10		0.20		0.10		0.10		0.10	
	0.10	**0.18**	0.20	**0.27**	0.10	**0.17**	0.08	**0.09**	0.20	**0.13**	0.10	**0.10**
	0.15		0.50		0.20		0.10		0.10		0.10	
Boiling water (10 mL)	9.70		11.30		8.30		10.20		11.90		11.10	
	9.80	**9.80**	11.00	**11.33**	7.80	**8.40**	10.40	**10.03**	12.00	**12.20**	11.40	**11.47**
	9.90		11.70		9.10		9.50		12.70		11.90	
Water (50 mL)	8.90		10.00		10.10		12.00		11.20		9.20	
	9.30	**9.23**	9.30	**9.47**	9.40	**10.00**	11.50	**11.47**	11.60	**11.43**	10.10	**9.87**
	9.50		9.10		10.50		10.90		11.50		10.30	

Table 2. Extraction data of licorice

Solvents	5 min		10 min		20 min		30 min		40 min		60 min	
	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)	Ext. (mg)	Avg. (mg)
Water (10 mL)	5.90		8.50		6.20		7.10		7.00		4.50	
	5.90	**6.50**	8.40	**8.53**	6.90	**6.87**	7.40	**7.10**	6.30	**6.87**	4.70	**4.67**
	7.70		8.70		7.50		6.80		7.30		4.80	
Ethanol (10 mL)	0.40		0.40		0.60		0.30		0.40		0.40	
	0.30	**0.33**	0.10	**0.47**	0.10	**0.37**	0.40	**0.37**	0.20	**0.33**	0.30	**0.40**
	0.30		0.90		0.40		0.40		0.40		0.50	
N-hexane (10 mL)	2.20		2.50		0.10		0.10		0.30		0.30	
	2.60	**2.63**	2.90	**2.73**	0.10	**0.23**	0.10	**0.10**	0.50	**0.43**	0.90	**0.57**
	3.10		2.80		0.50		0.10		0.50		0.50	
Boiling water (10 mL)	3.90		4.20		2.00		4.30		4.10		4.10	
	3.60	**3.90**	3.40	**3.73**	3.20	**2.70**	4.00	**4.23**	4.50	**4.37**	4.00	**3.67**
	4.20		3.60		2.90		4.40		4.50		2.90	

Table 3: Antioxidant activity data of two plants (where GAE refers to Gallic Acid Equivalent and AAE; Ascorbic Acid Equivalent)

Antioxidant activity methods	*Momordica charantia L.*	*Glycyrrhiza glabra*
DPPH, IC50 (µg/mL)	1212 ± 19.46	1169.39 ± 25.23
TPC (mg GAE/g)	6.59 ± 0.16	25.41 ± 0.25
FRAP (mM Fe(II)/g)	1174.8 ± 30.25	1800 ± 12.58
CUPRAC (mM AAE/g)	267.77 ± 7.63	523.54 ± 10.92
FIC (mg EDTAE/g)	10.81 ± 2.39	37.33 ± 3.64

* Each value is the mean of three experiments.

Antioxidant molecules usually take part in electron transfer activities, which prevent the oxidation of other chemicals that are vulnerable to it when associated with them. Organic compounds were often evaluated for their antioxidant properties, and several of these compounds were employed as protective agents.[34,35]It is possible to assess the antioxidant properties of plant extracts using a variety of techniques; in fact, using at least two separate techniques is advised.[36]

The antioxidant potential of the two chosen species was demonstrated in our investigation using five different techniques to measure their antioxidant activity (Table 3). Additionally, there was a strong link between all of the approaches. With its apparent introduction about half a century ago, the DPPH technique is frequently employed to assess a compound's antioxidant properties as well as its potential to operate as a hydrogen donor or free radical scavenger. A 50% reduction in DPPH activity (color) is the substrate concentration known as the IC50 parameter, which is utilized to interpret DPPH method findings. In comparison to ascorbic acid, the plant extracts were shown to have higher IC50 values. Momordica charantia was shown to have a lower antioxidant capacity than Glycyrrhiza glabra due to the negative relationship between the IC50 concentration and antioxidant capacity.

The Folin–Ciocalteu reagent was used to quantitatively determine the total phenolic content. The TPC was determined as mg GAE/g dry extract weight using a calibration line (y = 0.0087x - 0.0297, R2 = 0.9948) of standard gallic acid. It has been discovered that licorice contains more phenolics.

When comparing the plant extracts' ferric ion-reducing activity to their DPPH scavenging activity, there was little variation seen. Licorice had modest ferric ion reduction activities, which were consistent with the outcomes of radical scavenging experiments. One of the most important ways to assess antioxidant activity is to see whether a molecule can chelate metal ions. This test demonstrates how the molecule interacts with specific ions that are physiologically active. Licorice showed better chelating ability with both iron and copper, according to the results.

Experimental studies have demonstrated that licorice has a higher antioxidant content compared to bitter melon. Also, when considering the frequency of consumption among the public, licorice consumption is more widespread and deeply rooted in Kahramanmaraş. Bitter melon, on the other hand, is consumed among individuals with a health-conscious mindset, particularly for specific health issues however it does not form as prevalent a consumption habit as licorice.

Statistical Analysis

Antioxidant activity was found to be highly significant ($P < .0001$) with DPPH and FRAP techniques for both plants when compared with ascorbic acid (Figure 2A). When compared to disodium EDTA, the FIC method was significant ($P < .01$) for *Momordica charantia L.* and highly significant ($P < 0.0001$) for *Glycyrrhiza glabra L.* There is a significant correlation between five methods of antioxidant activity for *Glycyrrhiza glabra L*. at $p < 0.001$ (Figure 2C). For *Momordica charantia L*., except FRAP other four methods were found to provide strong significance of antioxidant potential (Figure 2B).

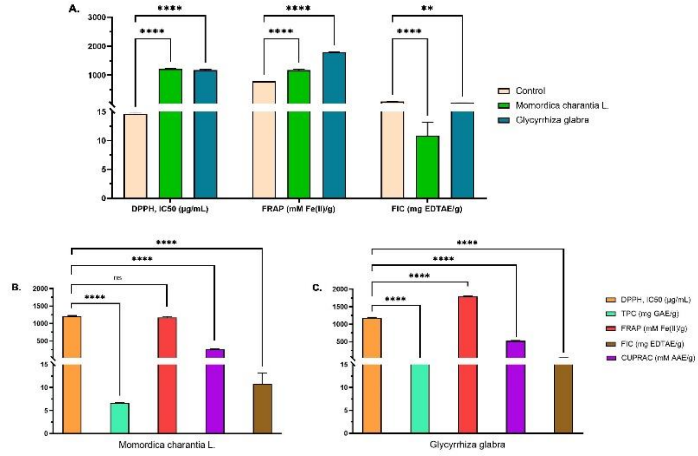

Figure 2. Comparison of antioxidant activity among *Momordica charantia L.* and *Glycyrrhiza glabra*. The antioxidant potential of the two chosen species compared with ascorbic acid using three different techniques (A), data are represented as ± SE, $N = 3$. Statistical analysis is two-way ANOVA $^{**}P < .01$; $^{****}P < .0001$. Comparison of antioxidant potential of *Momordica charantia L.*(B) and *Glycyrrhiza glabra L*. (C) using five different techniques, data are represented as ± SE, $N = 3$. Statistical analysis is one-way ANOVA $^{****}P < .0001$.

DISCUSSION

The research of plant species' antioxidant activity has increased dramatically in recent years due to the fact that many of them are used as sources of phytotherapeutic substances.[26-29] Since phenols feature an aromatic ring that permits the stability and relocation of unpaired electrons within their structure, they are the primary chemicals in plants that exhibit antioxidant action. This property facilitates the transfer of hydrogen atoms and electrons from the hydroxyl groups of their structure.[30,31] The species, tissue, stage of development, and environmental elements—such as temperature, stresses caused by water, and light conditions—all affect the total phenol content of plants.[32,33]

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It is possible to assess the antioxidant properties of plant extracts using a variety of techniques; in fact, using at least two separate techniques is advised.[36] The antioxidant potential of the two chosen species was demonstrated in our investigation using five different techniques to measure their antioxidant activity (Table 3). Additionally, there was a strong link between all of the approaches. With its apparent introduction about half a century ago, the DPPH technique is frequently employed to assess a compound's antioxidant properties as well as its potential to operate as a hydrogen donor or free radical scavenger. A 50% reduction in DPPH activity (color) is the substrate concentration known as the IC_{50} parameter, which is utilized to interpret DPPH method findings. In comparison to ascorbic acid, the plant extracts were shown to have higher IC_{50} values. *Momordica charantia* was shown to have a lower antioxidant capacity than *Glycyrrhiza glabra* due to the negative relationship between the IC_{50} concentration and antioxidant capacity.

The Folin–Ciocalteu reagent was used to quantitatively determine the total phenolic content. The TPC was determined as mg GAE/g dry extract weight using a calibration line ($y = 0.0087x - 0.0297$, $R^2 = 0.9948$) of standard gallic acid. It has been discovered that licorice contains more phenolics. When comparing the plant extracts' ferric ion-reducing activity to their DPPH scavenging activity, there was little variation seen. Licorice had modest ferric ion reduction activities, which were consistent with the outcomes of radical scavenging experiments. One of the most important ways to assess antioxidant activity is to see whether a molecule can chelate metal ions. This test demonstrates how the molecule interacts with specific ions that are physiologically active. Licorice showed better chelating ability with both iron and copper, according to the results.

Experimental studies have demonstrated that licorice has a higher antioxidant content compared to bitter melon. Also, when considering the frequency of consumption among the public, licorice consumption is more widespread and deeply rooted in Kahramanmaraş. Bitter melon, on the other hand, is consumed among individuals with a health-conscious mindset, particularly for specific health issues however it does not form as prevalent a consumption habit as licorice.

In conclusion, this study reveals the antioxidant potential of two plants which are consumed commonly in Kahramanmaraş, Turkey, could protect against free radical damage.

The information and data obtained from this study are considered beneficial for the development of food products and additives with appropriate antioxidant properties. The identification of natural antioxidant sources and their appropriate consumption in the daily diet or the use of isolated compounds in clinical practices would be beneficial for a healthy life. From a broader perspective, the data obtained from this study will be crucial for individuals who rely herbal treatment methods based on natural sources, enabling them to make more informed consumption decisions. However, it is essential to remember that the effectiveness of herbal treatments depends on using the right plant, for the right person, in the right way, and at the correct dosage.

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