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ORIGINAL ARTICLE

Evaluation of the Antidepressant-like Activity of the Aqueous Extract of *Crataegus aronia*

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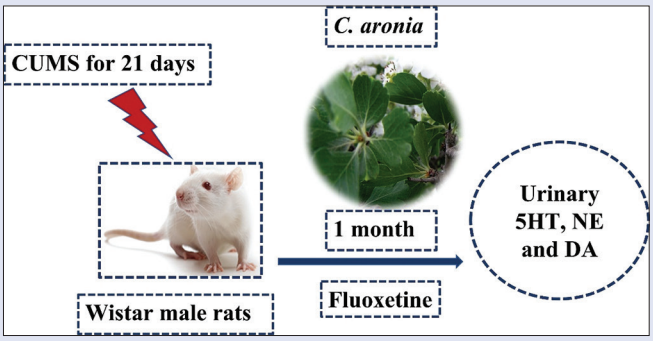
ABSTRACT

Background: *Crataegus aronia* L. Syn: *Azarolus* (*C. aronia*) is a hawthorn species and a perennial bush native to Mediterranean regions. It is recognized for its high polyphenol content and potent antioxidant effects. Research was conducted to test the antidepressant activities of an aqueous extract of *C. aronia* in a chronic, unpredictable, mild stress-induced rat model. **Materials and Methods:** Thirty-two adult Wistar male rats were divided into four groups: A control group (no stress and no treatment), stress-model group (stress and no treatment), fluoxetine-treated stress group (stress and fluoxetine treatment), and *C. aronia*-treated stress group (stress and *C. aronia* treatment). Urine samples were collected at 0, 21, 36, and 51 days. Enzyme-linked immunosorbent assay kits were used to assess serotonin, norepinephrine (NE), and dopamine (DA) levels. **Results:** There was a decrease in serotonin levels 3 weeks after stress exposure, but urinary NE and DA levels increased. *C. aronia* significantly ($P < 0.001$) reversed the depressive-like symptoms in the rats according to the increased urinary levels of serotonin. Moreover, *C. aronia* also reduced urinary levels of urinary NE and DA. The neuromodulatory effects of *C. aronia* were comparable to that of fluoxetine. **Conclusion:** With its active ingredients of anthocyanins and procyanidins, *C. aronia* exhibits significant antidepressant-like activity in validated stressed rats, which may be related to its neuromodulatory effects on central monoamines.

Key words: Antidepressant, aqueous extract, chronic unpredictable mild stress, *Crataegus aronia*, fluoxetine, urinary neurotransmitters

SUMMARY

- Based on the findings of this investigation, *Crataegus aronia* showed antidepression-like action in chronic unpredictable mild stress-induced depression in rats. These effects were probably due to the modulation of central neurotransmitter levels and inhibition of oxidative stress by the synergistic action of various phytoconstituents of *C. aronia*. Further investigations *in vivo* are required to support these findings and to investigate the exact mechanisms of these antidepressant activities.



Abbreviations used: CUMS: Chronic unpredictable mild stress; CNS: Central nervous system; 5HT: 5-Hydroxytryptamine; DA: dopamine; NE: Norepinephrine; AECA: Aqueous extract of *Crataegus aronia*; BDNF: Brain-derived neurotrophic factor; MDD: Major depressive disorder; ELISA: Enzyme-linked immunosorbent assay; WHO: World Health Organization; dBA: Decibel A scale; ECM: Research Ethics Committee; DDDW: De-ionized double distilled water; SEM: Standard error of the mean; ANOVA: Analysis of variance.

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INTRODUCTION

Depression is a major concern, and the World Health Organization (WHO) predicts that almost 450 million individuals suffer from depressive disorders worldwide.[1] The WHO rated major depressive disorder (MDD) as third among the major causes of healthcare burden in 2008, and it is expected to rise to first by 2030.[1] It is a complex illness that expresses in a variety of ways at the psychological, behavioral, and physiological levels.[2] The severity of depression is linked to increased treatment costs, treatment efficiency, unemployment, disability, and decreased work performance.[3] The underlying pathology of MDD development is still unknown, and existing treatments are ineffective in many patients. A full grasp of the pathophysiological process is necessary for efficient therapy and disease resolution.[4] The failure of most antidepressant medications highlights the enormous burden and negative impact of depression on individuals, communities, and economies.

Researchers confront major challenges due to the complex nature of MDD, which is frequently linked to other medical conditions. Over the last two decades, antidepressant therapy for MDD has grown in quantity and popularity. However, this tendency has sparked some debate as the therapies' long-term safety and effectiveness have been questioned. Currently available antidepressants have significant downsides, including moderate efficacy, resistance, a slow onset of effect, significant withdrawal symptoms, issues with overdose safety, and dangers during pregnancy and breastfeeding.[5,6]

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The growing understanding of mood disorders and interest in developing more potent and safer antidepressants have led to a search for many natural products.[7] In the history of psychiatry, natural products have played a significant role and appear to be reasonable options for depressed patients who prefer an approach based on natural products.[7] Natural medications such as S-adenosyl-L-methionine, omega-3 fatty acids, and St. John's wort may be valuable supplements to the pharmaceutical arsenal for mood disorders as both monotherapies and adjuvant therapies.[8,9]

Crataegus is a plant genus in the Rosaceae family and is commonly known as hawthorn. It includes several hundred species of bushes and trees that are native to the temperate climatic zones of the Northern Hemisphere in Asia, North America, North Africa, and Europe. *Crataegus* species are recognized for having high polyphenol content and some of the most powerful antioxidant effects of any plant species.[10,11] Correspondingly, there has been a resurgence in scholarly interest in *Crataegus* in recent years, resulting in numerous research reports on its antioxidant effects.[12]

Despite the many species of hawthorn found worldwide, only a few have been investigated and used clinically for treating depression, including *Crataegus pinnatifida* and *Crataegus monogyna*.[13-16] *Crataegus aronia* syn. *Azarolus* (L) (*C. aronia*) is one of the most widespread types of hawthorn and inhabits the mountains of the Mediterranean region. It has been used in folk medicine for many conditions, such as impotence, cardiovascular disease, diabetes, and cancer.[10,17] Various extracts of *C. aronia* leaves reveal the presence of different triterpenoids (euscaphic acid, jacaranoic acid, 2oxopomolic acid, and arjunic acid) and flavonoids (epicatechin, 4'"acetylvitexin2"Orhamnoside, vitexin2"O-rhamnoside, and vitexin).[18-20]

Polyphenols are abundant in *C. aronia*, and a growing number of epidemiological studies suggest their use in treating neuropsychiatric and neurodegenerative disorders. In addition, animal experiments have revealed that foods high in polyphenols can improve cognitive performance.[21] To the best of our knowledge, there has been no research on the antidepressant effects of *C. aronia*. Therefore, we designed an experiment to evaluate the impact of an aqueous extract of *C. aronia* (AECA) on urinary neurotransmitter levels in experimental rats using a chronic stress-induced model to induce depression.

MATERIALS AND METHODS

Materials

Enzyme-linked immunosorbent assay (ELISA) assay kits for serotonin 5-hydroxytryptamine (5-HT), norepinephrine (NE), and dopamine (DA) were purchased from Abnova (CA, USA). Fluoxetine (Prozac, fluoxetine hydrochloride equivalent to 20-mg fluoxetine) was purchased from a local pharmacy. Whole specimens of fresh *C. aronia* (flowers, leaves, and stems) were bought from a farmers' market in Jordan. The location where it was collected place is situated at 31.963158° N and 35.930359° E, and the specimen was identified by an expert taxonomist. An authentic voucher specimen (067-CA/KKU/2020) has been deposited in the Herbarium and Biological Specimen Department of King Khalid University for future reference. The experiments were done at the physiology labs of King Khalid University, Saudi Arabia.

Preparation of aqueous extract of *Crataegus aronia*

The entire plant of *C. aronia* was air-dried, and an extract was obtained in the pharmacognosy laboratory. The dried plant material was ground into a powder and soaked for 3 days at 37°C with distilled water (100 g/300 mL, w/v). The AECA was filtered through Whatman No. 1 paper under vacuum and evaporated under reduced pressure in a rotary evaporator. The resulting residue (40 g) was then stored at 4°C. The residue was reconstituted in double-distilled de-ionized water, filtered through 0.2-μM filters, and then stored in a refrigerator until the experimental study.[22] The aqueous extract was subjected to preliminary phytochemical analysis to detect plant components using standard chemical tests.[23]

Experimental animals

The experimental animals were 32 adult Wistar male rats from a similar lineage and genetic pool, which were obtained from the animal house at King Khalid University. Their age was 6 months, and they weighed 230–250 g. The animals were housed in polypropylene mouse cages with standard dimensions (50 cm × 26 cm × 16 cm) in groups of 4 rats per cage. The cages were kept at 25°C ± 1°C with a standard 12-h day/night cycle. All experimental protocols were approved by King Khalid University's Research Ethics Committee (#20-0862) and carried out while following the National Institutes of Health's guidelines for the care and use of laboratory animals. Every effort has been made to ensure minimal animal suffering and a minimal number of animals used.

Chronic unpredictable mild stress-induced depression model

Chronic unpredictable mild stress (CUMS) was used to induce depression in rats [Table 1] as follows: Cage tilting and damp sawdust for 24 h (200 mL of water per individual cage, which is enough to make the sawdust bedding wet); 5 min of cold swimming in water at 4°C; noise for 1 h (alternative periods of 60-Decibel A scale noise for 10 min and 10 min of silence); 5 min of thermal stimulation in an experimental room at 50°C; 48 h of food deprivation and 24 h of water deprivation; 15 electric shocks to the foot (15 mA, one shock/5 s, 10-s duration); a 1-min tail pinch; and restricted movement for 4 h. On each day, one stressor was applied, and the complete stress procedure lasted 3 weeks, with the stressors being delivered in entirely random order.[24] The healthy control group of rats was accommodated undisturbed in another experiment room under the same circumstances.

Experimental design

After 2 weeks of habituation, all of the rats were divided at random into four groups ($n = 8$). The control group received distilled water and normal rat diet for 51 days (no stress and no treatment), and the stress-model group was stressed for 21 days and then received distilled water and normal rat diet for the next month. The fluoxetine-treated stress group was stressed for 21 days, received fluoxetine daily (2 mg/kg/day, i.p.) for the next month, and was kept on a normal rat diet. The *C. aronia*-treated

Table 1: Types of chronic unpredictable mild stress applied to experimental rats in random order (each day, one stressor was used, and the complete stress procedure lasted 21 days)

Order of CUMS applied	Types of CUMS applied
1	Cage tilting and dampness (200 mL of water per individual cage, which is enough to make the sawdust bedding wet)
2	5 min of cold swimming in cold water at 4°C
3	Noise for 1 h (alternative periods of 60-dBA noise for 10 min and 10 min of silence)
4	5 min of thermal stimulation in an experimental room at 50°C
5	48 h of food deprivation and 24 h of water deprivation
6	15 electric shocks to the foot (15 mA, one shock/5 s, 10-s duration)
7	A 1-min tail pinch and restricted movement for 4 h

CUMS: Chronic unpredictable mild stress, dBA: Decibel A Scale

stress group was stressed for 21 days and then received the AECA orally (10 mg/kg/day, p.o.) for the next month. The pharmacological dose was selected from a previous experimental study, which reported that whole-plant AECA has no toxic effects when administered at 2000 mg/kg.[25]

Urine collection and biochemical analysis

Urine samples were collected from rats at days 0, 21 (after stress induction), 36 (15 days after stress induction), and 51 (1 month after stress induction) using metabolic cages (1 rat/cage). Collected urine was filtered using 0.2-µm filters, and then special ELISA kits (Abnova, USA) were used for the determination of 5-HT (KA 1894), NE (Cat No. KA1891), and DA (Cat NO. KA1887).[26]

Statistical analysis

The data were analyzed using GraphPad Prism version 8.0 for Windows (*GraphPad* Software, San Diego, CA, USA), and the results are reported as the mean ± standard error of the mean. Two-way analysis of variance using Tukey's multiple comparison test was employed to evaluate the statistically significant changes between the treatments and intervals, and $P < 0.001$ was considered statistically significant.

RESULTS

Phytochemical analysis of *C. aronia* indicated the presence of flavonoids, polysaccharides, terpenoids, tannins, catechins, monoterpenes, proanthocyanidins, and steroids. The levels of 5-HT, NE, and DA were evaluated as potential urinary biomarkers for CUMS-induced depression in rats. The ELISA method was used to measure the urinary neurotransmitter levels. The concentration of 5-HT was significantly lower in the urine samples of CUMS-induced animals than in normal rats, as shown in Figure 1. Lower urinary levels of 5-HT were also observed in the CUMS-exposed treatment groups than in controls. However, long-term therapy with AECA significantly increased the concentration of 5-HT after 36 and 51 days ($P < 0.001$) compared with CUMS-stressed rats. The 5-HT concentration in the fluoxetine group was also significantly higher after 36 and 51 days ($P < 0.001$) than in the CUMS control group.

Figures 2 and 3 show a significant rise in the urinary levels of NE and DA after 21 days of CUMS induction compared to the unstressed group. When comparing the CUMS-stressed group to the treatment group, we found a sustained and substantial increase in NE and DA levels. Both AECA and fluoxetine-treated groups exhibited a significant decrease in NE [Figure 2, $P < 0.001$] and DA levels [Figure 3; $P < 0.001$] after 36 and 51 days.

DISCUSSION

Urine is widely considered the recommended body fluid for measuring neurotransmitters because of its non-invasive collection technique and the fact that it is the main mechanism of neurotransmitter excretion.[27] In this study, neurotransmitters were detected with commercially available ELISA kits. Compared to blood sampling, urinary samples are more appropriate for collection, especially for small experimental animals. The non-invasive sampling method could prevent interference in neuropharmacological studies of experimental animals.[28]

ELISA provides an appropriate and robust way of investigating urinary monoamines, including 5-HT. A recent literature review indicates that neurotransmitters released in urine could be used as biomarkers of nervous system function.[29] Several studies have consistently shown a significant relationship of depressive disorders with urinary monoamine excretion levels, thus suggesting that they could help in diagnosing and managing MDD patients.[30]

CUMS is the most widely used, reliable, and effective model currently available to induce depression in animals. Several experimental studies have confirmed that the CUMS model can cause depression-like behavior in experimental rats.[31-33] In the current experiment, CUMS-induced

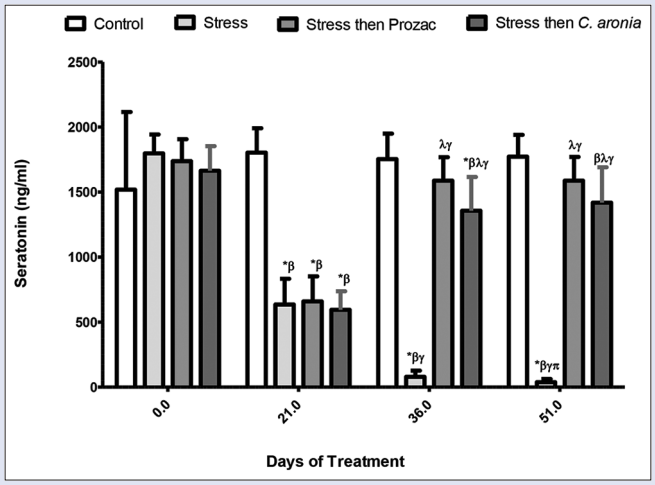

Figure 1: Effect of chronic oral administration of aqueous extract of *Crataegus aronia* on urinary 5-hydroxytryptamine content in chronic unpredictable mild stress-induced depression in rats. The values are expressed the mean ± standard error of the mean. Statistical analyses were performed between various groups and treatment intervals. Two-way analysis of variance followed by Tukey's multiple comparison test was used to analyse the data. *, β, λ, γ, π $P < 0.001$ are considered as statistically significant. *: significantly different from control group at day 0.0. β: Significantly different from control group on same day. λ: Significantly different when compared to stress group on same day. γ: Significantly different when compared to day 21

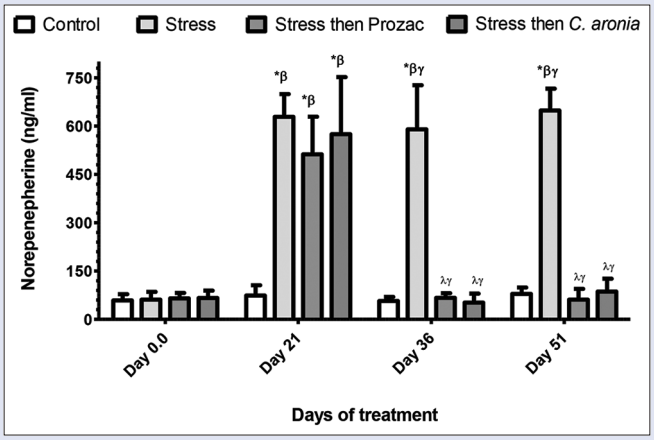

Figure 2: Effect of chronic oral administration of aqueous extract of *Crataegus aronia* on urinary norepinephrine content in chronic unpredictable mild stress-induced depression in rats. The values are expressed as the mean ± standard error of the mean. Statistical analyses were performed between various groups and treatment intervals. Two-way analysis of variance followed by Tukey's multiple comparison test was used to analyze the data. *, β, λ, γ, π $P < 0.001$ are considered as statistically significant. *: Significantly different from control group on day 0.0. β: Significantly different from control group on same day. λ: Significantly different when compared to stress group on same day. γ: Significantly different when compared to day 21

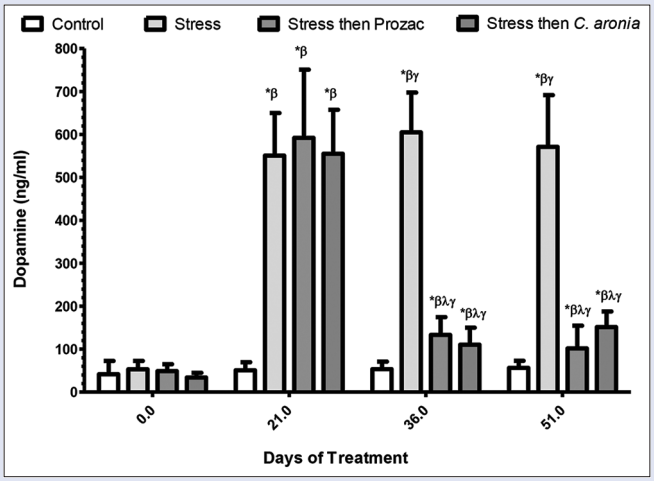

Figure 3: Effect of chronic oral administration of aqueous extract of *Crataegus aronia* on urinary dopamine content in chronic unpredictable mild stress-induced depression in rats. The values are expressed as the mean ± standard error of the mean. Statistical analyses were performed between various groups and treatment intervals. Two-way analysis of variance followed by Tukey's multiple comparison test was used to analyze the data. *, β, λ, γ $P < 0.001$ are considered as statistically significant. *: Significantly different from control group on day 0.0. β: Significantly different from control group on same day. λ: Significantly different when compared to stress group on same day. γ: Significantly different when compared to day 21

depressive rats showed higher levels of DA and NE and lower levels of 5-HT. After 21 days of applying the CUMS procedure, the altered levels of these urinary monoamines could be associated with CUMS-induced depression.[34]

The administration of AECA can effectively reduce elevated levels of DE and NE while increasing 5-HT levels, and the changes were consistent with previous studies in animal models of depression. CUMS has a significant effect on monoaminergic activity in rats.[35] The brain monoamine systems play a significant role in depressive disorders and are supported by antidepressants' mechanisms of action.[36] Reduced levels of monoamine neurotransmitters in the central nervous system, especially with dysfunction of the serotonin system, have a major role in the pathogenesis of depression.[37] The concept of using antidepressants as the first-line clinical treatment of MDD involves enhancing levels of neurotransmitters and monoamine receptors.[38]

In the current research, monoamine neurotransmitter levels were significantly altered in the CUMS-stressed rats, but oral AECA administration significantly reversed the clinical observations generated by CUMS and reversed the altered levels to levels comparable to those of fluoxetine-administered rats. As hypothesized, this observation suggests that these normalizations of neurotransmitter expression may produce the antidepressant-like effects of AECA through increased central monoamine neurotransmitters levels. The use of phytotherapy-based nutraceuticals to treat MDD is an important strategy for potentially improving clinical outcomes.[39] One possible mechanism of action of AECA is the attenuation of oxidative stress produced during CUMS-induced depression by polyphenolic compounds, such as flavonoids (mainly anthocyanins and procyanidins).[40,41] Polyphenols have potent neuroprotective effects and are suggested to improve depression by increasing monoamine neurotransmitter levels, which may occur through the up-regulation of brain-derived neurotrophic factor expression.[42,43]

The antidepressant-like effect of AECA in this study is consistent with other investigations demonstrating the antidepressant and antioxidant effects of *Crataegus* species, which are rich in polyphenols.[44,45] Lim *et al.* showed that 1 month of freely drinking phenolic-rich *Crataegus* extract up-regulates central 5-HT levels but not noradrenaline, which may be associated with anxiolytic-like and antidepressant-like effects.[15] Finally, the current study suggests that the antidepressant effect of AECA is partly due to the modulation of 5-HT, DA, and NE. However, the exact mechanism needs more examination, and the exact targets of the antidepressant-like effects of AECA remain unknown, thus requiring further research.

CONCLUSION

The present study provides the first proof that AECA, which has active ingredients of anthocyanins and procyanidins, exhibits significant antidepressant-like activity in a validated CUMS-induced depression model. These effects may be related to its neuromodulatory effects on central monoamine levels such as NE, DA, and 5-HT. The high polyphenolic contents in *C. aronia* could be linked to its mechanism of action, which reduced CUMS-induced oxidative stress. For further study, the active phytoconstituents should be isolated and identified from the aqueous extract to determine what is responsible for the effects. In addition, other potential mechanisms could be implicated, and safety studies are required. The current study indicates that *C. aronia* deserves further investigation as a potential antidepressant and could pave the way to develop a new phytotherapy.

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Conflicts of interest

The author declares no conflicts of interest.

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