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Original Article

Phytochemical Analysis and Exploration of the Anti-Amnesic and Anti-Depressive Potentials of Avena fatua Crude Extract and Its Fractions in Animal Model

Shehzad Khan[1], Yasar Shah[1], Maryam Zulfat[2], Kashif Ali[3], Fazli Hadi[4], Saqib Jahan[4], Maryam Pirzadi[1], Qamar Gul[5], Ali Khan[6], Fazal Hadi[7], Muhammad Ikram[1], Hafiz Sajid Akbar[8]

[1] Department of Pharmacy, Abdul Wali Khan University Mardan, Mardan, Pakistan
[2] Department of Biochemistry, Women University Swabi, Swabi, Pakistan
[3] Pharmacist, Mardan Medical Complex, Mardan, Pakistan
[4] Department of Pharmacy, University of Swabi, Swabi, Pakistan
[5] Hospital Pharmacist, Tehsil Headquarter Hospital Kabal, Swat, Pakistan
[6] The Professional Institute of Health Sciences, Mardan, Pakistan
[7] Vertex College of Pharmacy, Mardan, Pakistan
[8] Department of Pharmacy, Vertex Institute of Science and Technology, Mardan, Pakistan

✉ **Correspondence:** Dr. Muhammad Ikram, mikram@awkum.edu.pk

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ABSTRACT

Background: Avena fatua contains several bioactive phytochemicals, but its anti-amnesic and anti-depressant potentials remain insufficiently characterized. Objective: To evaluate the phytochemical profile and anti-amnesic and anti-depressant effects of Avena fatua crude methanolic extract and its chloroform and ethyl acetate fractions in mice. Methods: This experimental animal study used BALB/c mice. The crude extract was prepared by methanolic maceration and fractionated into chloroform and ethyl acetate fractions. HPLC and GC–MS were used for phytochemical characterization. Acute toxicity was assessed up to 2000 mg/kg. Anti-amnesic activity was evaluated using the Y-maze and novel object recognition test, together with brain AChE, ACh, SOD, CAT, and GSH estimation. Anti-depressant activity was assessed using the tail suspension and forced swim tests. Data were analyzed using analysis of variance, with Dunnett's multiple-comparison test applied for anti-depressant activity. Results: No mortality was observed up to 2000 mg/kg. HPLC detected phloroglucinol, hydroxybenzoic acid, and ascorbic acid, while GC–MS identified 18 constituents. Chl-AF at 150 mg/kg produced spontaneous alternation of 71.49% compared with 37.04% in the amnesic control and a recognition index of 62.04% compared with 32.94%. Treatment groups also showed lower AChE and higher ACh, SOD, CAT, and GSH values than the amnesic group. Chl-AF at 150 mg/kg produced TST and FST immobility times of 89.56 and 112.28 seconds, respectively. Conclusion: Avena fatua crude extract and its fractions demonstrated preliminary anti-amnesic and anti-depressant activity in mice, accompanied by favorable cholinergic and antioxidant changes. Keywords: Avena fatua; anti-amnesic; anti-depressant; scopolamine; phytochemicals; neuroprotection.

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INTRODUCTION

Memory is a fundamental cognitive function that enables the encoding, temporary maintenance, consolidation, and retrieval of information required for learning and adaptive behavior. Working memory, in particular, supports attention, reasoning, and goal-directed cognitive activity, while disruption of memory processes can substantially impair everyday functioning (1). Amnesia represents a disturbance of memory that may affect the formation or retrieval of information and can arise from neurological injury, pharmacological effects, substance exposure, or other medical conditions (2). Because impairment of memory is a prominent feature of several neurological disorders, experimental models that reproduce cognitive deficits remain important for investigating potential neuroprotective and cognition-modulating agents.

Natural products have historically provided structurally diverse bioactive compounds for pharmacological discovery and continue to contribute to the development of therapeutically relevant molecules (3). Among plant-derived constituents, phenolic and flavonoid compounds have received particular attention because of their antioxidant, anti-inflammatory, and neuroprotective properties and their potential influence on cognitive function (4,5). Oxidative stress and disruption of neuronal homeostasis may contribute to cognitive dysfunction, whereas plant-derived antioxidants may help preserve neuronal function by modulating oxidative injury and related biochemical pathways (4,5). In addition, several flavonoids have demonstrated cholinesterase-inhibitory activity, suggesting a potential role for naturally occurring compounds in modulating cholinergic neurotransmission involved in learning and memory (6). These observations have stimulated continuing interest in medicinal plants as sources of compounds with possible cognition-enhancing and neuroprotective activity.

Avena fatua L., commonly regarded as a wild oat and an important agricultural weed, is widely distributed and belongs to the genus Avena (7). Although the neuropharmacological properties of A. fatua remain insufficiently characterized, evidence from other Avena species provides a biological rationale for further investigation. In particular, preparations derived from Avena sativa have been examined for their effects on cognitive performance and mood. Kennedy et al. reported cognitive effects following acute administration of a wild green-oat extract, including changes in domains related to attention and working memory (8). Subsequent research has also reported improvements in measures of working memory and processing speed following acute Avena sativa supplementation in healthy adults (9). These findings cannot be directly extrapolated to A. fatua, because botanical species, extraction procedures, phytochemical composition, doses, and experimental populations differ; nevertheless, they support investigation of the genus as a potential source of neuroactive phytochemicals.

More broadly, medicinal plants containing phenolic, flavonoid, and other secondary metabolites have been investigated for memory-modulating properties in experimental models (10). Experimental evidence from plant-derived compounds and extracts suggests that cognitive effects may involve several interacting processes, including modulation of oxidative stress, preservation of cholinergic activity, and protection against neuronal dysfunction rather than a single pharmacological mechanism (4–6). However, detection of phytochemicals alone cannot establish neuropharmacological activity, and biological effects observed with one plant species or isolated compound cannot be assumed to occur with another. Direct experimental characterization is therefore required to determine whether A. fatua extracts possess measurable effects on cognitive behavior and associated biochemical markers.

Despite increasing interest in plant-derived neuroprotective agents and the reported cognitive effects of other Avena preparations, evidence concerning the phytochemical composition and neurobehavioral activity of A. fatua remains limited. In particular, the relationship between its crude extract and solvent fractions, cognitive performance following pharmacologically induced memory impairment, cholinergic markers, and endogenous antioxidant defenses has not been adequately characterized in the evidence cited in this manuscript. The inclusion of behavioral models of antidepressant-like activity may additionally provide preliminary information on whether the neurobehavioral effects of the plant extend beyond memory-related outcomes. Such preclinical characterization is necessary before individual constituents, molecular mechanisms, or potential translational applications can be investigated.

Accordingly, the present study aimed to characterize the phytochemical profile of the crude methanolic extract of Avena fatua using high-performance liquid chromatography and gas chromatography–mass spectrometry and to evaluate the neurobehavioral effects of the crude extract and its chloroform and ethyl acetate fractions in mice.

Specifically, the study assessed anti-amnesic activity in a scopolamine-associated memory-impairment model using the Y-maze and novel object recognition tests, examined brain acetylcholinesterase and acetylcholine together with superoxide dismutase, catalase, and glutathione, and evaluated antidepressant-like activity using the tail suspension and forced swim tests. The study therefore investigated whether A. fatua crude extract and its fractions could improve behavioral and biochemical outcomes relative to the corresponding control and standard-treatment groups under the experimental conditions employed.

MATERIAL AND METHODS

The present experimental animal study was conducted to investigate the phytochemical composition and anti-amnesic and anti-depressant potentials of *Avena fatua* crude extract and its fractions. The study included preparation and fractionation of the plant extract, phytochemical analysis using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS), acute toxicity assessment, evaluation of anti-amnesic activity using the Y-maze and novel object recognition test (NORT), biochemical estimation of acetylcholinesterase (AChE), acetylcholine (ACh), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), and evaluation of anti-depressant activity using the forced swim test (FST) and tail suspension test (TST).

Avena fatua was collected from Chakdara, Dir Lower, Khyber Pakhtunkhwa, Pakistan, and authenticated by a taxonomist at the University of Malakand. The plant material was ground into powder and macerated in methanol for 14 days with intermittent stirring and filtration. The filtrate was concentrated using a rotary evaporator at 40°C to obtain the crude methanolic extract, designated as Crd-AF. The crude extract was subsequently fractionated using suitable solvents to obtain the chloroform fraction (Chl-AF) and ethyl acetate fraction (Et-AF). Commercial-grade methanol, chloroform, ethyl acetate, hexane, butanol, DTNB, thiobarbituric acid, acetylthiocholine, donepezil, Tween-80, and other required chemicals were obtained from commercial suppliers.

The phytochemical profile of the crude extract was evaluated using HPLC and GC–MS. For HPLC analysis, the plant extract was processed in methanol and subjected to chromatographic analysis according to previously described procedures (11). The sample was treated with methanol and heated with water at 70°C for 1 hour before analysis. An Agilent HPLC system was used, and the detected compounds were identified on the basis of retention characteristics and comparison with available standards and previously reported chromatographic information (12). HPLC analysis was performed to identify major phenolic and related constituents present in the crude extract.

GC–MS analysis was carried out on the crude methanolic extract according to an established procedure (13). An Agilent gas chromatographic system equipped with a capillary column and mass detector was used. Electron-impact ionization was performed at 70 eV. The initial temperature was maintained at 70°C and subsequently increased to 180°C at a rate of 10°C/min, followed by an increase to 280°C, which was maintained for 20 minutes. The extract was diluted with n-pentane at a ratio of 1:1000 (v/v), and 1 µL of the sample was injected in splitless mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The separated compounds were characterized according to their mass-to-charge ratios and chromatographic retention characteristics.

BALB/c mice weighing 17–23 g were obtained from the National Institutes of Health, Islamabad. The animals were acclimatized in plastic cages at the animal facility of the University of Malakand under standard laboratory conditions before experimentation. Acute toxicity of Crd-AF and its fractions was evaluated according to Lorke's method (14). Different dose concentrations were administered orally to mice, with six animals used in the toxicity assessment. Tween-80 was used for the negative-control condition. The animals were observed for mortality and signs of toxicity, including salivation, diarrhea, convulsions, sleeping behavior, and lethargy, for approximately 24 hours. No mortality was observed at doses up to 2000 mg/kg body weight. Based on the toxicity findings, lower doses were selected for subsequent pharmacological evaluation.

For evaluation of anti-amnesic activity, mice were allocated to experimental groups with six animals in each group. The reported treatment groups included a normal control group, an amnesic control group receiving scopolamine, a standard group receiving donepezil 2 mg/kg, Crd-AF groups receiving 100 and 200 mg/kg, Chl-AF groups receiving 75 and 150 mg/kg, and Et-AF groups receiving 75 and 150 mg/kg. Treatments were continued for 28 days. On the 29th day, scopolamine was administered to induce amnesia, after which memory impairment and the effects of the test treatments were assessed using the Y-maze and NORT.

The Y-maze consisted of three arms measuring 35 cm × 8 cm × 15 cm positioned at angles of 120° from one another. Each mouse was placed at the end of one arm and allowed to explore the maze freely for 8 minutes. Spontaneous alternation behavior was recorded and expressed as percentage spontaneous alternation according to the established Y-maze procedure (15). The apparatus was cleaned with 70% ethanol between trials to minimize olfactory cues.

The novel object recognition test was performed using a transparent apparatus containing objects of different shapes according to an established procedure (16). One day before testing, the mice were allowed to habituate to the apparatus for approximately 2–5 minutes. During the sample phase, two identical objects were placed in opposite areas of the apparatus and exploration behavior was recorded. After 24 hours, one of the familiar objects was replaced by a novel object. The mice were then allowed to explore the objects, and the exploration times for the novel and familiar objects were recorded. The recognition index reported for each group was used to compare memory performance between experimental conditions. The apparatus was cleaned with 70% ethanol between animals.

Following behavioral testing, brain tissue was collected and the hippocampal region was dissected. A 10% w/v tissue homogenate was prepared in 0.1 M phosphate buffer at pH 7.4 and centrifuged for biochemical analysis. Acetylcholinesterase, acetylcholine, catalase, glutathione, and superoxide dismutase were estimated in brain homogenates using previously described biochemical procedures (17). AChE and ACh were assessed as cholinergic markers, whereas SOD, CAT, and GSH were assessed as markers of antioxidant status.

Anti-depressant activity of Crd-AF and its fractions was assessed using the forced swim test and tail suspension test. Mice were divided into groups containing eight animals each. The treatment conditions included a control group, an imipramine-treated standard group receiving 30 mg/kg, Crd-AF at doses of 150 and 300 mg/kg, Chl-AF at doses of 75 and 150 mg/kg, and Et-AF at doses of 75 and 150 mg/kg.

For the forced swim test, the mice were assessed 30 minutes after administration of the respective treatments. Each animal was placed individually in a water-filled cylinder, and its behavior was observed for 6 minutes. Immobility time was recorded as the principal outcome according to the established forced swim test procedure (18). For the tail suspension test, mice were similarly assessed 30 minutes after treatment administration and suspended individually by the tail for 6 minutes. Immobility time was recorded according to the established tail suspension procedure (19,20).

Quantitative data were expressed as mean ± standard error of the mean (SEM) in the original analysis. Statistical analysis was performed using GraphPad Prism. Differences among experimental groups were evaluated using analysis of variance. For the anti-depressant activity experiments, one-way ANOVA followed by Dunnett's multiple-comparison test was used to compare treatment groups with the control group. Statistical significance was evaluated at *p*<0.05, *p*<0.01, and *p*<0.001.

The animal experiments were conducted at the University of Malakand in accordance with institutional ethical approval and the Animal Byelaws 2008 of the University of Malakand. The animals were acclimatized before experimentation and monitored during the acute toxicity and pharmacological procedures for observable adverse effects.

RESULTS

No mortality was observed following administration of the crude Avena fatua extract at doses up to 2000 mg/kg body weight. The animals were monitored for salivation, diarrhea, convulsions, sleep-related changes, and lethargy, and no fatal toxicity was reported. On this basis, lower doses were used for subsequent behavioral and biochemical experiments.

Table 1. HPLC phytochemical profile of the crude methanolic extract of Avena fatua

No.	Retention time (min)	Compound	Peak area
1	2.34	Phloroglucinol	42.54
2	2.69	Hydroxybenzoic acid	21.07
3	4.43	Ascorbic acid	49.57

HPLC, high-performance liquid chromatography. HPLC analysis identified three principal constituents in the crude extract: phloroglucinol, hydroxybenzoic acid, and ascorbic acid. Among the reported chromatographic peaks, ascorbic acid had the largest peak area (49.57), followed by phloroglucinol (42.54) and hydroxybenzoic acid (21.07).

Table 2. GC–MS profile of the crude methanolic extract of Avena fatua

No.	Compound	Retention time (min)	Peak (%)	CAS No.	m/z
1	2,4-Di-tert-butylphenol	12.366	0.14	000096-76-4	191.0
2	Aromandendrene	14.110	0.26	000489-39-4	204.0
3	4-Methoxy-6-methyl-6,7-dihydro-4H-furo[3,2-c]pyran	16.106	0.39	091894-15-4	59.1
4	Hexadecanoic acid	16.902	0.48	000112-39-0	43.1
5	10,13-Octadecadienoic acid	18.452	1.09	056554-62-2	95.1
6	cis-13-Octadecenoic acid	18.533	0.37	1010333-58-3	83.1
7	Phytol	18.707	0.37	000150-86-7	55.1
8	Oleic acid	18.914	6.10	000112-80-1	69.1
9	9,12-Octadecadienoic acid (Z,Z)	19.063	0.51	000060-33-3	81.1
10	1,8,11-Heptadecatriene	21.538	4.59	056134-03-3	95.1
11	trans-13-Octadecenoic acid	21.826	6.01	000693-71-0	98.1
12	Linoleaidic acid	23.164	0.30	000506-21-8	81.1
13	Curan-17-oic acid	30.054	19.99	056053-15-7	69.1
14	E,E-3,13-Octadecadien-1-ol	30.081	2.06	1000131-08-9	81.1
15	1,8,11-Heptadecatriene	30.110	2.71	056134-03-3	95.1
16	Tridecanedial	31.500	1.12	063521-76-6	69.1
17	cis-13,16-Docasadienoic acid	31.762	2.15	1000333-60-3	83.1
18	4-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(20),7(16)-diene	31.985	2.89	1000155-84-7	69.1

GC–MS, gas chromatography–mass spectrometry; CAS, Chemical Abstracts Service; m/z, mass-to-charge ratio. GC–MS profiling detected 18 constituents. The largest reported chromatographic contribution was observed for curan-17-oic acid (19.99%), followed by oleic acid (6.10%), trans-13-octadecenoic acid (6.01%), and 1,8,11-heptadecatriene at a retention time of 21.538 min (4.59%). The chromatographic profile is presented in Figure 1.

Table 3. Spontaneous alternation in the Y-maze after treatment with Avena fatua extract and fractions

Group	n	Spontaneous alternation, mean ± SD (%)	Mean difference vs amnesic control	95% CI
Normal control	6	78.15 ± 3.55	41.11	36.15 to 46.07
Amnesic control	6	37.04 ± 4.12	—	—
Crd-AF 100 mg/kg	6	56.12 ± 3.16	19.08	14.32 to 23.84
Crd-AF 200 mg/kg	6	61.45 ± 3.58	24.41	19.44 to 29.38
Chl-AF 75 mg/kg	6	69.75 ± 3.53	32.71	27.76 to 37.66
Chl-AF 150 mg/kg	6	71.49 ± 4.09	34.45	29.17 to 39.73
Et-AF 75 mg/kg	6	57.17 ± 3.45	20.13	15.22 to 25.04
Et-AF 150 mg/kg	6	62.96 ± 2.87	25.92	21.28 to 30.56
Donepezil 2 mg/kg	6	80.05 ± 2.94	43.01	38.34 to 47.68

CI, confidence interval; Crd-AF, crude Avena fatua extract; Chl-AF, chloroform fraction; Et-AF, ethyl acetate fraction. SD values were derived from the reported SEM using SD = SEM × $\sqrt{n}$. Mean differences and 95% CIs compare each group with the amnesic control. Scopolamine-associated memory impairment was accompanied by a reduction in spontaneous alternation from 78.15% in the normal control group to 37.04% in the amnesic control group. All extract and fraction groups had higher spontaneous-alternation values than the amnesic control. Crd-AF produced mean differences of 19.08 percentage points (95% CI 14.32 to 23.84) at 100 mg/kg and 24.41 percentage points (95% CI 19.44 to 29.38) at 200 mg/kg. Among the tested fractions, Chl-AF produced the largest differences, reaching 34.45 percentage points (95% CI 29.17 to 39.73) at 150 mg/kg. Donepezil produced a spontaneous-alternation value of 80.05 ± 2.94%, corresponding to a difference of 43.01 percentage points (95% CI 38.34 to 47.68) relative to the amnesic control.

Table 4. Novel object recognition performance after treatment with Avena fatua extract and fractions

Group	n	Sample A1, mean ± SD (s)	Sample A2, mean ± SD (s)	Novel object, mean ± SD (s)	Familiar object, mean ± SD (s)	Recognition index, mean ± SD (%)	Difference vs amnesic control	95% CI
Control	6	18.96 ± 1.57	18.29 ± 1.76	18.72 ± 1.13	16.97 ± 1.32	52.41 ± 4.58	19.47	13.50 to 25.44
Amnesic	6	8.98 ± 1.74	7.78 ± 1.86	6.41 ± 1.22	13.03 ± 1.76	32.94 ± 4.70	—	—
Crd-AF 100 mg/kg	6	14.72 ± 1.94	14.99 ± 1.94	14.32 ± 1.69	11.33 ± 1.79	55.81 ± 4.31	22.87	17.06 to 28.68
Crd-AF 200 mg/kg	6	15.10 ± 1.10	15.03 ± 1.89	14.40 ± 1.98	10.67 ± 1.45	57.31 ± 3.94	24.37	18.76 to 29.98
Chl-AF 75 mg/kg	6	14.89 ± 2.08	15.03 ± 1.84	14.53 ± 1.94	9.29 ± 1.25	60.79 ± 4.31	27.85	22.04 to 33.66

Group	n	Sample A1, mean ± SD (s)	Sample A2, mean ± SD (s)	Novel object, mean ± SD (s)	Familiar object, mean ± SD (s)	Recognition index, mean ± SD (%)	Difference vs amnesic control	95% CI
Chl-AF 150 mg/kg	6	14.74 ± 2.42	15.15 ± 1.32	15.06 ± 1.22	9.25 ± 1.27	62.04 ± 4.75	29.10	23.02 to 35.18
Et-AF 75 mg/kg	6	14.92 ± 1.86	14.88 ± 1.79	14.44 ± 1.91	11.36 ± 1.81	57.80 ± 4.12	24.86	19.16 to 30.56
Et-AF 150 mg/kg	6	14.86 ± 1.64	14.85 ± 1.96	14.31 ± 2.13	10.22 ± 1.98	58.22 ± 4.61	25.28	19.29 to 31.27
Donepezil 2 mg/kg	6	14.97 ± 2.16	14.59 ± 1.37	14.97 ± 2.08	8.75 ± 1.42	64.12 ± 4.78	31.18	25.08 to 37.28

CI, confidence interval. SD values were derived from the reported SEM. The recognition-index values are those directly reported in the manuscript; comparisons and 95% CIs use the amnesic group as the reference. The amnesic group showed a recognition index of 32.94 ± 4.70%, compared with 52.41 ± 4.58% in the normal control group. Recognition indices were higher in all extract- and fraction-treated groups. Crd-AF produced indices of 55.81 ± 4.31% and 57.31 ± 3.94% at 100 and 200 mg/kg, respectively. Chl-AF showed the highest values among the tested fractions, with 60.79 ± 4.31% at 75 mg/kg and 62.04 ± 4.75% at 150 mg/kg. The corresponding difference for Chl-AF 150 mg/kg relative to the amnesic group was 29.10 percentage points (95% CI 23.02 to 35.18). Donepezil produced the highest reported recognition index, 64.12 ± 4.78%.

Table 5. Brain acetylcholinesterase and acetylcholine levels after treatment

Group	n	AChE, mean ± SD	Difference vs amnesic control (95% CI)	ACh, mean ± SD	Difference vs amnesic control (95% CI)
Control	6	11.90 ± 2.99	−15.96 (−20.00 to −11.92)	16.19 ± 3.04	9.98 (6.21 to 13.75)
Amnesic	6	27.86 ± 3.28	—	6.21 ± 2.82	—
Crd-AF 100 mg/kg	6	15.20 ± 2.67	−12.66 (−16.53 to −8.79)	13.11 ± 3.01	6.90 (3.15 to 10.65)
Crd-AF 200 mg/kg	6	15.12 ± 2.87	−12.74 (−16.71 to −8.77)	13.23 ± 3.48	7.02 (2.92 to 11.12)
Chl-AF 75 mg/kg	6	13.90 ± 2.62	−13.96 (−17.81 to −10.11)	13.51 ± 3.23	7.30 (3.39 to 11.21)
Chl-AF 150 mg/kg	6	13.58 ± 3.04	−14.28 (−18.35 to −10.21)	13.85 ± 3.80	7.64 (3.29 to 11.99)
Et-AF 75 mg/kg	6	14.95 ± 3.09	−12.91 (−17.01 to −8.81)	13.12 ± 3.16	6.91 (3.05 to 10.77)
Et-AF 150 mg/kg	6	15.10 ± 3.31	−12.76 (−17.00 to −8.52)	13.43 ± 3.31	7.22 (3.26 to 11.18)
Donepezil 2 mg/kg	6	11.98 ± 3.09	−15.88 (−19.98 to −11.78)	14.95 ± 3.04	8.74 (4.97 to 12.51)

AChE, acetylcholinesterase; ACh, acetylcholine; CI, confidence interval. AChE unit reported in the source manuscript: moles of substrate hydrolyzed/min/g tissue; ACh unit reported: mmol/min/mg protein. SD values were derived from the reported SEM. The amnesic group had an AChE value of 27.86 ± 3.28 compared with 11.90 ± 2.99 in the control group, whereas ACh was 6.21 ± 2.82 compared with 16.19 ± 3.04. All extract and fraction groups showed lower AChE and higher ACh values than the amnesic group. The largest treatment-associated AChE difference among the fractions occurred with Chl-AF 150 mg/kg [−14.28 (95% CI −18.35 to −10.21)], while the corresponding ACh difference was 7.64 (95% CI 3.29 to 11.99). Donepezil produced values closest to those of the normal control, with AChE of 11.98 ± 3.09 and ACh of 14.95 ± 3.04.

Table 6. Brain oxidative-stress biomarkers after treatment

Group	n	SOD, mean ± SD (U/mg protein)	Difference vs amnesic control (95% CI)	CAT, mean ± SD (U/mg protein)	Difference vs amnesic control (95% CI)	GSH, mean ± SD (µg/mg protein)	Difference vs amnesic control (95% CI)
Control	6	9.51 ± 3.60	6.35 (2.38 to 10.32)	42.25 ± 3.63	32.07 (27.72 to 36.42)	55.83 ± 3.92	25.64 (20.68 to 30.60)
Amnesic	6	3.16 ± 2.25	—	10.18 ± 3.09	—	30.19 ± 3.80	—
Crd-AF 100 mg/kg	6	9.12 ± 2.99	5.96 (2.52 to 9.40)	36.72 ± 3.28	26.54 (22.44 to 30.64)	45.24 ± 3.14	15.05 (10.55 to 19.55)
Crd-AF 200 mg/kg	6	9.19 ± 3.28	6.03 (2.34 to 9.72)	37.29 ± 2.96	27.11 (23.22 to 31.00)	45.70 ± 3.31	15.51 (10.92 to 20.10)
Chl-AF 75 mg/kg	6	9.29 ± 3.45	6.13 (2.29 to 9.97)	37.12 ± 3.63	26.94 (22.59 to 31.29)	47.42 ± 3.80	17.23 (12.35 to 22.11)
Chl-AF 150 mg/kg	6	9.75 ± 3.23	6.59 (2.95 to 10.23)	38.48 ± 2.96	28.30 (24.41 to 32.19)	49.26 ± 3.48	19.07 (14.38 to 23.76)
Et-AF 75 mg/kg	6	9.11 ± 2.57	5.95 (2.83 to 9.07)	35.73 ± 3.40	25.55 (21.36 to 29.74)	45.98 ± 3.60	15.79 (11.03 to 20.55)
Et-AF 150 mg/kg	6	9.31 ± 3.45	6.15 (2.31 to 9.99)	36.88 ± 3.72	26.70 (22.28 to 31.12)	47.45 ± 3.09	17.26 (12.78 to 21.74)
Donepezil 2 mg/kg	6	10.19 ± 3.72	7.03 (2.95 to 11.11)	39.12 ± 3.48	28.94 (24.70 to 33.18)	51.92 ± 3.72	21.73 (16.89 to 26.57)

SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; CI, confidence interval. SD values were derived from the reported SEM.

The amnesic condition was associated with lower antioxidant-marker values than the normal control, with SOD decreasing from 9.51 ± 3.60 to 3.16 ± 2.25 U/mg protein, CAT from 42.25 ± 3.63 to 10.18 ± 3.09 U/mg protein, and GSH from 55.83 ± 3.92 to 30.19 ± 3.80 µg/mg protein. All Avena fatua treatment groups showed higher values than the amnesic group. Chl-AF at 150 mg/kg produced SOD of 9.75 ± 3.23 U/mg protein, CAT of 38.48 ± 2.96 U/mg protein,

and GSH of 49.26 ± 3.48 µg/mg protein. The corresponding differences relative to the amnesic group were 6.59 U/mg protein for SOD (95% CI 2.95 to 10.23), 28.30 U/mg protein for CAT (95% CI 24.41 to 32.19), and 19.07 µg/mg protein for GSH (95% CI 14.38 to 23.76).

Table 7. Immobility time in the tail suspension and forced swim tests

Group	n	TST, mean ± SD (s)	Difference vs control (95% CI), s	FST, mean ± SD (s)	Difference vs control (95% CI), s	Reported p-value
Control	8	171.14 ± 4.02	—	181.43 ± 3.87	—	—
Imipramine 30 mg/kg	8	71.37 ± 4.33	−99.77 (−104.25 to −95.29)	83.96 ± 3.82	−97.47 (−101.60 to −93.34)	<0.001
Crd-AF 150 mg/kg	8	118.41 ± 4.21	−52.73 (−57.15 to −48.31)	138.12 ± 3.14	−43.31 (−47.11 to −39.51)	<0.001
Crd-AF 300 mg/kg	8	94.28 ± 3.59	−76.86 (−80.95 to −72.77)	121.94 ± 3.65	−59.49 (−63.53 to −55.45)	<0.001
Chl-AF 75 mg/kg	8	112.71 ± 3.79	−58.43 (−62.62 to −54.24)	118.61 ± 3.87	−62.82 (−66.98 to −58.66)	<0.001
Chl-AF 150 mg/kg	8	89.56 ± 4.27	−81.58 (−86.03 to −77.13)	112.28 ± 3.71	−69.15 (−73.22 to −65.08)	<0.001
Et-AF 75 mg/kg	8	141.18 ± 4.07	−29.96 (−34.30 to −25.62)	147.48 ± 4.64	−33.95 (−38.55 to −29.35)	<0.001
Et-AF 150 mg/kg	8	108.21 ± 4.21	−62.93 (−67.35 to −58.51)	125.94 ± 3.79	−55.49 (−59.60 to −51.38)	<0.001

TST, tail suspension test; FST, forced swim test; CI, confidence interval. SD values were derived from the reported SEM. Reported p-values are from one-way ANOVA followed by Dunnett's multiple-comparison test versus the control group. Imipramine produced the lowest immobility times in both behavioral assays. Relative to the control group, Crd-AF demonstrated a dose-related reduction in TST immobility from −52.73 s (95% CI −57.15 to −48.31) at 150 mg/kg to −76.86 s (95% CI −80.95 to −72.77) at 300 mg/kg. A similar pattern occurred in the FST, with corresponding differences of −43.31 s (95% CI −47.11 to −39.51) and −59.49 s (95% CI −63.53 to −55.45).

Among the solvent fractions, Chl-AF produced the largest reductions in immobility. At 150 mg/kg, TST immobility was 89.56 ± 4.27 s, a difference of −81.58 s (95% CI −86.03 to −77.13) relative to control, while FST immobility was 112.28 ± 3.71 s, a difference of −69.15 s (95% CI −73.22 to −65.08). Et-AF also reduced immobility at both tested doses, with larger differences at 150 mg/kg than at 75 mg/kg. All treatment comparisons in the original Table 7 were reported as $p<0.001$ versus control.

Overall, the behavioral data showed higher Y-maze spontaneous alternation and novel-object recognition indices in mice receiving the crude extract or solvent fractions than in the scopolamine-treated amnesic group. These behavioral patterns were accompanied by lower AChE, higher ACh, and higher SOD, CAT, and GSH values. Across several outcomes, Chl-AF at 150 mg/kg produced the largest numerical response among the tested Avena fatua fractions, while donepezil remained the strongest comparator for the cognition-related outcomes. In the antidepressant-like behavioral assays, reductions in immobility were observed with the crude extract and both fractions, with the largest fraction-associated reductions again observed with Chl-AF at 150 mg/kg.

DISCUSSION

The present study provides preliminary preclinical evidence that the crude methanolic extract of Avena fatua and its chloroform and ethyl acetate fractions were associated with improved performance in behavioral tests of memory, modulation of cholinergic markers, restoration of endogenous antioxidant biomarkers, and reduced immobility in two antidepressant-like behavioral assays. Among the tested fractions, Chl-AF at 150 mg/kg generally produced the largest numerical responses across the cognitive, biochemical, and behavioral outcomes, whereas donepezil and imipramine produced the strongest responses within their respective comparator paradigms. These findings support the possibility that neurobehaviorally active constituents are present in A. fatua, although the current study does not establish a specific active compound or molecular mechanism.

The improvement in Y-maze spontaneous alternation and novel-object recognition performance observed with the crude extract and fractions is consistent with previous evidence that plant-derived compounds can influence cognitive function through multiple neurobiological pathways. Flavonoids and other phytochemicals have been associated with neuroprotective activity, modulation of oxidative stress, and effects on neuronal signaling relevant to memory and learning (4,5). Flavonoids have also been reported to possess cholinesterase-inhibitory activity, providing a plausible biological link between phytochemical composition and cholinergic function (6). In the present study, lower AChE values and higher ACh values were observed in treated groups relative to the amnesic control, which is compatible with a cholinergic contribution to the observed behavioral responses. However, these data demonstrate association within the experimental model rather than direct proof that cholinesterase inhibition was the principal mechanism responsible for the behavioral effects.

The current findings are also broadly consistent with previous work involving other Avena preparations. Kennedy et al. reported changes in cognitive performance following acute administration of a wild green-oat preparation, including effects in domains related to attention and working memory (8), while Power et al. described improvements in working memory and processing speed following acute Avena sativa supplementation in healthy adults (9). These studies differ substantially from the present experiment in species, preparation, dose, experimental design, and outcome assessment, and therefore cannot be considered direct confirmation of the effects of A. fatua. Nevertheless, they provide supportive evidence that members of the Avena genus contain constituents capable of influencing neurobehavioral function.

The oxidative-stress findings provide an additional plausible explanation for the behavioral pattern. Scopolamine-treated mice showed lower SOD, CAT, and GSH values, whereas animals receiving Crd-AF, Chl-AF, or Et-AF showed values shifted toward those of the normal-control condition. Oxidative imbalance has been implicated in neuronal dysfunction, and several plant-derived flavonoids and phenolic compounds have demonstrated antioxidant and neuroprotective properties in experimental systems (4,5). Quercetin, for example, has been reported to exert neuroprotective effects in models of neurological injury and cognitive dysfunction (21). Similarly, Andrographis-derived preparations have been associated with improved cognitive performance accompanied by reductions in oxidative stress and acetylcholinesterase activity (22). The present biochemical findings are therefore biologically coherent with the broader literature, but they do not establish whether the antioxidant changes preceded, mediated, or simply accompanied the behavioral effects.

Other experimental studies of medicinal plants further support the concept that cognitive effects may arise from combined antioxidant and cholinergic mechanisms. The hydroalcoholic extract of Teucrium polium has been reported to improve cognitive performance while reducing oxidative-stress-related changes in rats (23), whereas Flos puerariae extract has been associated with restoration of antioxidant defenses and cholinergic activity in experimental cognitive impairment (24). Neurobehavioral effects have also been reported with extracts of Aloe vera and Withania somniferous in animal models of cognitive dysfunction (25). Although these findings cannot be directly extrapolated to A. fatua, they support the broader hypothesis that plant-derived mixtures containing multiple bioactive constituents may influence cognition through overlapping antioxidant, cholinergic, and neuroprotective pathways.

The phytochemical findings in the present study provide a preliminary chemical basis for these observations. HPLC detected phloroglucinol, hydroxybenzoic acid, and ascorbic acid, while GC–MS identified a broader range of constituents, including oleic acid, phytol, and several fatty-acid derivatives. Natural products constitute an important source of structurally diverse pharmacologically active compounds (3), and plant-derived phenolics and related constituents have been repeatedly investigated for potential effects on neuronal function and cognitive health (4). However, the present chromatographic analyses identified constituents in a complex extract and did not determine which compounds were biologically active. Consequently, the behavioral and biochemical effects should be attributed to the tested extract and fractions as whole preparations rather than to any individual phytochemical.

The reduced immobility observed in the FST and TST suggests antidepressant-like behavioral activity of the crude extract and fractions under the conditions tested. Both the forced-swim and tail-suspension paradigms are established screening methods for detecting behavioral responses to antidepressant agents (18–20). Nevertheless, reduced immobility in these assays should not be interpreted as evidence that the extract treats clinical depression, because these tests assess acute behavioral responses in mice rather than the full pathophysiology of human depressive disorders. The numerically greater responses observed with the higher doses of Crd-AF and Chl-AF may indicate dose-related pharmacological activity, but confirmation would require appropriately powered experiments designed specifically to test dose-response relationships.

From a translational perspective, the findings indicate that A. fatua deserves further preclinical characterization as a source of neuroactive phytochemicals. The combination of behavioral, cholinergic, and antioxidant outcomes provides a broader pharmacological profile than behavioral testing alone. However, these observations remain at an exploratory stage. Before any clinical relevance can be inferred, the active constituents would need to be isolated and chemically confirmed, their pharmacokinetic properties characterized, and their effects reproduced in independent experiments using more rigorous mechanistic and disease-relevant models.

Several limitations should be considered when interpreting the results. The behavioral groups were small, with six animals per group for the anti-amnesic experiments and eight per group for the antidepressant-like tests, and no formal sample-size justification was reported. Details of randomization, allocation concealment, and blinding were incomplete, which limits assessment of potential experimental bias. The study used crude extract and solvent fractions rather than isolated compounds, preventing attribution of activity to a specific constituent. The pharmacokinetic behavior, tissue distribution, and molecular targets of the extract were not assessed. In addition, the reported novel-object recognition index requires methodological clarification because the equation originally described in the manuscript does not mathematically reproduce the reported table values. Animal sex and several husbandry variables were also not fully reported, limiting assessment of external reproducibility. Finally, the FST and TST provide evidence of antidepressant-like behavior only and should not be interpreted as direct models of clinical depression.

Despite these limitations, the study has several strengths. It combined phytochemical characterization with multiple behavioral and biochemical outcomes, allowing the neurobehavioral findings to be examined alongside cholinergic and oxidative-stress markers. The use of both crude extract and solvent fractions also provided an initial indication that the biological activity may differ according to extract composition. Taken together, the findings support further experimental investigation of A. fatua, particularly studies designed to identify active constituents, verify dose-response relationships, clarify cholinergic and antioxidant mechanisms, and reproduce the observed effects using rigorously controlled preclinical designs.

CONCLUSION

The crude methanolic extract of Avena fatua and its chloroform and ethyl acetate fractions demonstrated preliminary anti-amnesic and antidepressant-like activity in mice, accompanied by lower acetylcholinesterase values, higher acetylcholine concentrations, and restoration of SOD, CAT, and GSH toward normal-control levels. Among the tested fractions, the chloroform fraction generally produced the strongest numerical responses, particularly at 150 mg/kg. These findings support the potential neuropharmacological relevance of A. fatua as a source of bioactive constituents, while remaining limited to the experimental animal models and outcomes assessed in this study.

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