http://doi.org/10.47626/1516-4446-2024-3541

Supplementary Table S1 Comparison of possible side effects attributable to the pharmacological intervention among NAC and placebo groups using SAFTEE in individuals with alcohol use disorder

	W1		W2		W3		W4		W5		W6		W7		W8	
SAFTEE	**Placebo (n=25)**	**NAC (n=23)**	**Placebo (n=23)**	**NAC (n=22)**	**Placebo (n=23)**	**NAC (n=21)**	**Placebo (n=22)**	**NAC (n=21)**	**Placebo (n=20)**	**NAC (n=19)**	**Placebo (n=20)**	**NAC (n=19)**	**Placebo (n=20)**	**NAC (n=17)**	**Placebo (n=18)**	**NAC (n=17)**
Question A - General Problem in the last week	14 (56.0)	9 (39.1)	15 (65.2)	10 (45.5)	8 (34.8)	10 (47.6)	8 (36.4)	5 (23.8)	4 (20.0)	5 (26.3)	4 (20.0)	3 (15.8)	6 (30.0)	7 (41.2)	3 (16.7)	5 (29.4)
Question B – Event: nausea	0	1 (4.3)	0	1 (4.5)	0	0	0	0	0	3 (15.8)	1 (5.0)	0	2 (10.0)	2 (11.8)	3 (16.7)	0
Question B – Event: vomiting	0	1 (4.3)	0	1 (4.5)	0	0	0	0	0	0	0	0	1 (5.0)	2 (11.8)	2 (11.1)	0
Question B – Event: diarrhea	1 (4.0)	5 (21.7)	0	1 (4.5)	1 (4.3)	2 (9.5)	3 (13.6)	1 (4.8)	1 (5.0)	0	5 (25.0)	1 (5.3)	3 (15.0)	2 (11.8)	1 (5.6)	1 (5.9)
Question B – Event: abdominal pain	1 (4.0)	4 (17.4)	0	3 (13.6)	1 (4.3)	2 (9.5)	1 (4.5)	1 (4.8)	0	0	4 (20.0)	0	1 (5.0)	1 (5.9)	1 (5.6)	3 (17.6)
Question B – Event: decreased appetite	3 (12.0)	2 (8.7)	3 (13.0)	3 (13.6)	3 (13.0)	2 (9.5)	3 (13.6)	1 (4.8)	1 (5.0)	1 (5.3)	1 (5.0)	1 (5.3)	1 (5.0)	3 (17.6)	0	2 (11.8)
Question B – Event: increased appetite	12 (48.0)	8 (34.8)	6 (26.1)	8 (36.4)	2 (8.7)	5 (23.8)	2 (9.1)	6 (28.6)	2 (10.0)	5 (26.3)	2 (10.0)	6 (31.6)	2 (10.0)	2 (11.8)	4 (22.2)	3 (17.6)
Question B – Event: headache	1 (4.0)	3 (13.0)	2 (8.7)	5 (22.7)	4 (17.4)	4 (19.0)	5 (22.7)	8 (38.1)	6 (30.0)	4 (21.1)	5 (25.0)	4 (21.1)	4 (20.0)	6 (35.3)	7 (38.9)	4 (23.5)
Question B – Event: dizziness	1 (4.0)	4 (17.4)	2 (8.7)	2 (9.1)	2 (8.7)	3 (14.3)	2 (9.1)	2 (9.5)	3 (15.0)	2 (10.5)	3 (15.0)	1 (5.3)	4 (20.0)	1 (5.9)	3 (16.7)	2 (11.8)
Question B – Event: fatigue	1 (4.0)	0	2 (8.7)	2 (9.1)	2 (8.7)	2 (9.5)	1 (4.5)	0	1 (5.0)	3 (15.8)	2 (10.0)	3 (15.8)	3 (15.0)	2 (11.8)	1 (5.6)	2 (11.8)
Question B – Event: anxiety	7 (28.0)	10 (43.5)	6 (26.1)	11 (50.0)	8 (34.8)	10 (47.6)	3 (13.6)	8 (38.1)	3 (15.0)	7 (36.8)	6 (30.0)	7 (36.8)	5 (25.0)	6 (35.3)	8 (44.4)	6 (35.3)
Question B – Event: insomnia	6 (24.0)	7 (30.4)	7 (30.4)	8 (36.4)	6 (26.1)	8 (38.1)	4 (18.2)	7 (33.3)	1 (5.0)	4 (21.1)	2 (10.0)	4 (21.1)	2 (10.0)	3 (17.6)	2 (11.1)	1 (5.9)
Question B – Event: drowsiness	4 (16.00)	5 (21.7)	2 (8.7)	3 (13.6)	4 (17.4)	2 (9.5)	6 (27.3)	6 (28.6)	3 (15.0)	3 (15.8)	3 (15.0)	3 (15.8)	4 (20.0)	7 (41.2)	2 (11.1)	4 (23.5)
Question B – Event: depression	3 (12.0)	4 (17.4)	4 (17.4)	2 (9.1)	3 (13.0)	4 (19.0)	3 (13.6)	1 (4.8)	2 (10.0)	3 (15.8)	2 (10.0)	4 (21.1)	2 (10.0)	4 (23.5)	2 (11.1)	5 (29.4)
Question B – Event: itching	1 (4.0)	4 (17.4)	1 (4.3)	4 (18.2)	5 (21.7)	7 (33.3)	4 (18.2)	7 (33.3)	4 (20.0)	2 (10.5)	2 (10.0)	3 (15.8)	2 (10.0)	3 (17.6)	0	3 (17.6)
Question B – Event: skin rash	0	0	0	1 (4.5)	1 (4.3)	1 (4.8)	1 (4.5)	1 (4.8)	0	3 (15.8)	2 (10.0)	2 (10.5)	0	0	1 (5.6)	2 (11.8)
Question B – Event: decreased Libido	1 (4.0)	1 (4.3)	1 (4.3)	4 (18.2)	0	3 (14.3)	1 (4.5)	1 (4.8)	0	2 (10.5)	2 (10.0)	1 (5.3)	1 (5.0)	1 (5.9)	1 (5.6)	4 (23.5)
Question B – Event: increased Libido	3 (12.0)	3 (13.0)	0	2 (9.1)	0	1 (4.8)	2 (9.1)	1 (4.8)	2 (10.0)	0	0	0	0	0	0	0

NAC = N-acetylcysteine; SAFTEE = Systematic Assessment for Treatment Emergent Effects; W = week.

http://doi.org/10.47626/1516-4446-2024-3541

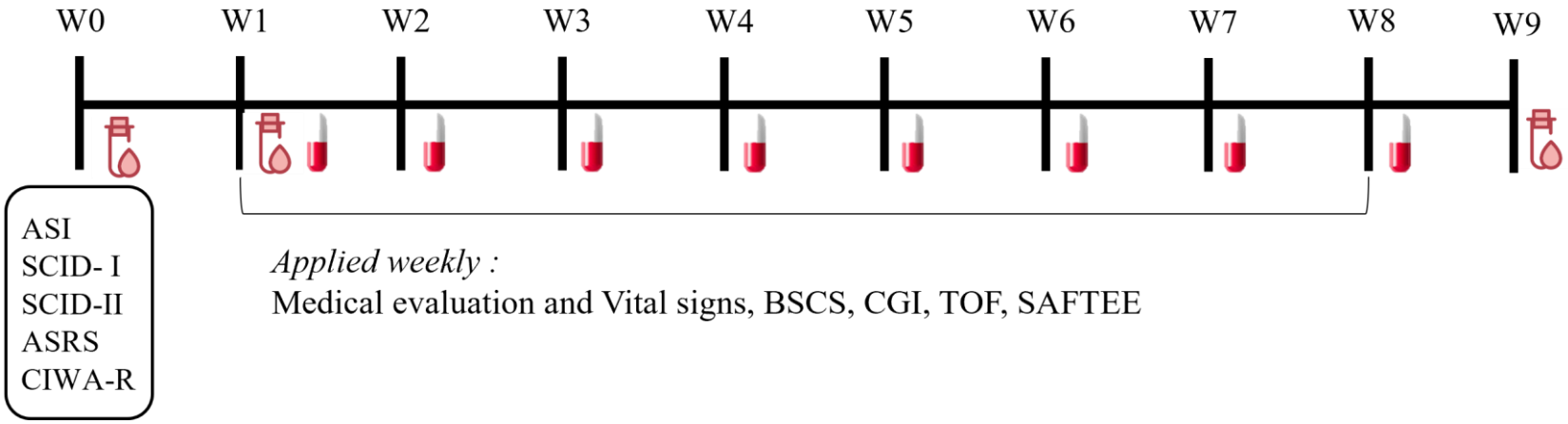
Supplementary Table S2 Assessment of the clinical blinding process with the TOF for individuals with alcohol use disorder

TOF - What treatment do you think the patient is receiving?	W1		W2		W3		W4		W5		W6		W7		W8	
	Placebo (n=22)	**NAC (n=23)**	**Placebo (n=23)**	**NAC (n=22)**	**Placebo (n=23)**	**NAC (n=20)**	**Placebo (n=22)**	**NAC (n=20)**	**Placebo (n=21)**	**NAC (n=20)**	**Placebo (n=20)**	**NAC (n=19)**	**Placebo (n=19)**	**NAC (n=17)**	**Placebo (n=18)**	**NAC (n=17)**
Researcher 1																
NAC	7 (31.8)	10 (43.5)	8 (34.8)	9 (40.9)	11 (47.8)	6 (30.6)	9 (40.9)	10 (50.0)	10 (47.6)	9 (45.0)	12 (60.0)	11 (57.9)	11 (57.9)	9 (52.9)	6 (33.3)	10 (58.8)
Placebo	15 (68.2)	13 (56.5)	15 (65.2)	13 (59.1)	12 (52.2)	14 (70.0)	13 (59.1)	10 (50.0)	11 (52.4)	11 (55.0)	8 (40.0)	8 (42.1)	8 (42.1)	8 (47.1)	12 (66.7)	7 (41.2)
	Placebo (n=22)	**NAC (n=20)**	**Placebo (n=22)**	**NAC (n=22)**	**Placebo (n=21)**	**NAC (n=21)**	**Placebo (n=21)**	**NAC (n=21)**	**Placebo (n=19)**	**NAC (n=18)**	**Placebo (n=20)**	**NAC (n=19)**	**Placebo (n=18)**	**NAC (n=15)**	**Placebo (n=15)**	**NAC (n=16)**
Researcher 2																
NAC	4 (18.2)	9 (45.0)	8 (36.4)	9 (40.9)	9 (42.9)	12 (57.1)	12 (57.1)	11 (52.4)	12 (63.2)	9 (50.0)	12 (63.2)	9 (56.3)	9 (50.0)	13 (86.7)	11 (73.3)	10 (62.5)
Placebo	18 (81.8)	11 (55.0)	14 (63.6)	13 (59.1)	12 (57.1)	9 (42.9)	9 (42.9)	10 (47.6)	7 (36.8)	9 (50.0)	7 (36.8)	7 (43.8)	9 (50.0)	2 (13.3)	4 (26.7)	6 (37.5)
	Placebo (n=23)	**NAC (n=23)**	**Placebo (n=23)**	**NAC (n=22)**	**Placebo (n=23)**	**NAC (n=21)**	**Placebo (n=22)**	**NAC (n=21)**	**Placebo (n=21)**	**NAC (n=20)**	**Placebo (n=20)**	**NAC (n=19)**	**Placebo (n=20)**	**NAC (n=17)**	**Placebo (n=18)**	**NAC (n=17)**
Patient																
NAC	19 (82.6)	14 (60.9)	20 (87.0)	14 (63.6)	19 (82.6)	14 (66.7)	19 (86.4)	15 (71.4)	18 (85.7)	15 (75.0)	17 (85.0)	13 (68.4)	17 (85.0)	15 (88.2)	16 (88.9)	14 (82.4)
Placebo	4 (17.4)	9 (39.1)	3 (13.0)	8 (36.4)	4 (17.4)	7 (33.3)	3 (13.6)	6 (28.6)	3 (14.3)	5 (25.0)	3 (15.0)	6 (31.6)	3 (15.0)	2 (11.8)	2 (11.1)	3 (17.6)

NAC = N-acetylcysteine; TOF = Treatment Opinion Form; W = week.

http://doi.org/10.47626/1516-4446-2024-3541

Supplementary Figure S1 Flowchart of clinical, psychiatric, and psychological assessments (W0 indicates the first week of hospitalization, W1 the second week of hospitalization and the first week of pharmacological treatment, and so on until W8)



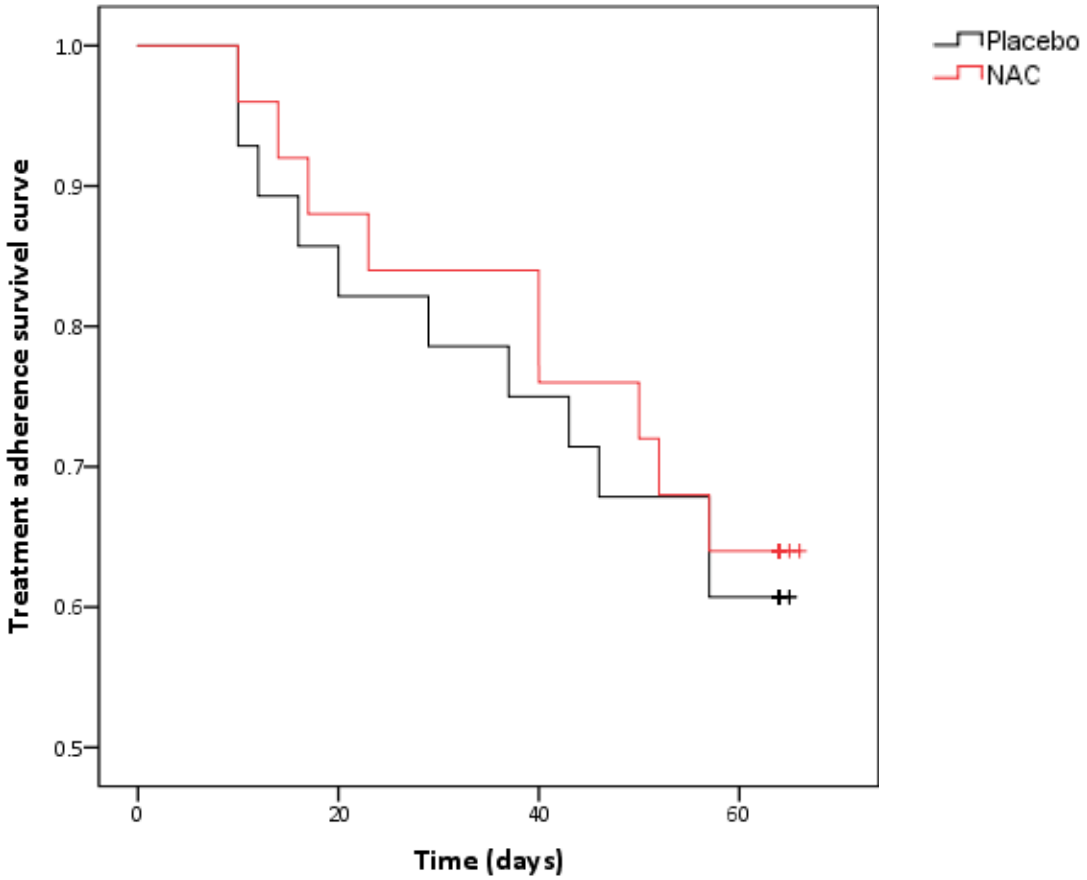
ASI = Addiction Severity Index; ASRS = Adult Self-Report Scale; BSCS = Brief Substance Craving Scale; CGI = Clinical Global Impression; CIWA-R = Clinical Institute Withdrawal Assessment for Alcohol Scale; SAFTEE = Systematic Assessment for Treatment Emergent Effects; SCID-I = Structured Clinical Interview for DMS-IV Axis I Disorders; SCID-II = Structured Clinical Interview for DMS-IV Axis II Disorders; TOF = Treatment Opinion Form.

Supplementary Table S3 Comparison of the intensity and frequency of craving symptoms in the NAC and placebo groups

Craving – BSCS	W0		W1		W2		W3		W4		W5		W6		W7		W8	
	Placebo (n=25)	NAC (n=24)	Placebo (n=24)	NAC (n=23)	Placebo (n=23)	NAC (n=22)	Placebo (n=23)	NAC (n=21)	Placebo (n=22)	NAC (n=21)	Placebo (n=21)	NAC (n=19)	Placebo (n=20)	NAC (n=19)	Placebo (n=20)	NAC (n=17)	Placebo (n=18)	NAC (n=17)
Intensity - mild-to-moderate in the last 24 hours	6 (24.0)	10 (41.7)	0	8 (34.8)*	2 (8.7)	4 (18.2)	1 (4.3)	4 (19.0)	2 (9.1)	3 (14.3)	4 (19.0)	3 (15.8)	4 (20.0)	5 (26.3)	6 (30.0)	6 (35.3)	9 (50.0)	4 (23.5)
Frequency - at least once in the last 24 hours	8 (32.0)	9 (37.5)	0	9 (39.1)**	3 (13.0)	4 (18.2)	3 (13.0)	5 (23.8)	4 (18.2)	4 (19.0)	3 (14.3)	3 (15.8)	5 (25.0)	5 (26.3)	6 (30.0)	6 (35.3)	10 (55.6)	4 (23.5)

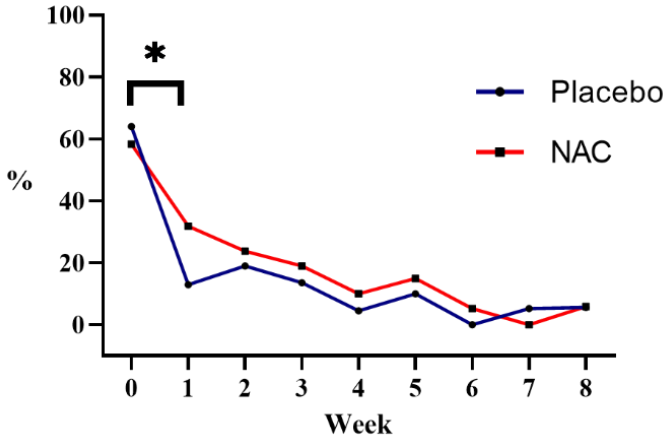
BSCS = Brief Substance Craving Scale; NAC = N-acetylcysteine; W = week.
* p = 0.002; ** p = 0.001.

Supplementary Figure S2 Kaplan-Meier survival curve for treatment adherence (p-value = 0.769)



NAC = N-acetylcysteine.

Supplementary Figure S3 Comparison of Clinical Global Impression scale results according to intervention group



NAC = N-acetylcysteine.
Values indicated the percentage of individuals with severe clinical condition.
* p < 0.001

http://doi.org/10.47626/1516-4446-2024-3541

Supplementary Table S4 Clinical Global Impression scale results in patients with alcohol use disorder

CGI		Severity, %			Global improvement, %		
	n†	Placebo	NAC	p-value	Placebo	NAC	p-value
Week 0	25/24	64.0	58.3	0.909	92.0	91.7	0.999
Week 1	23/22	13.0	31.8	0.248	100	95.5	0.982
Week 2	21/21	19.0	23.8	0.999	90.5	90.5	0.999
Week 3	22/21	13.6	19.0	0.946	95.5	85.7	0.566
Week 4	22/20	4.5	10.0	0.932	95.5	95.0	0.999
Week 5	20/20	10.0	15.0	0.999	100	95.0	0.999
Week 6	20/19	-	5.3	0.979	95.0	94.7	0.999
Week 7	19/17	5.3	-	0.999	89.5	88.2	0.999
Week 8	18/17	5.6	5.9	0.999	88.9	94.1	0.999

CGI = Clinical Global Impression; NAC = N-acetylcysteine.
The severity values indicate the percentage of patients with a moderately to extremely severe clinical condition. The global improvement values indicate the percentage of patients in slightly to much better clinical condition. Dashes indicate that no patients presented this clinical picture.
† n = sample size for the placebo/NAC groups.

http://doi.org/10.47626/1516-4446-2024-3541

Supplementary Table S5 Biomarker assessment in individuals with alcohol use disorder

	Placebo			NAC			p-value		
	Week 0 (n=28)	Week 1 (n=28)	Week 8 (n=18)	Week 0 (n=25)	Week 1 (n=28)	Week 8 (n=17)	Interaction	Group	Time
Creatinine (mg/dL)	0.86±0.03[1]	0.98±0.04[2]	0.88±0.04[1,2]	0.84±0.04[1]	0.98±0.04[2]	0.96±0.04[2]	0.268	0.640	**<0.001**
Urea (mg/dL)	23.33±1.1[1]	35.46±2.1[2]	26.58±1.8[1]	23.90±1.1[1]	33.63±2.0[2]	30.28±1.8[2]	0.129	0.670	**<0.001**
GGT (U/L)	125.52±21.9[1]	114.31±18.2[1]	41.24±5.9[2]	198.49±66.0[1]	132.00±33.3[1]	43.32±7.9[2]	0.216	0.408	**<0.001**
AST (U/L)	80.07±17.6[1]	57.43±10.8[1]	19.81±1.4 [2]	62.56±8.8[1]	37.06±4.6[2]	22.14±2.1[3]	0.154	0.185	**<0.001**
ALT (U/L)	60.68±15.0[1]	61.21±15.9[1]	20.17±2.4[2]	47.84±5.4[1]	55.27±8.1[1]	29.77±5.2[2]	0.407	0.761	**<0.001**
GPx (nmol/min/mL)	72.67±13.1	54.38±3.7	62.09±5.5	63.94±3.7	56.71±5.6	60.14±5.6	0.611	0.723	0.051
GSSG (µmol/L)	0.63±0.09[a,1]	0.52±0.08[1,2]	0.37±0.06 [2]	0.41±0.06[b]	0.47±0.08	0.35±0.07	0.230	0.241	**0.021**
GSH (µmol/L)	2.03±0.4	2.08±0.4	1.97±0.3	2.43±0.4	2.71±0.4	2.46±0.5	0.879	0.348	0.702
Total glutathione (µmol/L)	1.55±0.2	1.38±0.2	1.32±0.2	1.57±0.2	1.71±0.3	1.58±0.3	0.433	0.518	0.694
SOD (U/mL)	2.35±0.2	2.70±0.2	2.38±0.1[a]	2.34±0.1[1,2]	2.46±0.1 [1]	2.02±0.1[b,2]	0.210	0.212	**0.001**
TBARS (µM)	1.09±0.1	1.08±0.2	1.37±0.2	1.14±0.1	0.78±0.1	0.99±0.2	0.250	0.170	0.161
Carbonyl (nmol/mg)	5.88±0.5	5.60±0.4	5.33±0.3	4.78±0.4	5.34±0.5	4.94±0.5	0.417	0.269	0.576
BDNF (ng/mL)	10.71±1.0	11.41±1.0	10.85±1.6	11.18±1.3	9.93±1.3	8.94±1.5	0.360	0.519	0.606
NPY (pg/mL)	127.39±14.9	115.48±12.6	144.33±18.2	141.46±12.0[1]	128.22±14.5 [1]	169.83±17.0[2]	0.756	0.349	**0.001**
IL-1ß (pg/mL)	11.73±2.1	13.91±2.5	9.88±2.1	15.05±2.8	16.88±2.7	15.73±2.5	0.718	0.148	0.346
IL-2 (pg/mL)	13.43±2.5	15.75±3.1	12.28±2.3	21.70±4.6	20.52±3.8	12.29±2.5	0.342	0.223	0.066
IL-6 (pg/mL)	15.26±2.5	16.99±3.3	15.27±2.6	17.87±3.5	17.73±3.2	16.36±2.9	0.804	0.707	0.597
IL-10 (pg/mL)	4.44±0.6	4.56±0.7	3.99±0.6	6.08±0.8	5.68±0.8	5.08±0.5	0.456	0.172	0.078
TNF-α (pg/mL)	14.23±1.5	15.06±2.3	13.86±2.3	16.42±2.6	15.76±2.1	15.61±2.5	0.802	0.542	0.941

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BDNF = brain-derived neurotrophic factor; GGT = gamma-glutamyl transferase; GPx = glutathione peroxidase; GSH = reduced glutathione; GSSG = oxidized glutathione; IL = interleukin; NPY = neuropeptide Y; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive substances; TNF = tumor necrosis factor.
Data presented as mean and standard error.
[a,b] Superscript letters indicate differences between groups. [1,2,3] Superscript numbers indicate differences within groups.