Anti-Alcoholism Evaluation of Vachadi Ghrita

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Abstract:

The symptoms of alcohol withdrawal syndrome include irritability, aggression, irregularities in body posture and motor function, sensory hypersensitivity, and changes in the levels of different enzymes. In this situation, *Vachadi Ghrita* reduces alcohol cravings by crossing the blood-brain barrier. The Wistar rats received a liquid diet containing 7.2% v/v ethanol for 21 days. Animals in the control group were fed a regular diet and saline. Rats were monitored for anxiety and hyper locomotion, two of the most prominent withdrawal symptoms. With the aid of the raised plus maze, light, and dark models, withdrawal-related anxiety from ethanol was examined. The biochemical analyzer was used to determine the level of liver enzymes.

Keywords: *Vachadi Ghrita*, withdrawal syndrome, anxiety, hyperlocomotor activity, enzymes.

INTRODUCTION:

Worldwide, alcoholism continues to rank among the worst abuse issues. In psychiatric patients, depression and alcoholism are more prevalent. Many patients who are at risk for alcoholism have depressive symptoms. Alcohol dependency is a chronic, recurrent brain condition brought on by excessive consumption.**[1,2]** An ethanol withdrawal syndrome is brought on by stopping a chronic alcohol intake regimen. Alcohol withdrawal syndrome is a potentially fatal illness that develops when severe alcohol intake is abruptly stopped. After stopping drinking for 6 to 24 hours, withdrawal symptoms become apparent **[3].** Long-term alcohol consumption reduces the amount of inhibitory GABA receptors, and in response, there is an increase in the number of excitatory NMDA receptors additive individuals. These neuroadaptations are hidden by ethanol abstinence, which causes alcohol withdrawal symptoms. **[4,5]** The use of herbs as a kind of alcoholism treatment has a long history. In the past, people have used plants to cure diseases associated to alcohol consumption for many hundreds of years. Numerous plants, such as grapes, bitter gourd, apples, celery, dates, almonds, evening primrose, ginseng, milk thistle, dandelion, skullcap, and licorice roots, are among the herbal treatments for alcohol addiction. They offer effective treatments with few side effects **[6].** Since they work better with the human body, have fewer side effects, and provide more desirable results than conventional pharmaceuticals, herbal medicines are now widely acknowledged as the primary source of therapies **[7].** The present work deals to study the healing effects of *Vachadi Ghrita* on the withdrawal symptoms of alcohol.

2. MATERIALS AND METHODS

2.1. Preparation of Ghrita:

Vachadi ghrita was prepared as per standard Ayurvedic protocol. Fine powders of all herbal drugs were made into paste form by adding water, the solid mass is then added to cow ghee which was previously heated and cooled down. Mixture was stirred and then water was added as per quantity given in formula. Fine powders of all herbal drugs were made into paste form by adding water, the solid mass was then added to cow ghee which was previously heated and cooled down. Mixture was stirred and then water was added as per quantity given in formula.

Whole mixture was heated to evaporate total water content and to achieve extraction of active components of drugs in lipid base. *Ghrita* was then filtered and stored in dry containers.**[8,9]**

2.2. **Ghrita:** Following that, ghrita was extracted using 70% v/v ethanol at 50°C for two days. The alcoholic solvent was then concentrated using a rotary evaporator while under reduced pressure. All of the chemicals were procured from Sigma Aldrich Ltd. and used in PSIT's lab.

Animals: Male Wistar Rats weighing 150–200 grams were housed in the PSIT Animal House in Kanpur with a 12-hour day and night cycle provided and maintained. They were given a typical pellet diet along with enough water to drink at any time**.**

2.3. Acute Toxicity Studies:

The experiment's acute toxicity investigations, which dealt with doses ranging from 1000 to 4000 mg/kg, did not cause any anomalous behaviour or animal deaths until 14 days into the study's 14-day observation period. In Wistar rats, *Vachadi Ghrita* was shown to be harmless. To Wistar rats, doses ranging from 1000 to 4000 mg/kg were given. For the first four hours and then for the following 72 hours, they were monitored for mortality, eye pupil size, skin tone, and other factors.**[10,11]**They were watched for symptoms like as tremors, movements, body postures, absorbance, rearing, righting reflex, pain, touch response, etc. after taking the extract for seven days. They were examined after treatment for any obvious indications of aberration in behaviour, eating patterns, locomotor activity, and any other form of behaviour.**[13]**

2.4. Study Design:

Six groups of animals, as in table 1, each with six animals housed separately, were created: the normal group, the illness control group, the standard control group, and the three treatment groups. The rats were kept individually and fed ethanol as a modified liquid diet for chronic ethanol exposure. According to past studies, a liquid diet was used during alcohol treatment. **[14]** The liquid meal was produced in accordance with earlier research that were accessible, using 920 mL of cow's milk, 25–70 mL (95%), and 18 grams of sugar. At the start of the investigation, animals were given a liquid food without alcohol for 7 days. Then, for the following 3 days, 2.5% ethanol was administered along with the liquid diet. For the following four days, the ethanol concentration was increased to 5%.**[15,16]**

Table 1. Experimental Design for studying the healing effects of *Vachadi ghrita* on ethanol withdrawal syndrome in Wistar rats.

Group Number	**Dosing**
Group I (Normal Control)	Animals receiving saline (No alcoholism induced)
Group II (Disease Control)	Animals induced with alcoholism but not given any treatment
Group III (Standard)	Animals (induced with alcoholism) receiving diazepam (2 mg/kg, i.p) – Standard Drug
Group IV (Treated 1)	Animals (induced with alcoholism) receiving 370 mg of *Cow Ghrita*.
Group V(Treated 1)	Animals (induced with alcoholism) receiving ghrita dose of 200mg/kg.
Group VI ((Treated 3)	Animals (induced with alcoholism) receiving VG dose of 370 mg mixed with 370 mg Sugar (as per *Anupana* mentioned in Ayurvedic Formulary).

3.1. Elevated Plus-maze Model

The apparatus consists of a central platform elevated by 50 cm, two open and two closed arms positioned in opposition to one another, and the gadget. On the middle platform, each animal was kept separately and was facing the open arm. For 5 minutes, the total number of entries in the open arms, the total amount of time spent there, and the total number of entries in the closed arms were all recorded **[17,18].**

3.2. Locomotor Activity

Using a digital Actophotometer, the animals' individual spontaneous locomotion was captured for 5 minutes. Digital data was collected on the number of times the animal crossed a light beam that struck the photocell **[19].**

3.3. Ethanol Consumption and Body Weight Changes in Animals

In ethanol-fed rats, daily alcohol consumption ranged from (13.280.89) to (16.410.64) g/kg, with no discernible difference between the groups. At the beginning of the study, the control group's body weight was (225.714.56 gms) and the ethanol-fed group's was (231.305.49) gms, respectively. At the conclusion of the study, the control group's body weight was (262.345.06 gms) and the ethanol-fed group's was (242.146.01 gms). After the study, rats in the ethanol control group gained 4.6% of their baseline body weight, whereas control group rats gained 16.2%. As alcohol reduces the release of digestive enzymes and affects nutritional absorption, metabolism, and excretion, the change in the animals' body weight is consistent with past studies **[20,21,22].**

3.4 Forced Swim Test

After 21 days, the diet with ethanol in it was switched out for one without. Rats were administered with ghrita (100, 200, or 300 mg/kg, oral), diazepam, and saline 30 minutes before ethanol withdrawal tests. The animals were then checked for withdrawal symptoms, such as forced swim test models, at the sixth and twenty-fourth hours of the withdrawal period**. [23-25]** Animals were put back in their own cages after observation times. Each group received a second dose of the original medication 24 hours after the initial dose. Animals in the control group, which received a liquid diet devoid of ethanol, were likewise assessed for withdrawal symptoms. [**26]**

3.5. Changes in the Various Liver Enzyme Levels

The liver is the main organ in charge of metabolizing alcohol, and it is readily damaged by alcohol. Alcoholic hepatitis, an inflammation of the liver, is a more serious and lethal disorder that can be brought on by a pattern of heavy drinking**.[27]** The best indicators of the condition include heavy alcohol usage, specific physical symptoms, and positive laboratory results for liver disease. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) laboratory tests are frequently used for diagnosis. If the ratio of AST to ALT is more than 2, as it is in the majority of people with alcoholic liver disease, then liver disease is the most likely diagnosis. Alcohol can raise triglycerides (TG) in the body since it is high in calories and sugar**.[28,29]**

3.6. Serum Biochemical Assays

Blood samples were taken from the rats' retrobulbar blood arteries. After storing the samples at room temperature for an hour, the serum was separated using an R-4C Centrifuge from REM by centrifuging them at 1500 rpm for 10 minutes at 4 °C. After that, centrifugation was used to measure the serum ALT, AST, TG, and TC activity **[30-32].** Briefly, the samples were combined with a buffer solution to determine the levels of ALT and AST. The absorbance was measured after 5 minutes of incubation at room temperature. The final ALT and AST statistics were displayed as U/L. The materials were put into a 96-well plate containing substrates or a buffer solution for the evaluation of TG and TC. The plate was then examined after 10 minutes of incubation at 37 °C**.[33,34]**

4. RESULTS & DISCUSSION

4.1. Elevated Plus Maze Test

The time spent in open arms, as well as the number of entries into open arms and closed arms, significantly decreased in ethanol-fed rats when compared to the normal control group, while time spent in closed arms significantly increased on the sixth and twenty-fourth hours of the withdrawal period. In contrast to the disease control rats, treatment with *ghrita* at the sixth and twenty-fourth hours of alcohol withdrawal significantly increased the time spent in open arms and the number of entries in the open arms and closed arm, while it significantly decreased the time spent in closed arms, as in table 2.. **[35,36]**

Table 2 Effect of treatment of alcohol withdrawal anxiety using elevated plus maze model.

Group	No. of entries in open arm (6 hr)	No. of entries in open arm (24 hr)	Time spent in open arm (6 hr) in sec	Time spend in open arm (24 hr) in sec	No. of entries in closed arm (6 hr)	No. of entries in closed arm (24 hr)	Time spent in closed (6 hr) arm in sec	Time spent in closed (24 hr) arm in sec
Control	10±0.23	7	100±2.41	103±3.15	9±1.35	8±2.36	50±2.46	47±1.42
Disease Control	2±3.25 *	1±1.49 *	31±3.46 *	37±5.21 *	12±2.16 *	14±4.16 *	110±2.01 *	122±0.59 *
Standard (Diazepam)	10±0.84 **	8±1.5 **	90±4.12 **	100±2.25 **	8±1.36 **	7±1.56 **	54±1.36 **	56±1.25 **
Treated Group 1	7±1.36 **	6±1.29 **	90±3.39 **	91±3.12 **	5±2.35 **	4±2.34 **	55±1.45 **	64±1.38 **
Treated Group 2	8±2.63 **	7±3.15 **	94±2.16 **	96±3.55 **	7±2.13 **	6±3.26 **	54±2.16 **	54±2.16 **
Treated Group 3	9±1.16 **	7±2.94 **	98±1.35 **	100±2.56 **	8±3.48 *	7±2.49 *	51±2.46 **	45±3.23 **

Effect of *ghrita* on alcohol withdrawal anxiety using elevated plus maze model. Data expressed as mean ± SEM, n = 6. *Values were statistically significant (P<0.05) compared to control group.

**Values were statistically significant (P<0.05) compared to disease control group.

4.2. Locomotor Activity

When compared to the animals in the control group, rats fed alcohol displayed significantly higher levels of locomotor activity throughout the sixth and twenty-fourth hours of alcohol

withdrawal. At the sixth and twenty-fourth hours of alcohol withdrawal, *ghrita* (100, 200, and 300 mg/kg, oral) had notable suppressive effects on locomotor hyperactivity. The results of our investigation demonstrated the ghrita inhibitory effects on the withdrawal symptoms of ethanol in alcohol-dependent rats. **[37]** Previous research showed that daily ethanol consumption causes dependence in rats, and that cessation causes a variety of signs and symptoms. In this study, we noted several indicators of alcohol withdrawal, including. As indicated in Table 3, ghrita at doses of 100, 200, and 300 mg/kg significantly restored the actophotometer test's normal locomotor hyperactivity. **[38]** The glutamatergic system and NMDA receptors are key players in the symptoms of alcohol withdrawal. *Ghrita's* phytoconstituents may help mitigate the effects of ethanol deprivation and play a preventive role in the ethanol abstinence syndrome. The Student's t-test was used to analyse the changes in body weight between the rats in the alcohol control group and the animals in the normal group. Actophotometer was used to measure the effect of *ghrita* on Sensory Hyper Reactivity (locomotor activity). **[39]**

Table 3. Effect of *Ghrita* on the Sensory Hyper reactivity (locomotor activity) tested by using Actophotometer

Group	**Counts per 5 minutes**	
	6 hours	**24 hours**
Control	211±2.31	206±3.35
Disease Control	390±3.51∗	418±3.24∗
Standard (Diazepam treated)	244±2.41∗∗	205±3.38∗∗
Treated Group 1	250±2.93∗∗	240±2.35∗∗
Treated Group 2	245±1.35∗∗	234±1.39∗∗
Treated Group 3	220±2.13∗∗	214±3.42∗∗

Effect of *Ghrita* on **Sensory Hyper reactivity (locomotor activity) tested by using Actophotometer**. Data expressed as mean ± SEM, n = 6. *Values were statistically significant (P<0.05) compared to control group. **Values were statistically significant (P<0.05) compared to disease control group

5.3.4.1. . Alcohol intake and body weight deviations in animals

Student's t-test was applied to investigate body weight variations as shown in table 4, of alcohol control rats in comparison to normal group animals.

Table 4. Body Weight Changes at initiation and conclusion of study (values are in ±SEM)

Group	**Body Weight (initial) (in grams)**	**Body Weight (post treatment) (in grams)**
Control	200.41±3.26	237.20±2.52
Disease Control	205.13±2.81	220.23±3.24*
Standard (Diazepam)	201.23±1.33	234.12±1.37**
Treated Group 1	203.17±1.81	235.23±2.77**
Treated Group 2	206.21±1.24	240.52±1.52**
Treated Group 3	209.13±0.29	243.11±0.73**

Effect of *Ghrita* on Body Weight Changes.

Data expressed as mean ± SEM, n = 6. *Values were statistically significant (P<0.05) compared to control group. **Values were statistically significant (P<0.05) compared to disease control group.

Alcohol withdrawal symptoms:

As shown in table 5, in the form of tremors in ethanol dependent rats.

Table 5. Effects of *Ghrita* on tremors in ethanol dependent rats

Group	Observation Intervals		
	2 hr	4 hr	6 hr
Control	0.00 (0/8)	0.00 (0/8)	0.00 (0/8)
Disease Control	50.0 (4/8)*	50.00 (4/8)*	25.0 (2/8)*
Standard (Diazepam)	25.0 (2/8)**	12.5 (1/8)**	12.5 (1/8)**
Treated Group 1	12.5 (1/8)**	25.0 (2/8)**	12.5 (1/8)**
Treated Group 2	12.5 (1/8)**	12.5 (1/8)**	12.5 (1/8)**
Treated Group 3	12.5 (1/8)**	0.00 (0/8)**	0.00 (0/8)**

Effects of *Ghrita* on the incidence of the tremors in ethanol dependent rats on **Body Weight Changes**. Data expressed as mean ± SEM, n = 6. *Values were statistically significant (P<0.05) compared to control group. **Values were statistically significant (P<0.05) compared to disease control group.

Forced Swim Test :

Ethanol-fed rats showed a expressivel increased immobility time in the forced swim test as shown in table 6, on the 6th and 24th hour of the withdrawal period as compared with the normal saline group. Treated 1, Treated 2, Treated 3 and Diazepam at the 6th and 24th hour of ethanol withdrawal produced the significant decrease in the immobility time as compared to the disease control rats**.[40]**

Table 6. Effects of *Ghrita* on Immobility time in FST (values are in ±SEM)

Group	**Time in sec (6 hours)**	**Time in sec (24 hours)**
Control	45.21±1.14	46.13±1.21
Disease Control	192.23±1.43*	251.19±2.71*
Standard (Diazepam)	54.13±2.16**	53.01±2.13**
Treated Group 1	62.24±3.02**	61.12±1.31**
Treated Group 2	53.03±1.14**	54.03±0.29**
Treated Group 3	49.45±1.19**	47.21±2.31**

Data expressed as mean ± SEM, n = 6. Values were statistically significant (P<0.05) compared to control group. Values were statistically significant (P<0.05) compared to disease control group.

VG = D. *Ghrita*, NC = Normal control, DC = Disease control**.**

4.2. Serum Biochemical Enzymes

Early levels of ALT, AST, TG, and TC are present in the serum.

AST is a mitochondrial enzyme that is secreted by the body's liver and other organs, ALT is a cytosolic enzyme that is mostly found in the cytoplasm of cells. When liver cells are damaged, ALT and AST are released into the blood, raising serum transaminase. Meanwhile, the liver fat formation brought on by alcohol use is indicated by the levels of TG and TC **[41].** The serum levels of ALT, AST, TG, and TC in the ethanol group significantly rose in comparison to the control group, as shown in Table 7. AST and TG levels significantly decreased between low-dose and high-dose treatments**.[42]**

Table 7. Changes in bio-chemical enzymes (values are in ±SEM)

Group	**ALT (U/L)**	**AST (U/L)**	**TG (umol/L)**	**TC(umol/L)**
Control	41.23±2.02	90.21±3.14	4.2±2.02	7.1±1.34
Disease Control	62.12±1.07*	111.27±1.37*	6.5±1.21*	11.3±2.51*
Standard (Diazepam)	44.81±1.32**	97.02±1.81**	3.9±1.32**	7.9 ±2.12**
Treated Group 1	55.11±2.18**	98.01±2.12**	4.5±0.13**	8.2±1.01**
Treated Group 2	49.10±1.42**	96.1±0.21**	4.4±0.53**	7.9±0.32**
Treated Group 3	43.17±0.12**	92.13±1.1**	4.3±2.36**	7.3±2.81**

Effects of *Ghrita* on levels of ALT, AST, TG, and TC. Data expressed as mean ± SEM, n = 6. Values were statistically significant (P<0.05) compared to control group. **Values were statistically significant (P<0.05) compared to disease control group.

CONCLUSION

The current study concluded by demonstrating that the Ghrita treats depression-like behaviour and causes locomotor hyperactivity in alcohol-dependent rats. Therefore, by reducing the signs and symptoms of ethanol withdrawal, it may have therapeutic promise in the treatment of ethanol-type dependence. Rats exhibiting depressive-like symptoms of ethanol withdrawal respond favorably to the treatment. Ghrita also has benefits over chemically based alcohol addiction treatments because it is made from all-natural ingredients that don't build habits and have no negative side effects. As the dose is increased, data from the models mentioned above showed that the curative effects grow.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE Approval no. 1273/PO/Re/S/19/CPCSEA was granted to the study, approved by the Institutional animal ethical committee.

HUMAN AND ANIMAL RIGHTS No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION No humans were used in this study. Research work and all procedures carried out on the animals were according to OECD guidelines. Animals were used after proper approval by the Institutional animal ethical committee.

AVAILABILITY OF DATA AND MATERIALS All the findings and data of results are mentioned in the tables.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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