IJPSR (2023), Volume 14, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 May 2022; received in revised form, 10 August 2022; accepted, 19 November 2022; published 01 February 2023

AMELIORATIVE EFFECT OF *KALYANAKGHRITA* (KG) ON WITHDRAWAL SYMPTOMS IN ALCOHOL-DEPENDENT MICE

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Keywords:

Alcohol dependence, Withdrawal symptoms, *KalayanakaGhrita*, Addiction

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ABSTRACT: Introduction: Alcohol de-addiction is challenging due to the severe alcohol withdrawal symptoms. This article reports ameliorative effect of *KalyanakGhrita* (KG) on ethanol withdrawal symptoms in animal model. **Material and Methods:** After approval from Animal Ethics committee, alcohol dependence was developed in pre Rota road trained Swiss mice by intermitted access of 20% ethanol in 14 days. After confirmation of dependence, the animals were allocated in 6 groups, 6 animals in each group. Group I &II received distilled water & ethanol respectively. Group III to VI received cow ghee, KG in two dose level and Naltrexone respectively along with ethanol till next 14 days. Further alcohol abstinence was given but treatment drugs were continued for next 7 days. To note the effect of KG on withdrawal symptoms, animals was introduced to Rota rod on 0 day and 3rd day for muscular incoordination and to EPM on day 0 day & 7th day after abstinence of ethanol for anxiety assessment. **Results:** KG higher and lower dose demonstrated significant reduction in anxiety with the significance level of $p< 0.001$, $p<0.01$respectively whereas in muscular coordination KG showed reduction but statistically non-significant. General physical appearance and behavior of KG treated animals was similar to DW group. **Discussion:** The main measurable parameters of alcohol withdrawal are anxiety and muscular incoordination where KG showed ameliorative effect. The anticonvulsant, neuroprotective action of ingredients of KG may have contributed the results. **Conclusion:** KG effectively reduces the severity and duration of withdrawal symptoms in ethanol dependent Swiss albino mice.

INTRODUCTION: Alcohol is a curse to society from ancient period that has wreaked countless lives. Supporting traces of references mentioning physical and social ill health effects due to inappropriate consumption of alcohol are found in Ayurveda classics as well [1]. According to the National Survey on Drug Use and Health [NSDUH] conducted in 2019, among the reported 85,688 deaths due to liver disease, alcohol contributes 43 percent of deaths.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(2).756-65

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(2).756-65

The harmful use of alcohol is one of the leading risk factors for population health worldwide and has a direct impact on many health-related targets of the Sustainable Development Goals (SDGs), including those for maternal and child health, infectious diseases (HIV, viral hepatitis, tuberculosis), non-communicable diseases and mental health, injuries, and poisonings.

A research study suggests that, abstinence of alcohol in alcohol-dependent subjects led to greater mortality reduction than non-abstinence. Thus alcohol-dependent subjects can significantly reduce their mortality risk by reducing alcohol consumption [2]. The brain's reward system gets set into overdrive, causing the extremely high release of dopamine and other neurotransmitters. With repeated use, the reward system of the brain

becomes subservient to the need for the substance and in unavailability of dependence drug give rise to withdrawal symptoms. Thus, alcohol de-addiction treatment is very challenging due the occurrence of alcohol withdrawal symptoms. Many addicts quite the de-addiction treatment due to the intolerance to the severe withdrawal symptoms. Hence it is essential to reduce the intensity and duration of withdrawal symptoms to increase the rate of de-addiction treatment's success.

In conventional science Naltrexone, Acamprosate have been used to treat carving in alcohol dependence. Naltrexone belongs to a class of drugs known as opiate antagonists and acts on the brain chemicals mediating alcohols pleasurable and reinforcing effects. But chronic naltrexone administration permanently blocks the opiate receptors. The body produces more opioid receptors to compensate this blockade and maintains its normal level of opioid activity, rendering the endogenous opioid system more sensitive to alcohol's effect and enhancing alcohol preference [3]. The mechanism of action of acamprosate is uncertain, but the drug is thought to interact with neuronal NMDA receptors and calcium channels and these proteins are implicated in the induction of alcohol dependence [4]. But these drugs pose adverse symptoms affecting gastrointestinal and neuropsychiatric systems [5]. Hence, evaluation of alternative medicine to deal the withdrawal symptoms other than Naltrexone are necessary. In the purview of Ayurveda, Alcohol dependence may be considered as a *Manovaha Strotas* (psychologicaldisease). *Kalyanak Ghrita*, an Ayurveda fat based polyherbal formulation (KG) prescribed for cognition disordersin Ayurveda classics [6]. Present article reports the ameliorative effect of *KalyanakGhrita* (KG) on withdrawal symptoms in alcohol-dependent Swiss albino mice model.

MATERIALS:

Animals: Swiss albino mice procured from central animal house, Bharati Vidyapeeth (Deemed to be University) Medical College, Pune, India.

Drugs and Chemicals: *KalyanakGghrita*- KG (as study drug). The trial drug has 28 herbal drugs **Table 1** in was purchased from GMP approved pharmacy- AVS Pharmacy, Trissur.

The ingredients of the KG with botanical names have been tabulated in **Table 1.** Goghrita-(as a Vehicle control) market available Goghrita of company Patanjali Ayuved Ltd. was purchased. 20% ethanol- (to develop dependence) – purchased from a licensed chemical agency. 99.99% absolute alcohol. For 20% Ethanol was made up of 80ml water and 20 ml absolute alcohol. Naltrexone- (as a Standard comparator) –purchased from pharmacist with the brand name 'Naltrexone' with medical prescription for research.

Animal Model: Ethanol Dependence Swiss Albino Mice Model [7, 8].

Instruments: Elevated plus maze (EPM) for anxiety profile assessment. Rota rod Apparatus for muscular coordination & muscle spasm assessment

TABLE 1: INGREDIENTS OF KG

Sr. no.	Name of Herbal	Botanical Name
1	*Amalaki*	*Emblica officinalislinn.*
2	*Bibhitak*	*Terminalia belericaratz.*
3	*Haritaki*	*Terminalia chebularatz.*
4	*Vishala*	*Citrulus colocynthis L.*
5	*Bruhat Ela*	*Amomum subulatum Roxb.*
6	*Devdaru*	*Cedrus deodara*
7	*Elvaluk*	*Prunus cerasus L.*
8	*Sariva*	*Hemidesmus indicus R.*
9	*Krushna Sariva*	*Cryptolepis buchananii L.*
10	*Haridra*	*Curcuma longalinn.*
11	*Daruharidra*	*Berberis aristata L.*
12	*Shaliparni*	*Desmodium gangeticum L.*
13	*Prushniparni*	*Uraria pictajacq.*
14	*Priyangu*	*Callicarpa macrophyllaa. juss*
15	*Tagar*	*Valeria nawallichii L.*
16	*Bruhati*	*Solanum indicum L.*
17	*Kushtha*	*Saussurea lappa Falc.*
18	*Manjishtha*	*Rubia cordifolia L.*
19	*Nagkeshar*	*Mesua ferraelinn.*
20	*Dadim*	*Punica granatum L.*
21	*Vidanga*	*Embeliaribesburm F.*
22	*Talishpatra*	*Abies webbiana (D.Don) Spach*
23	*Sukshma Ela*	*Elletaria cardamomum L.*
24	*Malati*	*Jasminum grandiflorum L.*
25	*Kamal*	*Nelumbo nucifera* Gaertn.
26	*Danti*	*Baliospermummontanumd.don*
27	*Padmak*	*Prunus cerasoidesd.don*
28	*Raktacandan*	*Pterocarpus santalinusl. F*

METHODOLOGY:

Study Protocol: The permission of Institutional Animal Ethics Committee was obtained prior to initiation of experiment with Registration No:

BVDU/exam/494/2016-17. The study was conducted at CPCSEA approved Central Animal House following all the desired guidelines. Ideal housing and Feeding conditions were maintained throughout the experiment for all the animals with natural light and dark cycle of 12 hours and maintained on a standard pellet diet *ad libitum*.

36 Rota rod trained mice of either sex aged 6-8 weeks (approximately 20-30 gms) were selected for the study. Housing was done in metabolic cage (1 animal per cage) to create a socially deprived atmosphere for mice. Alcohol dependence was developed in 30 mice and 6 animals were provided with distilled water. To develop alcohol dependence, 30 animals were provided with access to two bottles, one containing 20% ethanol and the other containing distilled water. The 20% ethanol was provided intermittently with a gap of 24 hours (every alternate day). In the initial three days the animals were provided with continuous access to ethanol without any abstinence to make them habituated with ethanol consumption.

From fourth day, experiment schedule was maintained as intermittent access with 24 hrs of abstinence alcohol till day 14. Hence, the alcohol was provided on Monday, Wednesday and Friday and the abstinence of alcohol was on Tuesday, Thursday Saturday and Sunday. The confirmation of addiction was done with the withdrawal symptoms measurable in animal model [7, 8] in the form of occurrence of anxiety with EPM and muscular in coordination with Rota rod after 24 and 48 hrs from the abstinence of alcohol considering the dominance of occurrence of withdrawal symptoms [1]. Alcohol dependence was confirmed by assessment of entry and duration of stay in open & closed arm of plus maze and number of fall on Rota Rod.

Thus, 30 alcohol-dependent animals were randomly allotted into five groups having six animals in each group. All the animals from Gr II to Gr VI received 20% ethanol on an alternate day for next14 days with the respective treatment drugs concomitantly by oral route. Group II serves as the disease control and in group III clarified butter (cow ghee) was administered which was considered as vehicle control. The animals of group IV and V were treated with test drug KG in two dose level as higher and lower as per the defined extrapolation factor. In group VI Naltrexone was given as a standard comparator. Thus the study includes total six groups with respective treatment drugs with dose as shown in **Table 2.**

TABLE 2: EXPERIMENT GROUPS WITH TREATMENT DRUGS AND DOSAGE

Group	Drug	Dosage for 14 days
I	DW	-
II	20% Ethanol (EA)	Intermittent free Access
III	20% EA+ GO	5.2 gm/kg/d
IV	20% EA+ KG (X)	5.2 gm/kg /d
V	20% EA+ KG (2X)	10.4 gm/kg/d
VI	20% EA+ Naltrexone	1mg/kg/d[8]

The above animals were subjected to alcohol abstinence for next 7 days and the drug treatment was continued. Following parameters of assessment was measured to test efficacy of test drug-

Anxiety profile using Elevated Plus maze apparatus. Muscular coordination and Muscle spasms using Rota rod Apparatus. Behavioral assessment

Assessment on Elevated plus Maze (EPM): The elevated plus maze test is one of the most widely used tests for measuring anxiety and memory-like behavior. The test is based on the natural aversion of mice for open and elevated areas and their natural spontaneous exploratory behavior in novel environments [10].

The apparatus consists of 4 arms extended from center in right angle to each other 2 of them are closed and 2 are open crossed in the middle perpendicularly to each other, and a center area. The arms are elevated 30 cm. from ground. Mice are given access to all of the arms and are allowed to move freely between them. The number of entries into the open arms and the time spent in the open arms are used as indices of open space-induced anxiety in mice.

All the experimental mice are transferred to the behavior testing room 30 minutes prior to beginning the first trial to habituate to the condition of the behavior testing room. A mouse is placed in the center area of the maze with its head directed toward a closed arm. The animal's movement in open & closed arms is observed for 5 minutes. After five minutes, the animals are returned to its

home cage. After each trial, all arms and the center area are cleaned with distilled water, to remove the odor of excreta of previously tested animals to prevent a bias based on olfactory cues. Parameters recorded were time spent in open and closed arm and number of crossing in open and closed arm lines. Animal crossing cutting line by all the 4 limbs has been considered as an entry in respective arm. Precautions taken during assessment were, sterile condition confirmed; no distract of animals by sound, dark room, perfume, or other volatile liquid not allowed. Close arm were kept in contact with wall.

Assessment on Rota Rod Apparatus: In the experiment, the Rota rod was used to assess the drug effect in muscular incoordination resulting from the withdrawal tremors. Rota rod has a horizontal rod that can be rotated with controlled rotation per minute (RPM). It has a monitoring system at the base which can record fall & latency of fall of rodent. The method of using was apparatus was set to speed up from 4 to 20rpm in 300s, and animals were placed in separate lanes on rod initially rotating at 4rpm.

The trial begins when acceleration was started and ends when the animal falls off the rod. Recorded parameters were latency to fall and speed at fall. Before selecting animals for the experiments, the animals were exposed to Rota rod for two successive days. The animals which can adopt the muscular coordination to stay on a rotating Rota rod were selected for the experiment. The method of the whole experiment is summaries in **Fig. 1**

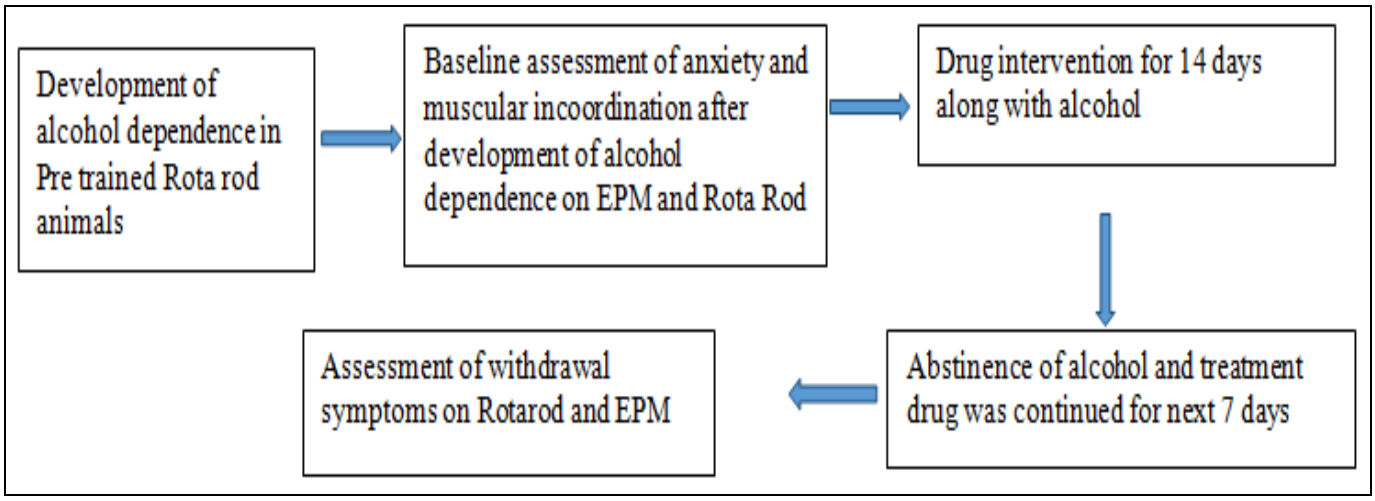

FIG. 1: FIGURE SHOWING THE METHODOLOGY OF THE EXPERIMENT

RESULTS:

Results of Anxiety Threshold Assessment by Elevated plus Maze (EPM): Anxiety, an essential feature of withdrawal of ethanol which is generally evident during 24 to 48 hours and completely gets cured after 3 to 7days [9].

Hence, to assess the effect of Test drug on the anxiety threshold, the animals were subjected to EPM on day 1 & 7 after abstinence of alcohol. To assess anxiety as feature of alcohol withdrawal following criteria was used-

1. Number of crossing by the individual animal in close arm in 05 minutes.
2. Number of crossing by the individual animal in open arm in 05 minutes.
3. Time Spend in open arms of EPM by the individual animal in 05 minutes.

EPM-Number of Crossing in Close Arm: It can be noted in **Table 3** that the number of crossings in control group I was unchanged to that of Day 1 and Day 7 whereas the no. of crossing in Disease control group II showed a decrease in closed arm on Day 1 as compared to control group. Further, on Day 7 the number of crossings did not show any increase in the number of crossing in Group II. However, all the drug-treated animals of group III to VI showed improvement in number of crossings. Still, the increase in no of crossings is statistically significant in animals of GO and KG- lower and higher intervention group.

TABLE 3: NUMBER OF CROSSING IN CLOSE ARM OF EPM

Group	Day-1 after abstinence of ethanol	Day-7 after abstinence of ethanol
I-DW	11±4.95	12.2±1.92
II-Ethanol	4.5±1.05	5±4.2
III-GO	6.17±1.17	11.5±1.52**
IV-KG-X	7.83±2.14	19.33±2.58**
V-KG-2X	6.33±1.03	16.67±1.86***
VI-Naltrexone	5.5±0.84	11.5±6.44

**P<0.01, ***P<0.001 as compared to Day-1 of withdrawal phase using 'paired T test'

EPM- Number of Crossing in Open Arm: As shown in **Table 4**, the animals of the control group showed same number of crossing on day 1 and day 7. Whereas in animals receiving ethanol of group II showed no increase in number of crossing of open arm and observed to be seated in one corner of closed arm with minimum activity on Day 1 and 7 as well. All the animals of groups III to VI with intervention drug GO, KG & Naltrexone in their respective groups showed no change in number of the crossing of open arm on day 1 and 7 but the open arm crossings were more than disease control group.

TABLE 4: NUMBER OF CROSSING IN OPEN ARM OF EPM

Group	Day-1 after abstinence of ethanol	Day-7 after abstinence of ethanol
I-DW	3(0-5)	4(0-10)
II-Ethanol	0(0-1)	0(0-1)
III-GO	1(0-2)	1(0-3)
IV-KG-X	1.5(1-3)	1(1-2)
V-KG-2X	1(0-2)	1(0-2)
VI-Naltrexone	0.5(0-2)	0.5(0-2)

EPM- Time Spend in Open Arms: As shown in **Table 5**, the animals receiving distilled water demonstrated increase in the time spend in open arm. The ethanol-treated group showed average zero time in the open arm in both assessments. The GO, KG and Naltrexone treated groups showed an increase in time spent in open arm after abstinence of ethanol on day 7 compared to day 1 reading but the increase was statistically non-significant.

TABLE 5: TIME SPEND IN SECONDS IN MINUTES IN THE OPEN ARMS OF EPM

Group	Day-1 after abstinence of ethanol	Day-7 after abstinence of ethanol
I-DW	0.045(0-0.3)	0.12(0-0.035)
II-Ethanol	0(0-0.01)	0(0-0.05)
III-GO	0.035(0.01-0.09)	0.05(0-0.2)
IV-KG-X	0.035(0.02-0.1)	0.07(0.01-0.11)
V-KG-2X	0.02(0-0.03)	0.06(0-0.2)
VI-Naltrexone	0.015(0-0.14)	0.02(0-0.04)

Muscular Incoordination and Muscle Spasms Assessment by Rota Rod:

Number of Fallon Rota Rod: The muscular coordination assessment was done on days 1, 3 & 7 after abstinence of alcohol; the results are shown in **Table 6**. The number of falls on day 7 was zero in all the group animals; hence the data is not shown.

TABLE 6: NUMBER OF FALLS ON ROTA ROD

Group	Day-1 after abstinence of ethanol	Day-3 after abstinence of ethanol
I-DW	0	0
II-Ethanol	2(1-3)	0(0-3)
III-GO	0	0
IV-KG-X	0(0-1)	0
V-KG-2X	0(0-1)	0
VI-Naltrexone	0(0-1)	1(0-1)

During the assessment for muscular coordination it was noted that GO and KG treated animals showed complete reduction in falls on 3rd day after alcohol abstinence.

General Behavioral Observation: It was noted that after developing dependence, the animals were inactive in cage and preferred to be in one corner of the cage with minimal teasing movements. Also the posture of animals was not healthy, and skin showed a blackish-white appearance due to pilo erection. Whereas the animals of the control group on distilled water maintain their normal behavior, posture and hair pattern till end of the experiment. After drug intervention, the animal receiving the study drug i.e. KG and GO was active compared to the disease control group. In tail holding test, animals of the disease control group gave up comparatively early than the animals of the intervention drug receiving groups. On teasing, animals showed an active response in all the groups except the disease control group.

DISCUSSION: Addiction is a term that has broad meaning, having been used in connection with obesity, sexual behavior, gambling, drugs of abuse, and an increasing list of motivated behaviors. Substance/drug addiction is a neuropsychiatric disorder characterized by a recurring desire to continue taking the drug despite harmful consequences. Addiction can be categorized in two types Substance and behavioral. Substance addiction is contributed by Tobacco, Alcohol, illicit drugs, and Prescription drugs, and behavior addiction is Gambling, Food, Sex, Internet, Video games, Work. Amongst substance addiction, the most commonly identified is alcohol addiction [12].

The global burden of disease attributable to alcohol is unacceptably high. According to a report released by the World Health Organization on Global status report on alcohol and health 2018 more than 3 million people died as a result of harmful use of alcohol in 2016. This represents 1 in 20 deaths. More than three-quarters of these deaths were among men. Overall, the harmful use of alcohol causes more than 5% of the global disease burden. Although the main target of alcohol is central nervous system, but the association of various systemic diseases and alcohol consumption is well established and represents a major public health burden. Ayurveda textual references has also mentioned the psychosomatic ill effects of alcohol [13]. Alcohol withdrawal is a syndrome manifested in the person having the habit of alcohol when the dose of alcohol consumption is reduced or stopped completely. Due to regular consumption of alcohol, the central nervous system (CNS) has adjusted to the constant presence of alcohol in the body and compensates for alcohol's depressive effects on both brain function and the communication among neurons. Consequently, the brain becomes hyperactive or hyperexcited when the alcohol level is suddenly lowered, causing withdrawal syndrome. Thus alcohol withdrawal syndrome is due to overactivity of the central and autonomic nervous systems, leading to tremors, insomnia, craving, nausea and vomiting, hallucinations, anxiety, and agitation [14].

The Clinical Institute for Withdrawal Assessment for alcohol revised scale (CIWA-Ar) is a tool used to assess the severity of alcohol withdrawal symptoms. The physical symptoms that are identified for the CIWA-Ar scale are the presence of Nausea and vomiting, Headache, Auditory disturbances, Agitation, Paroxysmal sweating, Visual disturbances Tremor, Orientation, Anxiety, Clouding of sensorium [15]. In the present experimental study, the symptom muscular incoordination and anxiety was used to confirm development of dependence and assess the study drug's efficacy in Swiss albino mice. Kalyanakghrita (KG) is a frequently used drug by Ayurveda physician to treat psychosomatic illness affecting cognition and memory. The base of the KG is cow ghee prepared by processing of clarified cow butter (Goghrita), which has been quoted as the best antitoxic drug [16] and a healthiest natural edible lipid drug (snehadravya).

According to Ayurveda, clarified cow butter protects the body from various diseases and promotes longevity. Cow ghee can transport the therapeutic properties of added herbs to all the tissues of body [17]. The lipophilic action of Cow ghee facilitates the transportation of properties of ingredients to a target organ and final delivery inside the cell since the cell membrane also contains lipid. Hence it can be assumed that the therapeutic actions of the ingredients of the KG can easily be transferred to the neurons. Besides, many medicated cow ghee formulations have been evidentially identified for their cognitive functions [18, 19]. During the experiment, after free access to alcohol for 14 days, the animals were given abstinence of alcohol and the presence of

withdrawal was tested after 48 hrs for anxiety, muscular coordination and general behavior in cage. The finding on EPM and Rota rod confirms the development of alcohol addiction. Then animals were treated with respective treatment drugs along with the administration of alcohol. The duration and severity of withdrawal symptoms were tested to know the effect of the intervention drug. The elevated plus maze test has a strong predictive validity for screening anxiolytic drugs and used widely in experiments [20, 21].

The anxiolytic drugs specifically increases, and anxiogenic drugs decreases, the number of entries into the open arms and the time spent there. The open and closed arms are considered to evoke the same exploratory drive, therefore avoidance of the open arms is considered to be a result of the induction of higher levels of fear. It is thought that mice's aversion to exploring the maze's open arms is caused by fear of open and elevated spaces. In the present experiment, the effect of KG on the anxiety threshold was tested on EPM by number of open & closed-arm crossing and time spent in open arm. The animals of control group maintained the three findingson baseline and end day exposure. This finding implies that the housing and feeding of animals have no impact on their behavior of animals.

The animals treated with KG and GO showed a significant increase in number of crossing of closed arm and increase of time spend in open arm. The increased activity of the animals implies reduced anxiety of animals showing effect of intervention drugs. Although all the animals receiving GO and KG showed increase in activity on EPM and free movement in cage but KG treated mice demonstrated remarkable improvement in behavioral patterns demonstrating effect of drug to reduce anxiety. On the other hand after abstinence of ethanol, animals of disease control group showed no increase in number of crossing of open and closed arm and preferred to be in one corner of closed arm with zero time spend in open arm. Hence the claim by Vaishali Murade *et.al* that untreated mice normally avoid the open arms of the EPM instead prefer to stay in the closed arms, whereas mice treated with antianxiety drugs show far less avoidance to open-arm20 was noticed during the experiment. This finding exhibits the ameliorative effect of KG on reducing anxiety caused due to withdrawal. The Rota rod was the other assessment instrument to measure the effect of the test drug. Rota rod is one of the most commonly used tests of motor incoordination and spasms Also has been well accepted that the Rotarod is sensitive to ethanol-like drugs that affect motor coordination [23]. The muscular coordination assessment was done on days 1, 3 & 7 after abstinence from alcohol.

KG and Go treated group showed a complete reduction in falls on 3rd day, indicating improvement in muscle coordination. The groups were comparable with the Naltrexone group, whereas the ethanol-treated group required 6 days to achieve normal muscular coordination. The results showed early decrease in tremors of the animals with KG. In ethanol-treated group showed 2(1-3) falls on day 1 and 0(0-3) fall on day 3 but no fall on days 7th [data not shown]. As reported by Simon Brooks *et. al* the deterioration of normal motor function within a disease state signals the progression of an underlying pathological process and identifies disease-sensitive time points. Owing to this fact and the experiment's finding, it can be claimed that complete recovery from muscular incoordination due to withdrawal is seen within seven days.

In general observation, animal on KG was active as compared to a disease control group receiving ethanol. Achievement of the normal health status of animals indicates the effect of KG on overall improvement in the health of animals. Thus anxiety reduction, and improvement in muscular coordination very important criteria of dependence showing significant result in KG group proving its efficacy in dependence. According to the Ayurvedic texual reference, the cumulative effect of Kalyanakaghritais to enhance physical & mental stability (Ojos & Manobalavardhak), to reduce confused state of mind and depression (Moha & Daivopahatcetasi) to increase the memory (smruti) and also detox the body tissue (Vishaghna) [6]. Some of the ingredients of Kalyanakaghrita have shown pharmacological activities related to central nervous system, Antioxidant, and anticholinesterase. The details about the reported pharmacological activities are as shown in **Table 7.**

TABLE 7: TABLE SHOWING THE PHARMACOLOGICAL ACTIVITIES OF INGREDIENTS OF KG

Herbal ingredient of KG	Pharmacological Activities
Emblica officinalis	Neuroprotective [25] Memory enhancer, Antioxidant and Anti-cholinesterase activity [26,] Amelioration of alcohol-induced oxidative stress [27] Antidepressant activity [28] Protection against alcohol-induced brain mitochondrial dysfunction [29]
Terminalia chebula	acetyl cholinesterase inhibitors [30]
Citruluscolocynthis	Antiepileptic activity [31]
Cedrus deodara	Anticonvulsant properties through the GABA levels in brain [32] Anxiolytic & Anticonvulsant activity [33]
Hemidesmus indicus	Neuroprotective [34]
Berberis aristata	Neuroprotective [35]
Desmodiumgangeticum	Neuroprotective [36], Memory enhancer [37]
Urariapicta	Anticholinesterase activities [38]
Valerianawallichii	Improves sleep quality and modulates brain monoamine [39] Anxiolytic, Sedative, Neuroprotective, Anticonvulsant [40]
Rubia cordifolia	Anticonvulsant [41], Neuroprotective [42]
Embeliaribes	Antianxiety activity [43] Anticonvulsant activity [44] Antidepressant activity [45] Neuroprotective activity [46] Anxiolytic activity [47]
Jasminum grandiflorum	Anticonvulsant Activity [48]
Prunus cerasoides	Neuroprotective [49]

As stated earlier, Kalyanak Ghrita is an Ayurveda fat-based polyherbal formulation for cognition disorders. Cow ghee is lipophilic in nature and used as carrier media to facilitate the transport of active principle across cell membranes which is permeable to lipid molecule *e.g.* BBB. Various medicated ghee is used to treat chronic diseases like anxiety [50].

It is well documented that the GABA neurotransmitters have a major role in expressing withdrawal symptoms. As shown in **Table 7** the KG is blended with the 28 ingredients exerting a variety of pharmacological activities including Neuro-protective, Antidepressant, Anxiolytic and Anticonvulsant and have action neurotransmitters like Ach & GABA as well. The combined effect of all these activities may have contributed to reducing withdrawal symptoms.

CONCLUSION: KG has an ameliorative effect on withdrawal symptoms, specifically on anxiety, tremors in the form of muscular in coordination, and behavioral changes caused due to abstinence from alcohol in the animal model. Further clinical studies are required to establish the ameliorative effect of KG on alcohol withdrawal symptoms manifested in humans.

ACKNOWLEDGMENT: Authors are very thankful to the authorities of Central Animal House of Bharati Vidyapeeth (Deemed to be University), Pune, for their support in conducting the whole experiment.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Chotai TM, Kapgate SM and Mate VH: Ameliorative effect of *KalyanakGhrita* (KG) on withdrawal symptoms in alcohol dependent mice. Int J Pharm Sci & Res 2023; 14(2): 756-65. doi: 10.13040/IJPSR.0975-8232.14(2).756-65.