-CONFIDENTIAL-

Research Project

Efficacy of Slow-Release L-cysteine in Prevention of Alcohol Hangover. Randomized Placebo-Controlled trial (RCT) with HDR-Help*

Executed by:

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*HDR-Help (Harm Dependence Reduction)(a provisional name of **a new combination formula**, with slow-release L-cysteine, B-vitamin complex, and vitamin-C)

SUMMARY

Background: A hangover is characterized by the constellation of unpleasant physical and mental symptoms that occur after a bout of heavy alcohol drinking. Physical symptoms of a hangover include fatigue, headache, increased sensitivity to light and sound, redness of the eyes, muscle aches, and thirst. Signs of increased sympathetic nervous system activity can accompany a hangover, including increased systolic blood pressure, tachycardia, tremor, and sweating. Mental symptoms include dizziness; a sense of the room spinning (i.e., vertigo); and possible cognitive and mood disturbances, especially depression, anxiety, and irritability. Hangover characteristics may depend on the type of alcoholic beverage consumed and the amount a person drinks. Typically, a hangover begins within several hours after the cessation of drinking, when a person's blood alcohol concentration (BAC) is falling. Symptoms usually peak about the time BAC is zero and may continue for up to 24 hours thereafter.

Research on alcohol hangover has suffered from **methodological shortcomings** and a lack of a systematic approach [16]. As a result, there is limited understanding of various basic issues, e.g. which biological processes cause alcohol hangover, and whether genetics play an important role. Also, it is unclear why, despite excessive alcohol consumption, there are great individual differences in the presence and **severity of alcohol hangovers.** Various research methodologies have been applied to examine alcohol hangover, each having their strengths and weaknesses, and potential confounders and bias that may reduce the credibility of alcohol hangover studies [16].

Recently, increasing number of spontaneous testimonials have started appearing on the websites, reporting that Biohit Oyj's **Acetium™ capsule** (100mg L-cysteine), designed for elimination of **acetaldehyde** in the stomach contents after alcohol intake, proved to be a highly **effective preventive measure in hangover**. According to these testimonials, hangover symptoms were completely prevented or significantly alleviated by the regular administration of Acetium capsules during the drinking bouts.

These spontaneous testimonials prompted us to formulate a novel study hypothesis that could possibly explain these dramatic effects of slow-release L-cysteine in prevention of alcohol hangover. This novel hypothesis is tackling on three key elements of hangover severity, including i) the widespread **toxic effects of acetaldehyde** as an intermediate metabolite of alcohol in various organs, ii) its established role as histamine liberator with bearings to **vascular-type headache** as part of hangover, as well as iii) confirmed mechanisms whereby **slow-release L-cysteine** administration could interfere with this cascade by **eliminating alcohol-derived acetaldehyde** from the stomach.

To fully exploit the effects of L-cysteine, a new formulation was designed, provisionally named as **HDR-Help** (HDR=harm dependence reduction). HDR-Help consists of a principal active compound (slow-release L-cysteine) with established effect against the key alcohol metabolite (acetaldehyde). To substantiate this main effect of acetaldehyde elimination by L-cysteine, the formulation contains the B-vitamin complex and vitamin-C. All these are interfered by alcohol intake, and many of the symptoms associated with depletion of these vitamins are shared by alcohol hangover state. Thus, an **adequate supplementation** of all these elements **after heavy alcohol intake** is the logical step to counteract these alcohol-associated effects, and has the potential to prevent or alleviate the hangover symptoms.

Objective: The present study is designed to validate this novel hypothesis that concomitant use of HDR-Help (2 capsules every 2 hours) during the whole drinking session is an effective means to decrease the symptoms and complaints of alcohol hangover.

Study design: The design of this double-blind, placebo-controlled trial (RCT) follows the **Consensus Statement on Best Practice in Alcohol Hangover Research**, recently elaborated by the Alcohol Hangover Research Group [16]. Into this **within-subject (cross-over)** design, 50 volunteers with frequent hangover symptoms will be enrolled by the Department of General Medicine, University of Tartu (Estonia). Importantly, the study design is a **naturalistic setting**, mimicking as closely as possible the regular drinking behaviour of the subjects. As the key research tool, the subjects will be administered **three validated hangover scores** (AHS, HSS, AHSS) recording the severity of hangover symptoms [30,41,55] after each drinking bouts. The

study protocol will be subjected for approval by the Regional Committee on Medical Research Ethics (University of Tartu).

Methods: To assess their **eligibility** for the study, all invitation-responders are asked to fill in a detailed pre-enrolment questionnaire, divided in three sections: Section 1 is a survey-type record of the issues related to alcohol intake during the past year, with altogether 10 questions. Section 2 is devoted to recording the experiences related to alcohol hangover, requesting the presence of 6 separate symptoms, stratified by the type of alcohol consumed. Section 3 is targeted to record the potential factors found by the subject to be restrictive for her/his alcohol intake, divided to those appearing during the drinking episode and those that appear during hangover. Only the subjects who i) drink alcohol, and who ii) experience regular and severe hangover symptoms are eligible. In addition to this eligibility assessment, these data might be useful as covariates of the study endpoint, i.e., severity of the hangover, analysed in multivariate models.

Each subject serves as her/his own control, undergoing 3 drinking sessions with Acetium and 3 sessions using placebo (double-blinded). These sessions are completed in naturalistic (non-laboratory) settings. On the occasion of each drinking episodes, the individuals record in detail the quantity and type of alcohol consumed, as well as the duration of the drinking session. The most important research tools are the three different **Hangover Severity Scores:** 1) **The Hangover Symptom Scale (HSS),** 2) **The Acute Hangover Scale (AHS)**, and 3) **The Alcohol Hangover Severity Scale (AHSS)** [16]. These documents are filled and submitted to the study monitor preferably after each drinking episode in electronic format, to enable better control for study compliance.

In statistical analysis, both conventional techniques (e.g. non-parametric paired-samples and repeated-samples test), and more sophisticated methods (e.g. reliability analysis with Cronbach's alpha, regression models) will be used. This within-subject design (n=50 in both arms) was found to be **adequately powered** to detect the true differences in hangover severity scores (HSS, AHS, AHSS) in power analysis using assumptions based on the effect size differences established in the original studies [30,41,55]. As determined from the final study compliance, data analyses might be necessary to run separately for i) Per Protocol (PP), and ii) Modified Intention-to-treat (mITT) groups.

Specific aims: The null hypothesis of this study implicates that slow-release L-cysteine is no better than placebo in prophylaxis of alcohol hangover symptoms. Rejection or not of the null hypothesis is based on comparison of the two strata (HDR-Help and placebo), and demonstration of a statistically significantly different scores in three **primary study endpoints** (hangover severity scores): 1) The Hangover Symptom Scale (HSS), 2) The Acute Hangover Scale (AHS), and 3) The Alcohol Hangover Severity Scale (AHSS). We also make an attempt to use headache as **the secondary endpoint**, because of its particular links with the study hypothesis, i.e., the role of histamine in vascular-type headache, histamine liberation by acetaldehyde, and acetaldehyde inactivation by Acetium capsules.

Study execution and time table: For execution of the study, the company shall make the contract with the Department of General Medicine, University of Tartu (Estonia). The execution of the study is monitored following the principles of good clinical practice (GCP) by a certified GCP monitor. The study protocol will be subjected for approval by the Regional Committee on Medical Research Ethics (University of Tartu). Enrolment of the study subjects will start only after the ethical approval has been obtained. Given that each study subject shall complete 3 drinking bout-pairs, 3 with Acetium and 3 with placebo (in a blinded fashion), to be completed in a naturalistic setting, we expect that the study completion will require between 6 months and one year.

Impact of the study: Given that i) L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, and ii) B vitamin complex and vitamin-C have several beneficial effects on alcohol-interfered metabolism, HDR-Help would comprise an ideal means to conduct alcohol hangover prophylaxis, without concern about the side effects that are inherent to many of the currently used treatment modalities. If the efficacy is proved in this formal RCT, the concept of using HDR-Help capsules in prophylactic treatment of alcohol hangover would represent a major step forward in a better control of these frequently intractable and incapacitating symptoms.

1. BACKGROUND

The alcohol hangover develops when blood alcohol concentration (BAC) falls considerably and peaks when it returns to almost zero [1]. The alcohol hangover may last up to 24 hours [1], and besides a feeling of general misery, several symptoms characterize the alcohol hangover including headache, tiredness, concentration problems, thirst, dizziness, nausea, cognitive impairment, and mood changes. At present, no theoretical model accounts for the pathology of alcohol hangover, nor have most studies systematically investigated the deleterious effects on daytime functioning without methodological confounds [2–4]. Among young adults, alcohol hangovers are reported as the most frequently occurring adverse effect of excessive alcohol consumption [5].

The impact of alcohol hangover on daily activities can be profound. A survey among Dutch university students [6] showed that more than half of them reported being unable to study when experiencing an alcohol hangover often or always. With an average hangover frequency of 2.7 days/month, 1 month a year is "lost" [6]. While one experimental study found no effects on academic performance [7], several experimental studies confirm that memory functioning is impaired during alcohol hangover [8, 9]. This is disturbing; especially since the core business of students is learning and remembering.

Alcohol hangovers are not limited to young adults. Hangovers are also common in the workplace. Frone [10] found that 9.23% (11.6 million workers) of the US workforce reported to work with a hangover in the past year, making it the most common form of alcohol-related workplace impairment in the survey. There is a significant relationship between alcohol consumption and next-day workplace absenteeism. A survey among 280 employees revealed a two-fold increased likelihood of absenteeism the day after alcohol consumption [11]. From the 173 days of absenteeism (of 5493 days at 'risk'), 74 days (43%) occurred the day after alcohol consumption. Interviews by Ames and colleagues [12] revealed that about half of interviewed workers reported being at work while having a hangover. During hangover, workers felt significantly sicker, had conflicts or fights with coworkers and their supervisor, problems in completing the job, and fell asleep at work. Reduced productivity is common when having a hangover at work. A recent Norwegian study [13] concluded that alcohol hangover is the largest substance abuse problem at the workplace. Employees reported that during the past year hangovers had resulted at least once in inefficient work (24.3%) and absence (6.2%).
Surprisingly, scientific evidence on the economic costs of alcohol hangover is scarce. A decade ago, Harwood [14] estimated the annual costs of alcohol hangover in U.S.A. at $185 billion, and although this amount was criticized for inaccuracy [15] it gives an impression of the economic impact of hangovers on society.

To convince policymakers of the profound impact of alcohol hangover on daytime functioning this information is, however, essential. Therefore, future studies should aim to determine the costs of alcohol hangover in terms of reduced productivity and absenteeism. If reduced productivity, increased accident risk and absenteeism rates

are translated into costs for society, this will likely increase the scientific and politic attention for alcohol hangover research.

Alcohol hangover has gained increased research attention the past decade, and in 2009 researchers from around the world united and founded the Alcohol Hangover Research Group (AHRG). At a satellite meeting of the Research Society on Alcoholism conference in San Antonio Texas, June 26th 2010, the AHRG conducted a symposium and consensus meeting to discuss potential guidelines for future alcohol hangover research. This study protocol follows, as far as feasible, the key outcomes of **these consensus statements** [16].

1.1.Potential pitfalls in alcohol hangover studies

Research on alcohol hangover has suffered from methodological shortcomings and a lack of a systematic approach. As a result, there is limited understanding of various basic issues, such as what biological processes cause alcohol hangover, and whether genetics play an important role. Also, it is unclear why, despite excessive alcohol consumption, there are great individual differences in the presence and severity of alcohol hangovers. Various research methodologies have been applied to examine alcohol hangover, each having their strengths and weaknesses, and potential confounders and bias that may reduce the credibility of alcohol hangover studies.

2.METHODS USED IN HANGOVER STUDIES

2.1.Preclinical studies

Currently, there is a lack of an animal model that establishes a physiological correlate of one or more of the hangover symptoms. Nonetheless, several preclinical studies conducted in rats have studied behavioral changes following administration of ethanol doses that are considered intoxicating in humans. For example, York and Regan [17] documented reduced operant activity and motor performance up to 16 hours after an acute ethanol administration. Morse and colleagues documented the induction of post-intoxication conditioned place aversion 10 hours after similar alcohol challenge [18]. Jung et al. [19] have reported reduced social interaction and overall social activity 18 hours after ethanol challenge. Whether these behavioral changes can be considered as correlates of hangover in humans remains to be established. Additional animal studies using acute administration of intoxicating amounts of ethanol to rats and mice have tested the efficacy of potential hangover “cures” on the metabolism of ethanol, its first metabolite **acetaldehyde** and the enzymes that play a role in promoting this process [20–22]. These “cures” have been reported to promote a reduction of blood alcohol and acetaldehyde concentrations after an acute or chronic alcohol challenge. **Since alcohol hangovers may be accompanied by an increased acetaldehyde concentration, the authors suggest that these cures may therefore be effective in preventing alcohol hangover**.

Studies with chronic alcohol administration should be interpreted with caution, because it is likely that alcohol withdrawal effects are measured rather than hangover effects. Animal **models for alcohol withdrawal** are **not**

useful for studying hangover effects, because in withdrawal develops after receiving alcohol for days or weeks, while hangover occurs after a single episode and different CNS systems are involved [4]. The development of an animal model that enables testing alcohol hangover effects after a single alcohol challenge is essential to enhance our knowledge on the pathology of alcohol hangover.

2.2.Genetics and individual susceptibility to hangover

Some studies have focused on alleles associated with **aldehyde dehydrogenase (ALDH)** and **flushing phenotypes** in Asians [23–26]. It must be concluded that genetic research on alcohol hangover is still **in its infancy**. It is likely that genetics play an important role, especially if one takes into account the great individual differences in hangover severity and the fact that about 25% of heavy drinkers claim that they have never had a hangover [27].

Individual differences in susceptibility have occasionally been estimated by examining the residual variance in a hangover frequency measure after co-varying measures of drinking quantity and frequency [28–30]. The assumption is that residual variance in hangover not accounted for by individual differences in drinking behavior may more clearly reveal individual differences in propensity to develop a hangover. Although this approach is defensible, it is recommended that more direct assessments of individual differences in hangover susceptibility be administered when this is a central focus of the research. For example, a questionnaire might assess the typical number of drinks required to produce a hangover [26] or the likelihood of experiencing a hangover at a given number of drinks [24–25]. Further experimental research involving repeated alcohol administration to the same individuals is needed to estimate the proportions of the population which are: (1) consistently resistant to hangover; (2) consistently susceptible to hangover; and (3) variably susceptible to hangover.

2.3.Survey-type of studies

Survey methods are essential for certain tasks, such as establishing the prevalence of hangovers in epidemiologic samples. Survey methods are also valuable for identifying the correlates of naturally occurring hangovers, including antecedent patterns of alcohol consumption and other potential contributory causes, individual differences, and consequences. Such information can be used to probe theoretical questions and also to identify phenomena worthy of closer scrutiny in laboratory-based experimental studies.

The current lack of well-validated, comprehensive instruments for assessing hangover-related information has probably contributed to the slow growth of hangover research [30]. Researchers have frequently devised their own assessments for survey studies, and so the literature contains a remarkable diversity of measurement strategies. Existing approaches include the use of face-valid, single-item assessments concerning the occurrence or frequency of hangover [31–36], multi-item assessments of occurrence or frequency of experiencing specific hangover symptoms [26, 29–30, 37], questions about the severity or duration of

symptoms during a typical hangover, a recent hangover, or following a recent drinking episode [26, 35, 38–40], and questions asking respondents to estimate their likelihood of experiencing a hangover after consuming a specified amount of alcohol [24–25]. A number of the measures include items that were not found to be valid in hangover induction studies or **that measure withdrawal or intoxication effects** [41]. **The development and evaluation of survey instruments should be regarded as a valued activity in hangover research.**

2.4.Clinical studies

2.4.1.Naturalistic vs. standardized (laboratory) studies

Most experimental data on alcohol hangover comes from studies in humans. Two different approaches are used to study alcohol hangover: **the naturalistic** and **experimental design** in laboratories. In **experimental studies**, a standard amount of alcohol is administered and consumed in a fixed (and often short) period of time. Factors affecting hangover severity such as food intake, time of going to bed, activities during the evening and sleep can be standardized and controlled. On the other hand, "moral" hangover symptoms experienced in real life such as guilt and shame are examples of response domains potentially undercut by hangovers induced in a controlled laboratory setting [42]. Using an electronic diary design, Muraven and colleagues [43] found that when drinkers felt they had violated a self-imposed drinking limit, they were more likely to report feeling bad and guilty about their drinking the next morning. Guilt reactions predicted the amount of alcohol consumed later in the day and two days later. Hangover was not a focus of this research, but hangover symptoms were measured. Hangover was related to the amount of alcohol consumed the night before and the amount of guilt experienced the next morning (suggesting guilt might be regarded as part of the syndrome). Controlling for physical hangover symptoms weakened but did not eliminate the limit violation effects. Guilt reactions - symptoms unlikely to be observed in laboratory research - may play a vital role in linking "morning after" processes to subsequent drinking.

In **the naturalistic design** the amount and type of alcoholic beverages are not under experimental control [44, 45]. The participant's activities are not under control and ingestion of alcohol is usually done over a longer period of time. Both study designs have their advantages and disadvantages. Important points to consider about the naturalistic approach are the fact that drinking time is under personal control, the place of consumption (i.e. the pub) is familiar to participants, and the rate of consumption and type of beverage can change during the evening. While this naturalistic approach has the advantage of being ecologically valid and mimicking a normal pattern of alcohol consumption, researchers may however prefer to control some of these factors that are left to the participant's discretion for some studies. In studies where these can affect the outcomes being studied, choosing a controlled experimental design can be more appropriate. Future studies should make a direct comparison between both types of designs to determine to what extent they are complementary or distinct.

3.MEASUREMENT OF HANGOVER PRESENCE AND SEVERITY

Scientific communication and integration of survey data would be facilitated by increased precision in writing about the dimensions of hangover actually assessed in a given study. The consensus group recommends that, to the extent feasible, survey investigators use descriptive terminology (e.g., "hangover frequency", "hangover susceptibility" "hangover symptom count", "hangover severity") rather than simply referring to "hangover" when reporting their findings [16]. It is recommended that the term **hangover severity** is reserved to describe measures of symptom intensity or magnitude and the term **hangover symptom count** is used to refer to tallies of the number of discrete symptoms endorsed. This distinction will promote clarity in scientific communication while allowing investigators to amass data using both approaches and conduct empirical tests of their overlap. Such tests might be profitably conducted using data from both retrospective surveys and ratings of acute hangover collected during experimental studies [41].

3.1.Hangover frequency

For many research applications, it is desirable to gather information about the frequency of hangover over some period of time, such as the past month or past year. Items that assess hangover frequency can, of course, be readily re-scored to indicate simple presence vs absence of any hangover during the same period. It is possible to achieve a given number of hangovers via multiple routes, such as by drinking frequently but being relatively invulnerable to hangover or by drinking rarely but being very sensitive to hangover effects. To be maximally informative, assessments of **hangover frequency** should be constructed so as to be able to distinguish a) the overall number (or range) of hangover events during the time period and b) the percentage of drinking occasions followed by hangover. An assessment of drinking practices during the same time frame, such as the frequency of excessive drinking, is a valuable adjunct for descriptive purposes or selecting subgroups for focused analysis (e.g., respondents matched on drinking frequency or intensity but differing in percentage of occasions followed by hangover).

3.2.Hangover severity

Severity of hangover can be construed in two ways. One approach defines severity in terms of the **magnitude or intensity of hangover** or individual hangover symptoms during a typical hangover or a designated hangover event [38, 40]. This use of the term closely accords with the way "severity" is indexed in a typical laboratory investigation. The second approach uses a **count of the number of distinct symptoms** endorsed as an index of the diversity of hangover experiences [30]. This approach is similar to the strategy of using a count of the total number of diagnostic criteria met by an individual as an index of disorder severity in psychiatric epidemiology.

Hangover may include a wide array of symptoms, and it is often impractical or undesirable to attempt to assess all possible hangover symptoms. For many investigations, it will be sufficient to assess the core set of symptoms that are most reliably associated with hangovers in the laboratory. However, when feasible, it would be valuable to include items tapping additional, less common symptoms. Rarely reported symptoms could be

important if they mark cases that are especially severe or that arise from unique causal pathways. Alternatively, infrequently reported symptoms may contribute error to an assessment. Rare symptoms may be too infrequently observed to study productively in laboratory settings, but may be more easily investigated in survey investigations with larger samples.

It is possible to rate overall hangover severity directly using one simple question that can be rated numerically from 'no hangover' to 'extreme or severe' hangover and to use this as the primary measure in some cases. The outcome of this question can then be used as the primary measure to relate to cognitive and psychomotor effects of alcohol hangover or biological correlates. Individual items (e.g., headache, fatigue, nausea) further allow insight in the nature of alcohol hangover experienced by a subject. These secondary outcomes may also permit calibration of the overall hangover severity score across samples or cultures if it turns out to be variable. On the other hand, many assessment researchers argue that a reliable **multi-item measure** is more valid than any single item, as discussed extensively elsewhere [56]. Thus, use of reliable and valid scales as the index of hangover seems more appropriate.

In the past, researchers made their own lists of symptoms to compose hangover scales and calculate overall hangover severity. Ylikahri [1] constructed some of the first hangover scales for use in laboratory studies, with one comprising physical signs rated by observers and the other composed of self-reported symptoms. His research group used this method in several publications, but most other researchers did not adopt these because the scales had no psychometric development work. The observer-rated physical signs had a very low score, no data were presented on the value of individual signs, and Seppälä et al. [57] reported that the physical signs were not valid. Chapman [46] also validated a number of individual hangover symptoms in his experimental studies but no scale development work was done.

Until recently, no psychometrically established hangover severity scale was available. Current research typically makes use of three hangover scales: Slutske et al. [30] developed the 13-item **Hangover Symptom Scale (HSS)** for use in survey studies, and Rohsenow et al. [41] developed the 8-item **Acute Hangover Scale (AHS)** for use acutely in experimental administration studies. Another more recent scale is called **Alcohol Hangover Severity Scale (AHSS),** introduced by Penning et al. in 2013 [55]. One notable difference between these scales, at least as originally published, is that the HSS assesses past year frequency of 13 symptoms whereas the AHS assesses the severity of currently experienced symptoms, and AHSS includes 12 items: fatigue, clumsiness, dizziness, apathy, sweating, shivering, nausea, heart pounding, confusion, stomach pain, concentration problems, and thirst. Each list of items, though, could clearly be re-worded to cover a variety of time frames. Surprisingly, both scales include somewhat different hangover symptoms. Nevertheless they seem to predict overall hangover severity in a similar manner. While other hangover symptoms exist that are not included in these scales, this does not limit the reliability of these scales. Reliable and valid scales do not require that all possible items be included, just the ones that most reliably represent the construct [58].

Rohsenow [41] argued that it is important to include only items that were validated in controlled experimental administration studies and to exclude withdrawal symptoms.

There is debate about some items included in these scales. For example, Slutske's scale includes an item concerning "trouble sleeping" (i.e., something that is experienced before having a hangover), whereas Rohsenow's scale includes an item rating "overall hangover severity" (i.e., similar to the overall construct measured by the scale). **The AHS** can be scored without including the "overall hangover severity item", without significantly lowering its reliability or validity [41]. Slutske et al. [30] argued that a rating of hangover includes people's attributions about the cause of their discomfort and therefore may be biased, so some researchers may prefer to use this measure without that one item. On the other hand, a person may report that they have hangover when their discomfort is due to other causes (e.g., lack of sleep per se) or not report hangover when in fact they have all the symptoms, due to their belief that alcohol is not the cause. **The HSS** [30] deliberately omitted a question concerning "hangover" per se. This approach is similar to common practices in psycho-diagnosis: patients are diagnosed on the basis of reported symptoms, not asked to rate whether or not they have the target disorder. The disadvantage of this approach is that, at least at present, there are not established thresholds for determining the presence of hangover on the basis of symptom scores. This makes it difficult to count hangover events. It is thus recommended that investigators assess about "hangover" per se in addition to individual symptoms. Depending on investigator preference, scoring of survey responses may exclude the "hangover" item.

Gathering information about "hangover" will provide a simple index that permits direct comparisons across samples and instruments and will foster empirical tests of the relations between ratings of individual symptoms and the "hangover" response. It is notable that, when participants are asked to rate the severity of currently experienced "hangover" in the laboratory, this item correlates strongly with ratings of other common hangover symptoms [41]. Although current scales may have their limitations, both are useful in determining **which hangover symptoms are present, and the overall hangover severity.**

Taken together, the severity of hangover can be measured using a single item scale or by rating several symptoms [16]. At present, too little is known about the symptomatic presentation of hangover to evaluate whether these two approaches measure the same latent construct. If there are wide individual differences in symptomatic profile, the two approaches might not be interchangeable. For example, some individuals might experience only one or two symptoms, but experience them very intensely, whereas other individuals might develop a diffuse set of low-grade hangover symptoms. Each presentation might be counted as "severe" in one scoring scheme but not the other. Both approaches may contribute to the generation of important descriptive information. Although consensus was not reached on this issue at the 2010 Alcohol Hangover Research Group meeting [16], **more research and validation of a uniformly accepted measure for the presence and severity of hangover** is required [59].

4.CAUSES OF ALCOHOL HANGOVER

Hangovers are a frequent, though unpleasant, experience among people who drink to intoxication. Despite the prevalence of hangovers, however, this condition is not well understood scientifically. Multiple possible contributors to the hangover state have been investigated, and researchers have produced evidence that alcohol can directly promote hangover symptoms through its effects on urine production, the gastrointestinal tract, blood sugar concentrations, sleep patterns, and biological rhythms. In addition, researchers postulate that effects related to alcohol's absence after a drinking bout (i.e., withdrawal), alcohol metabolism, and other factors (e.g., biologically active, non-alcohol compounds in beverages; the use of other drugs; certain personality traits; and a family history of alcoholism) also may contribute to the hangover condition. Few of the treatments commonly described for hangover have undergone scientific evaluation.

Hangover is a complex state that probably cannot be understood by a unitary explanation. Understanding the hangover condition, however, will lead to a better comprehension of the physiological effects of alcohol and the adaptive responses that alcohol engenders. Despite its long history, however, hangover has received relatively scant formal attention from researchers. Little is known about the physiology underlying the hangover condition. For example, it is unclear whether hangover signs and symptoms are attributable to alcohol's direct effects on the body, its aftereffects, or a combination of both. Similarly, investigators are uncertain about the degree to which hangover affects a person's thinking and mentally controlled motor functions, a question with serious implications for activities such as job performance and driving.

In addition, researchers know little **about hangover prevention and treatment**. Although folk remedies for hangovers abound, their efficacy in reducing the intensity and duration of a hangover has not received systematic study. In fact, **some researchers and clinicians question whether finding an effective treatment for hangovers is desirable, given that the hangover experience may deter some people from engaging in subsequent episodes of heavy drinking**. Although gaps clearly remain in scientific knowledge about hangovers, research has elucidated several aspects.

4.1.What is alcohol hangover?

A hangover is characterized by the constellation of unpleasant physical and mental symptoms that occur after a bout of heavy alcohol drinking [60]. Physical symptoms of a hangover include fatigue, headache, increased sensitivity to light and sound, redness of the eyes, muscle aches, and thirst [30,41,54]. Signs of increased sympathetic nervous system activity can accompany a hangover, including increased systolic blood pressure, rapid heartbeat (i.e., tachycardia), tremor, and sweating. Mental symptoms include dizziness; a sense of the room spinning (i.e., vertigo); and possible cognitive and mood disturbances, especially depression, anxiety, and irritability. The particular set of symptoms experienced and their intensity may vary from person to person and from occasion to occasion. In addition, hangover characteristics may depend on the type of alcoholic

beverage consumed and the amount a person drinks. Typically, a hangover begins within several hours after the cessation of drinking, when a person's blood alcohol concentration (BAC) is falling. Symptoms usually peak about the time BAC is zero and may continue for up to 24 hours thereafter.

Overlap exists between hangover and the symptoms of **mild alcohol withdrawal (AW),** leading to the assertion that hangover is a manifestation of mild withdrawal. Hangovers, however, may occur after a single bout of drinking, whereas withdrawal occurs usually after multiple, repeated bouts. Other differences between hangover and AW include a shorter period of impairment (i.e., hours for hangover versus several days for withdrawal) and a lack of hallucinations and seizures in hangover. People experiencing a hangover feel ill and impaired. Although a hangover may impair task performance and thereby increase the risk of injury, equivocal data exist on whether hangover actually impairs complex mental tasks.

4.2.Physiological factors contributing to hangover

Hangover symptoms have been attributed to several divergent causes [30,41,54,60], including the i) **direct physiological effects of alcohol** on the brain and other organs; ii) the effects of the **removal of alcohol** from these organs after alcohol exposure (i.e., withdrawal); iii) the physiological effects of compounds produced as **a result of alcohol's metabolism** (i.e., metabolites), **especially acetaldehyde**; and iv) **non-alcohol factors**, such as the 1) toxic effects of other biologically active chemicals (i.e., congeners) in the beverage, 2) behaviors associated with the alcohol-drinking bout (e.g., other drug use, restricted food intake, 3) disruption of normal sleep time), and 4) certain personal characteristics (e.g., temperament, personality, and family history of alcoholism). There exist a current consensus that **more than one factor** most likely contributes to the overall hangover state [16]. The present study is designed to explore the role of alcohol metabolites in pathogenesis of hangover (iii), with special reference to the **prophylactic effect** of **acetaldehyde removal** during the drinking session, thus leaving out the other potential causes in this text.

4.2.1.Effects of alcohol metabolites

Alcohol undergoes a two-step process in its metabolism. First, an enzyme (alcohol dehydrogenase, ADH) metabolizes alcohol to an intermediate product, **acetaldehyde.** The acetaldehyde is then attacked by another enzyme, acetaldehyde dehydrogenase (ALDH), and another substance called **glutathione**, which contains high quantities of cysteine (a substance that is attracted to acetaldehyde)[60]. Together, the acetaldehyde dehydrogenase and the glutathione form the nontoxic acetate (a substance similar to vinegar). This process works well, leaving the acetaldehyde only a short amount of time to do its damage **if only a few drinks** are consumed.

Unfortunately, the liver stores of glutathione quickly run out when **larger amounts of alcohol** enter the system. This causes the acetaldehyde to build up in the body as the liver creates more glutathione, **leaving the toxin**

in the body for long periods of time. In studies that blocked ALDH with a drug called Antabuse (designed to fight alcoholism), acetaldehyde toxicity resulted in headaches and vomiting so bad that even alcoholics were wary of their next drink. Although body weight is a factor, part of the reason **women** should not keep up with men drink-for-drink is because women have less ALDH and glutathione, making their **hangovers worse** because it takes longer for the body to break down the alcohol.

Acetaldehyde is a chemically reactive substance that binds to proteins and other biologically important compounds [60]. At higher concentrations, it causes **toxic effects**, such as a rapid pulse, sweating, skin flushing, nausea, and vomiting. In most people, ALDH metabolizes acetaldehyde quickly and efficiently, so that this intermediate metabolite does not accumulate in high concentrations, although small amounts are present in the blood during alcohol intoxication. In some people, however, **genetic variants of the ALDH** enzyme permit acetaldehyde to accumulate. Those people routinely flush, sweat, and become ill after consuming small amounts of alcohol. Because of the similarity between the acetaldehyde reaction and a hangover, some investigators have suggested that **acetaldehyde causes hangovers** [16,60]. Although free acetaldehyde is not present in the blood after BAC's reach zero, the **toxic effects of acetaldehyde** produced during alcohol metabolism may persist into the hangover period.

4.2.2.Alcohol and headache

In a large epidemiological survey of headache in Danish 25- to 64-year-olds, the lifetime prevalence of hangover headache was 72%, making it the most common type of headache reported [61]. Alcohol intoxication results **in vasodilatation**, which may induce headaches. Alcohol has effects on **several neurotransmitters** and hormones that are implicated in the pathogenesis of headaches, including **histamine,** serotonin, and prostaglandins [62]. However, the etiology of hangover headache remains unknown, and the role of histamine has not been firmly confirmed.

4.2.2.1.Histamine and headaches

Common to all vascular-type headaches is that substances inducing swelling and dilatation of the blood vessels among susceptible individuals can provoke an attack of these vascular headaches [63]. Thus, substances that cause blood vessel swelling can provoke an acute attack during a series period. Nitroglycerin or **histamine**, smoking or even **minimal amounts of alcohol** can precipitate or increase the severity of the attacks as the sufferer's blood vessels seem to change and become susceptible to the action of these substances [63]. The blood vessels are not sensitive to these substances during headache-free periods.

4.2.2.2.Acetaldehyde: a potent liberator of histamine

Most histamine in human body is generated in granules of the **mast cells** (in tissues) or in white blood cells called basophils. Mast cells are especially numerous at sites of potential injury, including the nose, mouth, **gastrointestinal tract**, skin, and **blood vessels**. Non-mast cell histamine is found in several tissues, including

the brain, where it functions as **a neurotransmitter**. Another important site of histamine storage and release is the enterochromaffin-like (ECL) cells of the gastric mucosa.

Given the fact that also alcohol intake causes, especially in Oriental individuals (with hereditarily deficient ALDH), **hypersensitivity** symptoms that can be blocked by antihistamines, as well as urticaria and anaphylactoid reactions in Caucasian individuals as well, led Koivisto et al. (1996) to speculate the possibility that **acetaldehyde** (the first metabolite of alcohol) might enhance these reactions by directly affecting the tissue **mast cell** functions [64]. Using purified rat peritoneal mast cells incubated with different concentrations of acetaldehyde and ethanol at body temperatures, they demonstrated that **acetaldehyde,** at relatively low concentrations (50μM), directly **induces histamine release** from the mast cells. Ethanol did the same, but only at higher (molar) concentrations [64].

4.2.3. Acetaldehyde in the stomach and saliva can be eliminated by slow-release L-cysteine

Cysteine is a non-essential amino acid, which was shown (almost 40 years ago) to be capable of eliminating the toxicity of acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA)[65]. MTCA is an inert and non-toxic compound that is eliminated from the body through feces and urine, without being absorbed into the blood circulation. This simple principle was used in the recent innovation of **Biohit Oyj's Acetium™ capsule,** which contains 100mg L-cysteine.

In the proof-of-concept study, oral administration of Acetium was confirmed to effectively bind **acetaldehyde** originated from **ethanol metabolism** in acid-free stomach [66]. In that setting, the mean acetaldehyde level of gastric juice was 2.6 times higher with placebo than with l-cysteine (13 vs. 4.7 μM, $p<0.05$), implicating that L-cysteine can be used to decrease acetaldehyde concentration in acid-free stomach during alcohol exposure. In a more recent study, the authors showed that Acetium capsule with slow-release L-cysteine effectively eliminated acetaldehyde from the gastric juice of PPI-treated ALDH2-active and ALDH2-deficient subjects [67].

This led the authors to examine the concept, whether it would be possible to eliminate **alcohol-derived acetaldehyde** also from the **saliva**, using L-cysteine slowly released from a special buccal (Acetium) tablet [68]. Indeed, this was shown to be the case in tested volunteers, in whom, up to two-thirds of acetaldehyde (after alcohol intake) could be removed from the saliva with a slow-releasing buccal L-cysteine formulation. This offers yet another means for reduction of the total acetaldehyde exposure and load during alcohol drinking [68]. The same applies to elimination of acetaldehyde during smoking, as demonstrated in another study with Acetium lozenges [69].

5.STUDY HYPOTHESIS

The present study was designed to test the validity of an intriguing novel hypothesis, suggesting that **slow-release L-cysteine as the active ingredient of HDR-Help capsules** (supplemented with vitamin-B complex and vitamin-C) **consumed during the entire drinking episode is an effective prophylactic means to prevent or alleviate the symptoms and complaints of alcohol hangover** next morning.

5.1.Testimonials from Acetium users

As described above, Acetium capsules and lozenges were originally developed for inactivation of acetaldehyde in the stomach and in the saliva after alcohol intake and smoking, respectively. This novel concept on the potential **prophylactic effect** of L-cysteine against **alcohol hangover** (including histamine-provoked vascular headache) is based on elimination of alcohol-derived acetadehyde and blocking the acetaldehyde-induced histamine liberation from the tissue mast cells, and emerged purely by chance. In fact, the trigger to formulating this hypothesis is derived **from the Acetium™ capsule users** suffering from severe hangover symptoms who initiated (some years ago) a lively discussion on the websites. Indeed, several hundred people (different age and gender) have recorded their experience on the efficacy of Acetium™ capsule intake during the drinking episodes and found a substantial relief or total prevention of alcohol hangover symptoms [70,71,72]. These testimonials are found an increasing number of supporters on the web.

These spontaneous testimonials given in writing by an increasing number of Acetium users [70,71,72] prompted us to consider, whether a plausible mechanism could be discovered to explain these dramatic effects experienced by most of these subjects soon after starting the use of Acetium capsules in the context of drinking episodes. Given that L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, it would comprise an ideal means to conduct hangover prophylaxis without concern about the side effects inherent to many current treatment modalities. If proven correct in a formal randomized controlled trial (RCT), the concept of using Acetium capsules in prophylaxis of alcohol hangover would represent a major step forward in a better control of these frequently intractable symptoms associated with substantial annual morbidity [16,30,41,54,60].

5.2.Components of the study hypothesis

The current study hypothesis is built up of several elements, all based on solid experimental and/or clinical evidence. These elements include both i) the widespread toxic effects of acetaldehyde as an intermediate metabolite of alcohol in various organs [16,60] ii) its established role as histamine liberator with bearings to vascular-type headache as part of hangover [61-63], as well as iii) confirmed mechanisms whereby slow-release L-cysteine administration could interfere with this cascade by eliminating alcohol-derived acetaldehyde both from the saliva and stomach [64-69]. From up-stream to down-stream, the sequence of events leading to hangover symptoms could be as follows.

5.2.1.Direct toxic effects of acetaldehyde

ADH and ALDH, the two key enzymes in alcohol metabolism, together with glutathione (another cysteine-rich molecule) convert alcohol to its non-toxic end metabolite, acetate in the liver [60,66-69]. As far as only a few drinks are consumed, this process works well keeping the acetaldehyde exposure as a short-lived event only. However, the liver stores of glutathione quickly run out when larger amounts of alcohol are taken. This leads to accumulation of acetaldehyde in the body while the liver creates more glutathione, resulting in exposure to this toxic substance for prolonged periods of time. This is equivalent to the situation where ALDH is blocked with Antabuse or inactive due to genetic ALDH mutation (common in Asia). In both situations, people get a flush, sweat, and become ill after consuming of even tiny amounts of alcohol. Because of the obvious similarity between the acetaldehyde reaction and a hangover, many investigators consider that **acetaldehyde causes hangovers** [16, 60]. It is true that free acetaldehyde is not present in the blood after BAC's reach zero, but the toxic effects of acetaldehyde produced during alcohol metabolism may well persist until the hangover period [60]

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5.2.2.Histamine induces vascular-type headache

In both the HSS [30] and AHS [41] hangover severity scales, headache is listed among the key symptoms. In fact, hangover-associated headache belongs among the most common types of headache reported [61]. Alcohol intoxication results in **vasodilatation**, which is typical to all vascular-type headaches (e..g. migraine and cluster headache). Alcohol has effects on several neurotransmitters and hormones that are implicated in the pathogenesis of headaches, including **histamine**, serotonin, and prostaglandins [62]. There is firm experimental and clinical evidence implicating that histamine induces the enzyme Nitric Oxide (NO) Synthase, making NO available to act locally on the vasculature as **a vasodilator**. Histamine is known to activate cerebral endothelial H1-receptors, leading to formation of NO [63,73].

5.2.3.Histamine is synthesized in tissue mast cells and basophils

Histamine is synthesized in tissue mast cells and basophils by histidine decarboxylase converting histidine to histamine. Another important source of histamine are **enterochromaffin-like (ECL) cell**s that are abundant in **gastric (corpus) mucosa**. Histaminic cephalalgia (=headache) is the old name for cluster headaches, implicating that histamine has been linked with the development of vascular headaches since their description. Mast cells are ubiquitous, and their activation (e.g. in the meninges) by migraine triggers is now believed to contribute to genesis of migraine headaches [74].

5.2.4.Histamine is liberated from the mast cells by acetaldehyde

Mast cells play a crucial role in hypersensitivity, allergic, and inflammatory reactions by secreting chemical mediators, e.g. **histamine**, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. One of the potent liberators of histamine from the mast cells is acetaldehyde, both in the

human and in experimental animals [64,75]. This could neatly explain the frequent occurrence of vascular-type headache associated with hangover due to excessive alcohol intake as an abundant source of acetaldehyde.

5.2.5.Acetaldehyde in the saliva and stomach is inactivated by L-cysteine

The phenomenon that L-cysteine eliminates free acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA)[65], was exploited by Biohit Oyj, while designing their **Acetium™ capsule** containing 100mg of L-cysteine in a slow-release formula. Both the capsule and the recently introduced Acetium lozenge (3 mg L-cysteine) effectively eliminate acetaldehyde derived from either alcohol or cigarette smoke [66-69].

5.2.6.The efficacy of slow-release L-cysteine in prophylaxis of alcohol hangover?

We are convinced that the dramatic prevention of hangover symptoms and complaints following a regular intake of Acetium capsules concomitantly with alcohol drinking documented by the numerous case testimonials [70,71,72] cannot be by change. Instead, we believe that the mechanism must be based on the capacity of slow-release L-cysteine to interfere with the above deciphered sequence of events, whereby elimination of acetaldehyde in the stomach could block 1) the direct toxic effects of acetaldehyde, and 2) inhibit acetaldehyde-induced histamine liberation from the tissue mast cells in the stomach, thus preventing the full-blown hangover symptoms to develop [16,30,41,55,60].

5.2.7.Rational for the inclusion of the supplementary components in the HDR-Help combination

The rational of the inclusion of the additional components in the HDR-help combination remedy is explained in detail for each of those components **in the ANNEX** of this study protocol.

The present study is designed to validate this novel hypothesis that concomitant use of HDR-Help (2 capsules every 2 hours) during the whole drinking session is an effective means to decrease the symptoms and complaints of alcohol hangover, as suggested by anecdotal case testimonials of several young people reporting complete symptom abortion.

6.STUDY DESIGN

This double-blind, placebo-controlled trial (RCT) is designed to test the efficacy of intervention by Acetium capsules (200mg, used at 2-h intervals during drinking) in reducing the frequency of (or completely aborting) the alcohol hangover symptoms after drinking episodes. The study design follows the **Consensus Statement on Best Practice in Alcohol Hangover Research**, recently elaborated by the Alcohol Hangover Research Group [16].

A cohort of 50 volunteer subjects with frequent hangover symptoms after alcohol drinking will be enrolled by the Department of General Medicine, University of Tartu (Estonia) utilising the different modes of invitation.

Each study subject will be his/her own control, blindly allocated to two study arms, administered either Acetium capsules or placebo during the alcohol intake episodes. Before enrolment, all subjects will be requested to fill in **a structured questionnaire** recoding their detailed history of alcohol hangover, as well as the usual drinking habits. The subjects will be administered **three validated hangover scores** (AHS, HSS, AHSS) recording the severity of hangover symptoms [30,41,55] in the morning following the drinking sessions. The trial will consist of 3 drinking sessions in both study arms (Acetium and Placebo). Importantly, the study design is a **naturalistic setting**, mimicking as closely as possible, the regular drinking behaviour of the study subjects.

This intervention trial is designed and conducted in conformity with the current guidelines of the Alcohol Hangover Research Group Consensus Statement on Best Practice in Alcohol Hangover Research [16], **jointly** by the clinical specialists in the Department of General Medicine, University of Tartu (Estonia), and the Clinical Research Department of Biohit Oyj (Helsinki). These guidelines give detailed recommendations for the selection of study subjects, trial design, as well as evaluation of the hangover severity, and closely followed in the study design.

6.1.Patient enrolment

A cohort of 50 volunteer subjects with frequent hangover symptoms after alcohol drinking will be enrolled by the Department of General Medicine, University of Tartu (Estonia) utilising the different modes of invitation. Each study subject will serve as his/her own control, blindly allocated to two study arms, administered either Acetium capsules or placebo during the alcohol intake episodes.

6.2.Criteria of eligibility surveyed by pre-enrolment questionnaire

Before enrolment in the study, a survey will be made among the volunteers who have agreed to participate in the trial. The issues related to this type of surveys have been discussed in the Consensus Statement on Best Practice in Alcohol Hangover Research [16], and considered in designing the **SURVEY QUESTIONNAIRE** used in this study **(APPENDIX 1).** It is emphasized that the current lack of well-validated, comprehensive instruments for assessing hangover-related information has probably contributed to the slow growth of hangover research [30]. Researchers have frequently devised their own assessments for survey studies, and so the literature contains a remarkable diversity of measurement strategies. Existing approaches include the use of face-valid, single-item assessments concerning the occurrence or frequency of hangover [31–36], multi-item assessments of occurrence or frequency of experiencing specific hangover symptoms [26, 29–30, 37], questions about the severity or duration of symptoms during a typical hangover, a recent hangover, or following a recent drinking episode [26, 35, 38–40], and questions asking respondents to estimate their likelihood of experiencing a hangover after consuming a specified amount of alcohol [24–25]. As emphasized by the Consensus Statement, the development and evaluation of survey instruments should be regarded as a valued activity in hangover research [16]. In the present study, a notice was taken of this comment while 1)

designing the SURVEY QUESTIONNAIRE, and 2) attempting to validate these data against the real-life hangover experience recorded in the trial drinking sessions (see Methods).

In principle, **eligible** are all subjects (women and men) between 20-70 years of age, who regularly use alcohol, and have a propensity to develop symptoms of hangover. **Excluded** shall be those i) who do not experience hangover symptoms, ii) pregnant women, and iii) those who refuse to consent for the study.

6.3.Age of the study subjects

Valuable points related to age of people experiencing hangover are discussed in the Consensus Statement [16]. Importantly, most of the published hangover research is performed on young adults. There is, however, no reason to assume that hangovers are not experienced by adults of all ages. Research does show that drinking patterns change across ages, and heavy drinking episodes that may result in hangover are much less often experienced as age increases [16].

It is **unknown**, whether hangover symptoms and **severity change with age** and this should be an important aim of future research [16]. As stated, more hangover research on populations ranging in age from young adult to elderly is needed to ascertain any age-related effects [16]. Accordingly, the present study is making an attempt to this direction by enrolling **50 volunteers aging between 20 and 70 years, stratified into 5 age-strata of equal size: 20-30-year-old (n=10), 30-40 (n=10), 40-50 (n=10), 50-60 (n=10), and 60-70 (n=10).**

6.4.Gender

Both **male** and **female** participants **shall be eligible in the present trial**. This is to avoid the bias of most of the previous studies published in the past, having been performed on men only [16]. Although some of the more recent studies included both men and women, the small sample sizes generally do not allow a direct comparison between the sexes [16].

During acute alcohol intoxication, sex differences are common on some measures, even when using sex-adjusted dosing [9], because women are more sensitive to the effects of alcohol. Results from recent surveys [39,53] show that hangover is significantly **more severe** and **lasts longer** among **women** when compared to men. However, the results of these studies could be biased by the fact that women are attaining **a higher BAC** at the same number of drinks compared to men. For adult women, adjusting for both the average weight differences and differences in response to alcohol, the number of drinks should be adjusted by 70% compared to men [16]. Following the recommendations of the Consensus Statement [16], the present study attempts to tackle with the **potential sex differences in hangover severity**, the nature of symptoms that are experienced, and residual effects of intoxication on cognitive and psychomotor performance.

6.5.Cross-over vs between-subjects study design

In a cross-over design, subjects are tested several times in different study arms (e.g., during hangover and after placebo). According to the Consensus Statement [16], the advantage of this design is that **within-subject variability** does not play an important role. In contrast, when applying a **between-subjects** design (e.g., comparing a hangover group with a placebo group) one risks comparing good performers with poor performers. Random allocation to different treatment groups minimizes this risk, but variability remains higher when compared to within-subject designs. Given the individual nature of experiencing hangover symptoms and severity, one may **prefer** to use a **within-subject** design [16].

This recommendation of using the **within-subject design** by the Consensus Statement [16] will be followed in the present study where **each study subject serves as his/her own control**. In the study setting, the subjects are NOT blinded to alcohol and non-alcohol, but to the two test substances (Acetium and placebo) evaluated for efficacy as hangover preventive measures.

6.6.Naturalistic setting

While weighting between the naturalistic setting and a laboratory (controlled) setting, the authors decided to select the **naturalistic setting** for the present study design. Both study designs have their advantages and disadvantages. Our selection in favour of the naturalistic design is based on several arguments addressed in the Consensus Statement [16]. Important points to consider about the naturalistic approach are the fact that i) the drinking time is under personal control, ii) the place of consumption (e.g. home, bar, pub) is familiar to participants, and iii) the rate of consumption and type of beverage can change during the evening. In the naturalistic design, however, the amount and type of alcoholic beverages are not under experimental control [44, 45]. The participant's activities are not under control, and ingestion of alcohol is usually done over a longer period of time. While this naturalistic approach has the **advantage** of being ecologically valid and mimicking a normal pattern of alcohol consumption, researchers may, however, prefer to control some of these factors that are left to the participant's discretion for some studies. In the present study, the participants are requested to keep accurate records on the **type and quantities** of alcoholic beverages consumed during each drinking episode, as well as record the start and end time points of each session.

6.6.1.Blinding

Due to the inherent study design, the subjects cannot be blinded to alcohol and non-alcohol beverages, but to the two test substances (Acetium and placebo) evaluated for efficacy as measures in hangover prevention. Each study subject will be provided with both test substances in identical packages and provided with a different label (e.g. K and M). Following the most stringent recommendations of the guidelines [16], this company-sponsored trial with Acetium capsules will be conducted in **triple-blinded fashion**; i.e., 1) study subjects are blinded, 2) investigators are blinded, and 3) the sponsor (=statistician evaluating the study results) is blinded, to avoid the possibility of an undue bias on the results at the stage of data analysis.

6.6.2.Placebo control

Placebo preparation with design and package identical to the test preparation (HDR-Help capsule) will be used in this trial, administered by all test subjects during 3 drinking episodes, exactly as the test preparation.

6.7.Study compliance

The cohort to be enrolled in this trial (n=50) is not particularly large, and does not allow many dropouts without losing the statistical power. Therefore, it is crucial to monitor patients' compliance with the test preparations during the entire study period. One such approach is to keep regular contacts to the study subjects, encouraging them to adhere to the pre-study schedule agreed for the drinking episodes, reminding about the importance of immediate delivery of the hangover severity scores (AHS, HSS, AHSS) to the monitors.

Irrespective what measures are taken, it can be anticipated that a small number of subjects is lost to follow-up, a few do not completely adhere to the study protocol, and maybe some will interrupt the trial for other reasons [16]. It is possible that the final analyses must be run separately for two groups: **1) Per Protocol (PP)**, and 2) **modified Intention-to-treat (mITT).** The former includes all subjects who have been compliant with the study protocol, without any major violations in i) taking the test preparations, and ii) in recording their hangover severity scores. The latter category includes all subjects who were not fully compliant with the protocol, but who completed all the drinking sessions and provided (at least) reasonable records of hangover scores.

7.METHODS

7.1.Pre-enrolment baseline data

Before final inclusion in the cohort, all subjects are requested to sign **a written consent**, after having been explained the details of the study and the commitment requested from each subject for the successful completion of their 3 drinking sessions in both study arms (Acetium and placebo)(6 sessions altogether).

To assess whether **eligible** for the study, all interested volunteers responding to the invitations are asked to fill in (with monitor's assistance) a detailed structured questionnaire (**APPENDIX 1**). This **Questionnaire** is recoding a detailed history of the i) drinking habits, ii) propensity to hangover episodes as well as iii) the factors restricting the alcohol intake. The Questionnaire is divided to three separate sections, preceded by a short section of personal data to start with. **Section 1** is a survey-type record of the issues related to **alcohol intake** during the past year, with altogether 10 questions. **Section 2** is devoted to recording the experiences related to **alcohol hangover**, requesting the presence of 6 separate symptoms, stratified by the type of alcohol consumed. **Section 3** is targeted to record the potential **factors** found by the subject to be **restrictive** for her/his alcohol intake, divided to those appearing during the drinking episode and those that appear during hangover.

This pre-enrolment questionnaire is designed to provide the data necessary to judge whether the individual is eligible for the study or not. Clearly ineligible are subjects who i) do not use alcohol, ii) who never (or only rarely) experience hangover, and iii) those with any factor severely restricting their alcohol intake. In addition to this eligibility assessment, these data might be useful as covariates of the study endpoint, i.e. severity of the hangover, when included in multivariate analysis.

7.2.Hangover severity scores

Current hangover research relies on the use of three hangover scales. Slutske et al. [30] developed the 13-item Hangover Symptom Scale (HSS) for use in survey studies, and Rohsenow et al. [41] developed the 8-item Acute Hangover Scale (AHS) for use acutely in experimental administration studies. Another more recent scale is called Alcohol Hangover Severity Scale (AHSS), introduced by Penning et al. in 2013 [54]. The present study makes use of all three, attempting to validate whether differences exist in their performance and utility in recording the severity of hangover.

7.2.1.The Hangover Symptom Scale (HSS)

The HSS was originally developed by Slutske et al. in 2003 [30], to record the past year frequency of 13 hangover symptoms (APPENDIX 2). The assessment of hangover symptoms contained 13 items that sampled from each of the eight domains (constitutional, pain, gastrointestinal, sleep and biological rhythms, sensory, cognitive, mood, and sympathetic hyperactivity symptoms), described by Swift and Davidson [60]. For each of the 13 hangover symptoms, the participants indicated the percentage of drinking occasions, on a 5-point scale ranging from never (0% of the time) to every time (100% of the time), that were followed the next morning by the symptom [30]. This response format was similar to that used in the HQ [29]. Assessing the percentage of drinking occasions after which hangover symptoms occur partially controls for differences in the frequency of drinking and allows the HSS item scores to be interpreted as hangover susceptibility or proneness. In the original study [30], the 13 hangover symptoms were assessed with reference to the first few times that participants ever drank alcohol, and then repeated with reference to drinking occasions that occurred in the past 12 months.

In the present study, the HSS is used to record the 13 symptoms on the occasion of each of the 6 drinking sessions, blinded by Acetium and placebo use. This enables direct comparisons in hangover prevention efficacy between the test substance and placebo in each session-pairs separately (number symptoms/13), and also collectively for all three session-pairs (mean frequencies of the 13 symptoms).

7.2.2.The Acute Hangover Scale (AHS)

The Acute Hangover Scale (AHS) is based on symptoms supported in experimental investigations of hangover, with psychometric determination of items to retain, reliability and validity [41]. The preliminary results were reported on a smaller separate set of maritime academy cadets, and the analyses in a larger sample with a

broad range of age and experience provided more stable estimates of the psychometric properties of hangover. The nine AHS (**APPENDIX 3**) items include all the validated items from Chapman (1970)[46] and Ylikahri et al., (1974)[1], except i) trouble sleeping (not an experience "right now") and ii) general malaise (not a term used in lay language but replaced by "hangover"). The answer format used in AHS is the 0-7 scale of Chapman [46], with four anchors: None (0), Mild (1), Moderate (4) and Intractable (7). The general instruction to fill is: "Please rate how you feel right now on the following rating scales" [41].

The AHS is particularly designed for recording the severity of acute hangover symptoms [16,41], and as such highly suitable for the present study. The AHS is used to record the severity of the 9 symptoms on the occasion of each 6 drinking sessions, blinded by Acetium and placebo. This enables direct comparison in the efficacy of hangover prophylaxis between Acetium and placebo in each session-pairs separately (mean AHS score), and also collectively for all three session-pairs (pooled mean AHS score). For reference, in the original study, the comparison was made between alcohol and placebo arms in a controlled (laboratory) setting, resulting in significant difference in the mean AHS scores [41].

7.2.3.The Alcohol Hangover Severity Scale (AHSS)

Prompted by the lacking evidence whether the AHS and HSS include all relevant symptoms that significantly contribute to predicting overall hangover severity, Penning et al. [55] developed the AHSS and compared its effectiveness with the AHS and HSS. In the AHSS item selection and scale development, factor analysis yielded 11 factors. If Cronbach's alpha was <0.7, the factor was not considered for development of the AHSS. For each remaining factor, linear regression analyses were performed to determine which item(s) had at least 80 % predictive validity of the total score on the factor. This item, or a combination of items, was included in the AHSS. Before combining items, it was also checked how scores on the items correlated with each other. When a choice had to be made between items, the most common (most frequently reported) was chosen. The validity of the AHSS was determined by correlating the score with those of the HSS, AHS, and drinking variables. Regression analysis revealed that 12 items—fatigue (being tired), clumsiness, dizziness, apathy, sweating, shivering, confusion, stomach pain, nausea, concentration problems, heart pounding, and thirst—significantly predicted the overall hangover severity score and form the AHSS (**APPENDIX 4**). Adding additional symptoms, including headache, did not significantly improve the predictive validity of the AHSS.

The AHSS is another important and validated measure for recording the severity of hangover symptoms [16,55], and will be tested in the present study by comparing with HSS and AHS. The AHSS is used to record the severity of the 12 symptoms (in 0-10 scale) after each 6 drinking sessions, blinded by Acetium and placebo. This enables direct comparison in the efficacy of hangover prophylaxis between Acetium and placebo in each session-pairs separately (mean AHSS score), and also collectively for all three session-pairs (pooled mean

AHSS score). In the original survey-type study, the correlation between AHSS, HSS and AHS was shown to be good [55].

7.3.Study endpoints

The single most important aim of this study is to establish whether L-cysteine (Acetium capsule) is an effective measure in decreasing the severity of the hangover symptoms following the alcohol intake sessions in a naturalistic setting, as strongly suggested by the spontaneous testimonials of an increasing number of Acetium users. The null hypothesis of the study implicates that L-cysteine is no better than placebo in hangover prophylaxis in this double-blind intervention trial.

7.3.1.Primary endpoints

Rejection or not of the null hypothesis is based on comparison of the two strata (HDR-Help and placebo), and demonstration of **a statistically significantly different scores** in **three primary study endpoints** (hangover severity scores): 1) **The Hangover Symptom Scale (HSS)**, 2) **The Acute Hangover Scale (AHS**), and 3) **The Alcohol Hangover Severity Scale (AHSS)**. For each of the three scales, the scores are compared between the two study arms following a drinking session in naturalistic settings. To minimize the effect of chance (or an outlier), the scores of three sessions each blinded by Acetium and placebo, will be pooled, completed by each study subjects.

7.3.2.Secondary endpoints

In addition to these primary efficacy endpoints (HSS, AHS, AHSS), the Consensus Statement on Best Practice in Alcohol Hangover Research discusses the role of alcohol headache as an independent item reflecting the severity of hangover [16]. In the present study, we make an attempt to use **headache** as **the secondary endpoint**, because of its particular link with the study hypothesis, i.e., the role of histamine in vascular-type headache, histamine liberation by acetaldehyde, and acetaldehyde inactivation by slow-release L-cysteine (Acetium capsules and lozenge)(See Section 5.2.1.-5.2.6.). In addition, headache is not included in the 12 items of AHSS, albeit among the items in the two other hangover severity scales (AHS, HSS).

7.4.Statistical analysis

All statistical analyses will be performed using the SPSS 23.0.0.2 for Windows (IBM, NY, USA) and STATA/SE 14.1 software (STATA Corp., Texas, USA). The descriptive statistics will be conducted according to routine procedures. Frequency tables will be analysed using the $\chi 2$-test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables (e.g. the items in HSS). The effects of test preparation and placebo can be analysed separately by the non-parametric paired samples t-test (Wilcoxon signed ranks test) to compare the hangover scores in the Acetium-placebo pairs. For the pooled data of 3 paired sessions, the differences in the mean scores (AHSS, AHS) are analysed using the non-parametric Friedman test for multiple paired

(repeated) samples. The statistics used to compare the two arms with regard to the secondary endpoint is straightforward. For categorical outcomes, conventional regression models can be used, where the results are expressed as crude OR (odds ratio), and their 95% confidence intervals (95% CI). The impact of each item in the overall hangover severity score (AHS, AHSS) can be assessed using the reliability analysis with Cronbach's alpha [41,55], or by linear regression analysis. Multiple regression models can be used to estimate whether any of the recorded pre-enrolment variables are significant covariates of alcohol hangover.

7.5.Power analysis

The importance of power analysis has been discussed in some detail by the Consensus Statement on Best Practice in Alcohol Hangover Research [16]. A major problem of past studies has been the low sample size, often n<10. The low power makes it hard to interpret results from these studies and explains why results from studies are often inconclusive. As repeatedly emphasized, the sample size calculation is essential to ensure sufficient power when setting up a hangover study [16]. The sample size and power should be based on a clear rationale about expected effects for the primary outcome measure of the study. As hangover studies are likely to experience drop-outs, the number recruited should be higher, so that the number of participants with complete data is sufficient to ensure the statistical power. An additional consideration might be that ethical constraints limit the doses of alcohol that would generally be administered in an experimental study. If you cannot elicit the hangover at "full strength" you may need to increase the number of enrolled study subjects to compensate for the inflated effect size. Also, Howland et al. [27] revealed that about 25% of people do not experience alcohol hangovers. If having experience with alcohol hangovers is not an inclusion criteria, the number recruited should be increased to cover these hangover-free subjects.

In the present study, three different hangover severity scores are used, each with different grading that necessitates a different approach for power analysis. Starting from **the HSS**, a total of 13 items are used as dichotomous categorical variables. Using the figures from the original report [30], where on the average, the participants reported 5/13 hangover symptoms. The simplest approach (for hangover scale reduction by HDR-Help treatment), the power can be calculated using the **paired proportions test,** marginal proportions (McNemar) option. Using the within-subject design (each study subject serving as a case and a control), and supposing that the use of Acetium shall reduce the average HSS scale from 5.0/13 (0.384) to 3.25/13 (0.250), the study is adequately powered (Type II error 0.80, type I error 0.05) **with n=50.** Except for the effect size difference, this study power is sensitive to correlation between the paired samples; e.g. only n=31 are needed with correlation 0.7, but increasing up to 118 at correlation 0.4.

As to **the Acute Hangover Scale (AHS)**, the approach for power analysis is different. In the AHS, 9 symptoms are recorded and graded for severity using the 4-tier scale (0=absent, 1=mild, 4=moderate, and 7-intractable)[41]. Using the figures from the original report [41], where the mean AHS score of 1.9 (SD=0.9) was calculated after alcohol (case) intake, as compared to 0.6 (SD=0.4) after non-alcohol administration

(placebo) [41], the power can be calculated using the **paired-means test**. With these assumptions (null difference 0.4, correlation 0.6), this within-subject design, with **50 subjects** serving as a case and a control, the study is **adequately powered** (type I error 0.05) with **Type II error >0.950 (>95%).** In this setting, the power is sensitive to i) effect size (null) difference between HDR-Help and placebo, as well as to ii) the magnitude of the AHS scores in the two arms, and iii) the correlation between the paired samples.

Finally, **the Alcohol Hangover Severity Scale (AHSS)** is built up of 12 individual items of hangover symptoms, scored from 1-10 [55]. In the naturalistic setting, the mean score on the AHSS was 2.41 during hangover and 1.10 on the control day [55]. If these figures are used as basic assumptions, the power can be calculated using the same **paired-means test** as for the AHS. With these assumptions (null difference 1.31 correlation 0.5), this within-subject design, with **50 subjects** serving as a case and a control, the study is adequately powered (type I error 0.05) with **Type II error 1.000 (100%).** With this magnitude in effect size difference, the power is robust to i) a wide range of AHSS scores, ii) wide spectrum of correlation between the paired samples, as well as iii) wide range in the SD values of the AHSS mean scores.

To calculate the power of the study for the pooled results derived from the **three repeated session-pairs** (Acetium-Placebo), a different approach is needed. In this case, **the repeated-measures analysis of variance (ANOVA)** test needs to be used. This within-subject setting with 50 study subjects is adequately powered (Type II error >0.80) also for this design, but sensitive to i) within-subject variance, ii) correlation, and iii) error variance between the two study arms. No previous data are available to be used as assumptions in these calculations.

8.STUDY EXECUTION AND TIME TABLE

For execution of the study, the company shall make the contract with the Department of General Medicine, University of Tartu (Estonia). The execution of the study is monitored following the principles of good clinical practice (GCP) by a certified GCP monitor. For this purpose, the company has employed a project coordinator, who will also assist the investigators in all necessary practical steps during the set-up and execution of the study.

The study protocol will be subjected for approval by the Regional Committee on Medical Research Ethics (University of Tartu). Enrolment of the study subjects will start only after the ethical approval has been obtained. Given that each study subject shall complete 3 drinking session-pairs, 3 with Acetium and 3 with placebo (in a blinded fashion), and in the naturalistic setting, we expect that the study completion will require between 6 months and one year.

9.PROJECTED COSTS

All project costs will be covered by the sponsor, Biohit Oyj. Stratified research budget is not yet available.

APPENDIX 1.

PRE-ENROLMENT QUESTIONNAIRE FOR ELIGIBILITY

Date: Male ☐ Female ☐ Age:

Weight (kg) Height (cm) ☐

I am healthy ☐ I am not healthy ☐
(diseases _____)

I am using medicines, hormonal preparations (other than contraceptive pill) or drugs? ☐

I am not using compounds mentioned above ☐

Only for women:

I am pregnant ☐ I am not pregnant ☐

What day did your last periods start? _____

Are you using contraceptive pill? No ☐ Yes ☐; Which brand?

_____

Are you using other hormonal preparations? No ☐ Yes ☐
which? _____

Questions related to alcohol consumption:

(Select the option that matches your situation the best)

Part 1 (AUDIT):

(applies only to the events of the past year if not otherwise defined)
(PLEASE NOTE! mild beers **(max. 2.8% Alc. vol.)** and others are considered as alcohol beverages: 1 bottle equals to half a dose.

1 How often do you drink beer, wine or other alcoholic beverages? Try to count in also the times when you consumed only small amounts of alcohol, for example a bottle of medium-strength beer or a sip of wine.

1 never
2 about once a month or less
3 2-4 times a month
4 2-3 times a week
5 4 time a week or more

2 How many **doses** of alcohol are you consuming during the days you are consuming alcohol?

One **dose** is a bottle of beer or cider, a glass of mild wine (12 cl), small glass of strong wine (8 cl) or a dose of strong alcohol (spirit) (4 cl). A pint (0.5 l) of medium-strength beer is considered as 1.5 doses and a pint of strong beer (4.8–5.8%) is considered as 2 doses. A bottle of mild wine equals to 6 doses and a bottle of strong spirit (0.5 l) equals to 12 doses.

1 1-2 doses
2 3-4 doses
3 5-6 doses
4 7-9 doses
5 10 or more

3 How often do you drink six or more doses during one drinking session?

1 never
2 less than once a month
3 once a month
4 once a week
5 daily or almost daily

4 How often during the past year you were not able to stop consuming alcohol once you started?

1 never
2 less than once a month
3 once a month
4 once a week
5 daily or almost daily

5 How often during the past year you failed doing something, what is normally expected of you, because of drinking?

1 never
2 less than once a month
3 once a month
4 once a week
5 daily or almost daily

6 How often during the past year you have needed a beer or another alcoholic beverage in the morning to get yourself going after heavy drinking?

1 never
2 less than once a month
3 once a month
4 once a week
5 daily or almost daily

7 How often during the past year you have felt guilt or remorse after drinking?

1 never
2 less than once a month

3 once a month
4 once a week
5 daily or almost daily

8 How often during the past year you were unable to remember what happened the night before because of drinking?

1 never
2 less than once a month
3 once a month
4 once a week
5 daily or almost daily

9 Have you injured yourself or have somebody else hurt or injured themselves because of your alcohol consumption?

1 no
2 yes, but not during the past year
3 yes, during the past year

10 Have your family or friends, a doctor or another healthcare professional been concerned because of your alcohol consumption or recommended that you reduce drinking?

1 no
2 yes, but not during the past year
3 yes, during the past year

Part 2 (questions related to hangover):

(tick the correct answers)

Have you ever had a hangover of any kind? Yes ☐ No ☐ move to P**art 3**

If you answered yes, a few more questions related to hangover:

Association with the drink type, does this occur with you? If yes, describe the strength of the symptoms

		Yes	No	Not know	Weak	Moderate	Strong
Nausea	Beer	☐	☐	☐	☐	☐	☐
	Strong spirit	☐	☐	☐	☐	☐	☐
	Whiskey	☐	☐	☐	☐	☐	☐
	Cognac	☐	☐	☐	☐	☐	☐
	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐
	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to a particular drink type	☐			☐	☐	☐

		Yes	No	Not know	Weak	Moderate	Strong
Headache	Beer	☐	☐	☐	☐	☐	☐
	Strong spirit	☐	☐	☐	☐	☐	☐
	Whiskey	☐	☐	☐	☐	☐	☐
	Cognac	☐	☐	☐	☐	☐	☐
	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐

	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to a particular drink type	☐			☐	☐	☐

		Yes	No	Not know	Weak	Moderate	Strong
Confusion	Beer	☐	☐	☐	☐	☐	☐
	Strong spirit	☐	☐	☐	☐	☐	☐
	Whiskey	☐	☐	☐	☐	☐	☐
	Cognac	☐	☐	☐	☐	☐	☐
	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐
	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to a particular drink type	☐			☐	☐	☐

		Yes	No	Not know	Weak	Moderate	Strong
”Drinker’s remorse”	Beer	☐	☐	☐	☐	☐	☐
	Strong spirit	☐	☐	☐	☐	☐	☐
	Whiskey	☐	☐	☐	☐	☐	☐
	Coqnac	☐	☐	☐	☐	☐	☐
	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐
	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to						

	a particular drink type	☐			☐	☐	☐

		Yes	No	Not know	Weak	Moderate	Strong
Vision disturbances	Beer	☐	☐	☐	☐	☐	☐
(momentary	Strong spirit	☐	☐	☐	☐	☐	☐
light phenomena	Whiskey	☐	☐	☐	☐	☐	☐
after closing	Cognac	☐	☐	☐	☐	☐	☐
eyes)	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐
	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to a particular drink type	☐			☐	☐	☐

		Yes	No	Not know	Weak	Moderate	Strong
Another symptom,	Beer	☐	☐	☐	☐	☐	☐
which?	Strong spirit	☐	☐	☐	☐	☐	☐
_____	Whiskey	☐	☐	☐	☐	☐	☐
	Cognac	☐	☐	☐	☐	☐	☐
	Red wine	☐	☐	☐	☐	☐	☐
	White wine	☐	☐	☐	☐	☐	☐
	Strong wine	☐	☐	☐	☐	☐	☐
	Cider	☐	☐	☐	☐	☐	☐
	Another drink, which? _____	☐			☐	☐	☐
	Not related to a particular drink type	☐			☐	☐	☐

Part 3 (factors limiting alcohol consumption):

(Applies to the factors that may have limited the amount of alcohol in a drinking session, when your alcohol consumption has been the most abundant and when you did not have to think about tomorrow's worries or other external constraints, such as money, the amount of alcohol at disposal, etc.)

The following lists a few unpleasant effects caused by alcohol. Please consider, how many of them apply to you. Tick the right answer.

1 – has clearly limited drinking
2 – has limited drinking to some extent
3 – has not limited drinking

During drunkenness:

-	headache	1	2	3
-	nausea	1	2	3
-	fatigue	1	2	3
-	allergy or hypersensitivity	1	2	3
-	another symptom, which? _____	1	2	3
	_____	1	2	3

During hangover:

-	headache	1	2	3
-	nausea	1	2	3
-	fatigue	1	2	3
-	"drinker's remorse"	1	2	3
-	another symptom, which? _____	1	2	3
	_____	1	2	3

Other factors that may limit your alcohol consumption:

-	concern about alcohol addiction	1	2	3
-	concern about other unhealthy effects, which?			
	_____	1	2	3
	_____	1	2	3
-	concern about drunken behaviour	1	2	3
-	other factor(s): _____	1	2	3
	_____	1	2	3

I have never experienced drunkenness ☐

I have never experienced alcohol hangover ☐

APPENDIX 2.

THE HANGOVER SYMPTOM SCALE (HSS)(Slutske et al. 2003)

Name:		Gender:	Weight:
Date:		Code of the Test substance:	No. of Capsules taken:
Drinking Start time:	End of drinking:		
No of *units consumed of:	Beer:	Spirit:	Whiskey:
Cognac or liquor:	Red wine:	White wine:	Strong wine:
Cider:	Other (specify):	Other:	Other:

*One unit is equivalent to one bottle (0.33 l) of beer or cider, class (12 cl) of wine, small class (8 cl) of strong wine, or 4cl of strong spirit or liquor.

Symptom or Complaint	**Present**	**Not present**
Felt extremely thirsty or dehydrated		
Felt more tired than usual		
Experienced a headache		
Felt very nauseous		
Vomited		
Felt very weak		
Had difficulty concentrating		
More sensitive to light and sound than usual		
Sweated more than usual		
Had a lot of trouble sleeping		
Was anxious		
Felt depressed		
Experienced trembling or shaking		

APPENDIX 3.

THE ACUTE HANGOVER SCALE (AHS)(Rohsenow et al. 2007)

Name:		Gender:	Weight:
Date:		Code of the Test substance:	No. of Capsules taken:
Drinking Start time:	End of drinking:		
No of *units consumed of:	Beer:	Spirit:	Whiskey:
Cognac or liquor:	Red wine:	White wine:	Strong wine:
Cider:	Other (specify):	Other:	Other:

*One unit is equivalent to one bottle (0.33 l) of beer or cider, class (12 cl) of wine, small class (8 cl) of strong wine, or 4cl of strong spirit or liquor.

Symptom or Complaint	**Score**			
	0 None	**1 Mild**	**4 Moderate**	**7 Intractable**
Hangover				
Thirsty				
Tired				
Headache				
Dizziness, faintness				
Loss of appetite				
Stomach ache				
Nausea				
Heart racing				

APPENDIX 4.

THE ALCOHOL HANGOVER SEVERITY SCALE (AHSS)(Penning et a. 2013)

Name:		Gender:	Weight:
Date:		Code of the Test substance:	No. of Capsules taken:
Drinking Start time:	End of drinking:		
No of *units consumed of:	Beer:	Spirit:	Whiskey:
Cognac or liquor:	Red wine:	White wine:	Strong wine:
Cider:	Other (specify):	Other:	Other:

*One unit is equivalent to one bottle (0.33 l) of beer or cider, class (12 cl) of wine, small class (8 cl) of strong wine, or 4 cl of strong spirit or liquor.

Symptom or Complaint	**Score**										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fatigue (being tired)											
Apathy (lack of interest/concern)											
Concentration problems											
Clumsiness											
Confusion											
Thirst											
Sweating											
Shivering											
Stomach pain											
Nausea											
Dizziness											
Heart pounding											

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ANNEX

HDR-Help (Harm-Dependence Reduction)

-Confidential Document for Pre-Market Survey-

Format
Capsule

Composition (subject to discussion)

1) Active ingredient:

L-cysteine (slow-release format)	100 mg

2) Additional active constituents:

B1-vitamin (Thiamine)	150 mg
B2-vitamin (Riboflavin)	6 mg
B3-vitamin (Niacin)	10 mg
B6-vitamin (Pyridoxin)	12 mg
B9-vitamin (Folic acid)	150 µg
B12-vitamin (Cyancobalamine)	12 µg
C-vitamin (Ascorbic acid)	200 mg

3) **Additional (inactive) compounds**:

Eudragit® RS-PO	100 mg
Calciumhydrophosphate	200 mg
Hypromellose	73.5 mg
Titanium dioxide	1.5 mg

Indication for use
Prevention and alleviation of alcohol hangover symptoms

Dosage
Two capsules every 2h during the whole drinking episode, starting at -1h (hour in advance)

1.Mechanisms behind hangover

1.1.Physiological factors contributing to hangover

Hangover symptoms have been attributed to several divergent causes (1,2, 3,4), including the i) direct physiological effects of alcohol on the brain and other organs; ii) the effects of the removal of alcohol from these organs after alcohol exposure (i.e., withdrawal); iii) the physiological effects of compounds produced as a result of **alcohol metabolism** especially **acetaldehyde**; and iv) non-alcohol factors, such as the 1) toxic effects of other biologically active chemicals (i.e., congeners) in the beverage, 2) behaviors associated with the alcohol-drinking bout (e.g., other drug use, restricted food intake), 3) disruption of normal sleep time), and 4) certain personal characteristics (e.g., temperament, personality, and family history of alcoholism). There

exist a current consensus that **more than one factor** most likely **contributes to the overall hangover state (5)**.

1.2.Effects of alcohol metabolites

Alcohol undergoes a two-step process in its metabolism. First, an enzyme (alcohol dehydrogenase, ADH) metabolizes alcohol to an intermediate product, **acetaldehyde.** The acetaldehyde is then attacked by another enzyme, acetaldehyde dehydrogenase (ALDH), and another substance called **glutathione**, which contains high quantities of cysteine (a substance that is attracted to acetaldehyde)(4). Together, ALDH and glutathione form **the non-toxic acetate**. This process works well as long as a few drinks only are consumed, leaving acetaldehyde exposure only a short-lived event.

Unfortunately, the liver stores of glutathione quickly run out when **larger amounts of alcohol** enter the circulation. This causes the acetaldehyde to build up in the body when the liver creates more glutathione, **leading to prolonged exposure of the body to this toxic agent (acetaldehyde).** When ALDH is being blocked with Antabuse, acetaldehyde toxicity results in intractable headaches and vomiting, making further alcohol intake practically impossible. Although body weight is a factor, part of the reason why **women** should not keep up with men drink-for-drink is because women have less ALDH and glutathione, making their **hangovers worse** because it takes longer for the body to break down the alcohol (4,5).

Acetaldehyde is a chemically reactive substance that binds to proteins and other biologically important compounds (4). At higher concentrations, it causes **toxic effects**, such as a rapid pulse, sweating, skin flushing, nausea, and vomiting. In most people, ALDH metabolizes acetaldehyde quickly and efficiently, so that this intermediate metabolite does not accumulate in high concentrations, although small amounts are present in the blood during alcohol intoxication. However, **genetic variants of the ALDH** enzyme (common in Asia) permit acetaldehyde to accumulate. Those people regularly flush, sweat, and become ill after consuming small amounts of alcohol. Because of the similarity between the acetaldehyde reaction and hangover, some investigators believe that **acetaldehyde is the leading cause of hangovers** [for review, see 4,5]. Although free acetaldehyde is not present in the blood after blood alcohol concentration (BAC) reaches zero, the **toxic effects of acetaldehyde** produced during alcohol metabolism may persist into the hangover period.

2.Rational for efficacy of the active ingredient (L-cysteine)

The active component of HDR-Help (slow-release L-cysteine) is capable of inactivating the key intermediate metabolite of alcohol (acetaldehyde), thus eliminating the exposure to this highly toxic alcohol metabolite from the pathogenetic pathway of hangover, which should have a prophylactic effect on the development and severity of the hangover symptoms.

2.1.Components of the study hypothesis

The current study hypothesis is built up of several elements, all based on solid experimental and/or clinical evidence. These elements include both i) the widespread toxic effects of acetaldehyde as an intermediate metabolite of alcohol in various organs (4,5), ii) its established role as histamine liberator with bearings to vascular-type headache as part of hangover (6-8), as well as iii) confirmed mechanisms whereby slow-release L-cysteine administration could interfere with this cascade by eliminating alcohol-derived acetaldehyde both from the saliva and stomach (9-14). From up-stream to down-stream, the sequence of events leading to hangover symptoms could be as follows.

2.1.1.Direct toxic effects of acetaldehyde

ADH and ALDH, the two key enzymes in alcohol metabolism, together with glutathione (another cysteine-rich molecule) convert alcohol to its non-toxic end metabolite, acetate in the liver (4,11-14). As far as only a few drinks are consumed, this process works well keeping the acetaldehyde exposure as a short-lived event only. However, the liver stores of glutathione quickly run out when larger amounts of alcohol are taken. This leads to accumulation of acetaldehyde in the body while the liver creates more glutathione, resulting in exposure to this toxic substance for prolonged periods of time. This is equivalent to the situation where ALDH is blocked with Antabuse or inactive due to genetic ALDH mutation. In both situations, people get a flush, sweat, and become ill after consuming of even tiny amounts of alcohol. Because of the obvious similarity between the acetaldehyde reaction and a hangover, many investigators consider that acetaldehyde causes hangovers (4,5). It is true that free acetaldehyde is not present in the blood after BAC's reach zero, but the toxic effects of acetaldehyde produced during alcohol metabolism may well persist until the hangover period (4).

2.1.2.Histamine induces vascular-type headache

In both the HSS (1) and AHS (2) hangover severity scales, **headache** is listed among **the key symptoms**. In fact, hangover-associated headache belongs among the most common types of headache reported (6). Alcohol intoxication results in vasodilatation, which is typical to all vascular-type headaches (e.g. migraine and cluster headache). Alcohol has effects on several neurotransmitters and hormones that are implicated in the pathogenesis of headaches, including histamine, serotonin, and prostaglandins (7). There is firm experimental and clinical evidence implicating that histamine induces the enzyme Nitric Oxide (NO) Synthase, making NO available to act locally on the vasculature as a vasodilator. Histamine is known to activate cerebral endothelial H1-receptors, leading to formation of NO (8,15).

2.1.3.Histamine is synthesized in tissue mast cells and basophils

Histamine is synthesized in tissue mast cells and basophils by histidine decarboxylase converting histidine to histamine. Another important source of histamine are entero- chromaffin-like (ECL) cells that are abundant in gastric (corpus) mucosa. Histaminic cephalalgia (=headache) is the old name for cluster headaches, implicating

that histamine has been linked with the development of vascular headaches since their description. Mast cells are ubiquitous, and their activation (e.g. in the meninges) by migraine triggers is now believed to contribute to genesis of migraine headaches (16).

2.1.4.Histamine is liberated from the mast cells by acetaldehyde

Mast cells play a crucial role in hypersensitivity, allergic, and inflammatory reactions by secreting chemical mediators, e.g. histamine, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. One of the potent **liberators of histamine** from the mast cells is **acetaldehyde**, both **in the human** and in experimental animals (9,17). This could neatly explain the frequent occurrence of **vascular-type headache** associated with hangover due to excessive alcohol intake as an abundant source of acetaldehyde.

2.1.5.Acetaldehyde in the saliva and stomach is inactivated by L-cysteine

The phenomenon that L-cysteine eliminates free acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA)(10), was exploited by Biohit Oyj, while designing their Acetium™ capsule containing 100mg of L-cysteine in a slow-release formula. Both the capsule and the recently introduced Acetium lozenge (3 mg L-cysteine) effectively eliminate acetaldehyde derived from either alcohol or cigarette smoke (11-14).

2.1.6.The efficacy of slow-release L-cysteine in prophylaxis of alcohol hangover?

We are convinced that the dramatic prevention of hangover symptoms and complaints following a regular intake of Acetium capsules concomitantly with alcohol drinking documented by the numerous **case testimonials (18-20) cannot be by change**. Instead, we believe that the mechanism must be based on the capacity of **slow-release L-cysteine to interfere with the above deciphered sequence of events**, whereby elimination of acetaldehyde in the stomach could block 1) the direct toxic effects of acetaldehyde, and 2) inhibit acetaldehyde-induced histamine liberation from the tissue mast cells (and ELC) in the stomach, thus preventing the full-blown hangover symptoms to develop, including the vascular headache (1,2,4,5,21).

3.Rational for the adjunct effects of the supplementary compounds

The composition of the additional constituents in the HDR-Help is designed to provide a maximum effect tackling the multiple other mechanisms contributing to the overall hangover state (4,5), thus providing synergistic effects to the active ingredient in this new formulation.

3.1.Vitamin-B1 (Thiamine)

Thiamine is a vitamin essential to two entirely separate processes in humans: 1) it is required for the oxygen-dependent part of the metabolism of carbohydrates (and alcohol) to produce energy, and 2) thiamine is also

required for the membrane polarisation / depolarisation step in nerve transmission (22). The effect of these two separate processes that both require thiamine means that alcohol consumption in cases where vitamin-B1 is limited, **diverts available thiamine away from the brain to break down the alcohol**. After this is exhausted, the remaining alcohol is converted to fat, but in the interim, **high levels of intermediate products of alcohol breakdown** (prior to the thiamine-dependent step) accumulate in the blood and enter the brain. In order to dilute the alcohol and its breakdown products, water is drawn out of the tissues (including the brain) into the blood, **dehydrating** these tissues (22-25).

Clinical data indicates that **3mg thiamine** per one bottle of beer (333mll) is more than enough to prevent Wernicke-Korsakoff syndrome and around 90% of the alcoholic brain damage (22). However, unpublished findings of this researcher indicate that **optimum hangover-eliminating doses** of vitamin-B1 are considerably higher (22). Speculation suggests that these higher doses of thiamine may substantially enhance the activity of alpha-ketoglutarate dehydrogenase, thus preventing the build-up of **glutamate** and hence GABA in the cerebellum of the brain. This hypothesis might account for the observation that **large thiamine doses** during alcohol intake tend to **reduce nausea and dizziness** during drinking as well as reducing or eliminating **headaches** and memory loss the next day (22,24).

This evidence would suggest that **a third thiamine-dependent process** may be a factor in hangovers. The most likely candidate appears to be **a multi-enzyme complex** called **alpha-ketoglutarate dehydrogenase**, which not only catalyzes a critical thiamine-dependent step in the energy-producing breakdown of alcohol, but is also at a point at which amino acid and carbohydrate metabolism interact (22). The substrate of this enzyme complex, alpha-ketoglutarate, can be converted to **glutamate** which in turn can be converted to **gamma-aminobutyric acid** (GABA), an inhibitory neurotransmitter and the active metabolite of gamma-hydroxybutyrate, (GHB) with the street drug name "Fantasy".

3.2.Vitamin-B2 (Riboflavin)

Riboflavin (vitamin-B2) is part of the vitamin B group. It is the central component of the cofactors FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) and as such required for a variety of flavoprotein enzyme reactions including activation of other vitamins (26). Glutathione reductase is an FAD-dependent enzyme that participates in the redox cycle of glutathione. The glutathione redox cycle plays a major role in **protecting** organisms from **reactive oxygen species**, such as hydroperoxides.

Riboflavin is one of several antioxidant B vitamins that your body needs simply for survival (26). The liver, kidneys and heart are all responsible for breaking down the nutrient absorbed from the food. Riboflavin is required for a wide variety of cellular processes, and like the other B vitamins, it plays a key role in energy metabolism, including proper metabolizing of fats, carbohydrates, ketone bodies and proteins.

Several groups of people are susceptible to a riboflavin deficiency (26). Riboflavin deficiency has been associated with increased oxidative stress (26,27,28). Studies show that by drinking large quantities of alcohol, one will reduce the riboflavin absorption by 50% (27,28). Thus, alcoholics may need five to 10 times the normal amount of vitamin-B2 needed by non-drinkers. Clearly, **alcoholics are at increased risk for riboflavin deficiency** due to i) decreased intake, ii) decreased absorption, and iii) impaired utilization of riboflavin. Interestingly, the elevated homocysteine levels associated with riboflavin deficiency rapidly decline during alcohol withdrawal (29).

Accordingly, supplementation of vitamin-B2 by a formulation intended for prevention of hangover after intense drinking has a rational in counteracting the **riboflavin-depleting effects of alcohol** intake (26-29).

3.3.Vitamin-B3 (Niacin)

Niacin, also known as vitamin-B3 and nicotinic acid, is an organic compound and, depending on the definition used, one of the 20 to 80 essential human nutrients (30). Insufficient niacin in the diet can cause nausea, skin and mouth lesions, anemia, headaches, and tiredness. The lack of niacin may also be observed in pandemic deficiency disease, which is caused by a lack of five crucial vitamins (niacin, vitamin C, thiamin, vitamin D, and vitamin A) and is usually found in areas of widespread poverty and malnutrition (30).

Other forms of vitamin-B3 include the corresponding amide and nicotinamide ("niacinamide"), where the carboxyl group has been replaced by a carboxamide group (CONH2)(31). Niacin cannot be directly converted to nicotinamide, but both compounds are **precursors** of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in vivo (30). NADP and NAD are coenzymes for many **dehydrogenases**, participating in many hydrogen transfer processes (32). In addition to **catabolism** of fat, carbohydrate, and proteins, NAD/NADP is essential in the metabolism of **alcohol** (by ADH and ALDH). High energy requirements (brain) or high turnover rate (gut, skin) organs are usually the most susceptible to their deficiency e.g. in chronic alcohol abuse (33).

Interestingly, acetaldehyde induces deficiencies of both niacin and NAD. NAD is normally the most plentiful **vitamin coenzyme** in the human brain, and as such an important catalyst in the production of many key, brain neurotransmitters, such as serotonin (30-33). As said, NAD is also **the coenzyme** that **activates ADH and ALDH; z**inc is also required to activate these two enzymes. Since the need for NAD in all cells is great, yet the supply is limited, **NAD is normally recycled** continually during cellular energy production (30).

However, when NAD is involved in **acetaldehyde** detoxification, this **recycling of NAD is blocked**, and an altered form of NAD called NADH accumulates, impairing cellular biochemistry in many ways. **Thus,**

chronic acetaldehyde exposure may produce a mild, functional, niacin/NAD deficiency, even in a person consuming a balanced diet which meets RDA (recommended dietary allowance) levels of vitamin-B3 intake (33).

Chronic exposure to **acetaldehyde** from any source (alcohol, cigarette smoke, alimentary) creates **a deficiency** of vitamin-B1, pantothenic acid, and **niacin** (and lack of NAD/NADH)(34). In subjects who have adequate amounts of glutamine, selenium, **niacin**, folic acid, vitamin-B6, -B12, iron, and molybdenum, aldehydes continue to be metabolized into acetic acid and acetyl coenzyme A. However, if these nutrients are in poor supply (like in chronic alcohol abuse), **aldehydes start accumulating in the tissues** (34). When the digestion is balanced, or with adequate supplements, the potential poison is transformed into a source of energy, i.e., aldehyde poison becomes acetyl coenzyme A (34).

3.4.Vitamin-B6 (Pyridoxine)

Vitamin B6, also called pyridoxine, is one of 8 B vitamins. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B-complex vitamins also help the body metabolize fats and protein, as well as make several neurotransmitters (35). Importantly, the body **needs vitamin-B6 also** in order **to properly absorb vitamin-B12.** Other general functions of pyridoxine include its role as an anti-oxidant, and also produces antibodies to help fight general disease. Red blood cell (RBC) formation depends on the presence of B6.

A deficiency of vitamin B6 alone is relatively uncommon and often occurs in association with other vitamins of the B complex. Deficiencies of vitamin-B6 can be subtle and may be readily dismissed as one of the nuisances of aging, a family trait or a personal idiosyncrasy. Symptoms of serious deficiency include: 1) muscle weakness; 2) nervousness; 3) irritability; 4) depression; 5) difficulty concentrating, and 6) short-term memory loss. The elderly **and alcoholics** have an increased risk of vitamin B6 deficiency, as well as other micronutrient deficiencies (36).

There is also evidence that ingestion of vitamin-B6 can **alleviate** some of the many symptoms of **an alcoholic hangover** and morning sickness from pregnancy. This might result from its mild diuretic effect (37). In 1973, Khan et al examined the effects of vitamin-B6 in people who were drinking to intoxication (38). During an evening of heavy drinking, 17 men and women received **1200mg** of either vitamin-B6 or placebo, in three equal doses of 400 mg each. The first at the beginning of alcohol consumption, the second 3 hours later, and the third at the end of the evening. Using a 20-symptom scale to assess hangover complaints, the researchers showed that the participants taking vitamin-B6 had a **50% reduction in their symptoms** compared with those taking placebo (38). This trial has not been repeated by other groups, however, but as placebo-controlled trial, this study provides evidence to suggest that vitamin-B6 might be beneficial in alleviating the cumbersome symptoms of alcohol hangover. These data clearly warrant inclusion of the vitamin-B6 in the new formulation.

3.5.Vitamin-B9 (Folic acid)

Folic acid is made and used in fortified foods and supplements on the theory that it is converted into folate (39). Folates occur naturally in many foods and, among plants, are especially plentiful in dark green leafy vegetables. However, folic acid is an oxidized form, not significantly found in fresh natural foods. To be used by the human, it must be converted to tetrahydrofolate (tetrahydrofolic acid) by dihydrofolate reductase (DHFR), the activity of which is low, however (40).

Vitamin-B9 is essential for numerous bodily functions. Humans cannot make folates; therefore, folic acid has to be supplied through the diet to meet their daily requirements. The human body needs folate to make DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions (41).

A lack of dietary folates can lead to **folate deficiency**. Common **symptoms of folate deficiency** include diarrhea, macrocytic anemia with weakness or shortness of breath, nerve damage with weakness and limb numbness, pregnancy complications, mental confusion, forgetfulness or other cognitive deficits, mental depression, sore or swollen tongue, peptic or mouth ulcers, headaches, heart palpitations, irritability, and behavioral disorders (42). Folate deficiency in not uncommon among **alcoholics**. Alcohol affects i) the body's ability to absorb folate and also ii) increases folate in the urine. Many alcohol abusers have poor quality diets that do not provide the suggested intake of folate. Increasing folate intake through diet, or folic acid intake through fortified foods or supplements, may benefit the health of alcoholics (42.).

There is no direct proof on the impact of folate on the severity of hangover symptoms in the literature. However, folic acid is an essential component of vitamin B complex, and as such a logical constituent in this new formulation targeted to prevent hangover symptoms.

3.6.Vitamin-B12 (Cobalamine)

Vitamin-B12, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and the formation of red blood cells (43).It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism. B12 is the largest and most structurally complicated vitamin and can be produced industrially only through a bacterial fermentation-synthesis. This synthetic B12 is used to fortify foods and sold as a dietary supplement.

Vitamin-B12 is important for a wide variety of physiological functions, ranging from the brain to the blood. Estimates of B-12 deficiency rates vary within wide range, but **alcohol consumption** is an established risk factor. More than moderate levels of alcohol consumption can significantly **reduce B-12 absorption** and may

deplete bodily stores. Common deficiency symptoms include anemia, fatigue, muscle weakness, loss of appetite, weight loss, mouth sores, numbness and tingling in the limbs, unsteady gait, low blood pressure, depression, confusion and poor short-term memory.

Alcohol, especially in large amounts, irritates the mucosal lining of the stomach and intestines. When the stomach lining is irritated it produces less hydrochloric acid and may secrete less intrinsic factor too, both of which contribute to reduced vitamin-B12 **absorption** from food. Alcohol not only impairs nutrient absorption by damaging the lining of the gastrointestinal system, but it also prevents nutrients from being fully utilized in the body by altering their transport, storage and excretion. Like other B vitamins, B12 is water soluble, get flushed quickly from the body, accelerated by the diuretic effect of alcohol. Alcohol hastens the B-vitamin flush, and even occasional drinkers should be mindful of replenishing their B vitamins after a drinking episode, and even moderate drinking can decrease vitamin B12 levels. Indeed, vitamin-B12 helps in converting carbohydrates into glucose which fights from fatigue and provide energy to our body.

Although formal studies on vitamin-B12 in **prevention of alcohol hangover** are few, there is abundant circumstantial evidence pointing to this direction (44). While B vitamins should not be relied upon as prevention of a hangover, they can **relieve symptoms** and make it easier to recover. Since alcohol is a diuretic, a lot of essential vitamins and minerals are being lost after a night of drinking heavily. Therefore, restoring these vitamins is important for hangover relief.

There are sound biological basis for this practice, making the use of B12-vitamin as a surefire method of **relieving hangover** symptoms. These issues include the following: 1) metabolizing alcohol requires B vitamins in the liver; 2) vitamin-B12 helps support a healthy nervous system; 3) increased urine production causes loss of critical vitamins; and 4) vitamin-B12 helps maintain healthy energy levels. Thus, taking vitamin-B12 helps the liver metabolize alcohol more effectively and keep the red blood cell count at a healthy level (44). Many people experience difficulty speaking, driving, and moving in any way while having hangover, in part due to a lack of vitamin-B12 in the system. B12 keeps the central nervous system operating more efficiently. Due to its diuretic effects, alcohol will also contribute to loss of many vitamins which can trigger fatigue and dehydration. Obviously, taking B12 vitamins before and after drinking helps healthy kidney function and keeps the bladder from storing too much water at one time (44). B12 plays a vital role in converting carbohydrates to sugar, or glucose, which is the main energy source for a healthy metabolism and overall healthy functions of the body. Since many people claim to have no energy after a night of drinking, taking B12-vitamin can relieve that symptom as well. All this contributes to the fact why many people notice that their hangover symptoms are much easier to handle with vitamin-B12 (44).

3.7.Vitamin-C (Ascorbic acid)

Vitamin C or L-ascorbic acid, is an essential nutrient for humans and certain other animal species. Vitamin C is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that, when

dysfunctional, cause the most severe symptoms of scurvy (45) Ascorbate also acts as **an antioxidant**, protecting against oxidative stress. Ascorbic acid is also widely used as a food additive to prevent oxidation. Vitamin-C is one of the essential nutrients **depleted by alcohol** consumption (47). Because it is the body's primary water-soluble dietary antioxidant, this depletion results in severe **oxidative stress** in daily drinkers (48). Vitamin-C is also an essential co-factor for many enzymes, and its depletion lowers levels of internally produced antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (49).

There is also some evidence that vitamin-C accelerates alcohol metabolism (46). Thus, having a healthy dose of vitamin-C in before and after drinking may **prevent** or **reduce** the severity of **hangovers** by speeding up the metabolism of alcohol by the liver. Alcoholics may also benefit from keeping up their vitamin-C intake as it can reduce alcohol cravings. These circumstantial observations justify the inclusion of vitamin-C also in the new formulation as a supplementary agent with potentially beneficial effects on hangover symptoms.

4.SUMMARY

There exists a current consensus that more than one factor contributes to the overall hangover state (1,2,5). However, the pathology of alcohol hangover still has not been fully elucidated. As a consequence, no validated and universally effective hangover remedies are available (5). A possible explanation for the lack of scientific interest may be that an effective cure is often regarded as undesirable by people who view alcohol hangover as an adequate punishment for excessive drinking. Moreover, effective hangover cures may even stimulate binge drinking (i.e., drinking continuously for days in a row)(5).

Given the complex pathogenesis of alcohol hangover, it is highly **unlikely** that **one single remedy** would be universally effective. This is well illustrated by the multitude of remedies attempted for prevention and treatment of hangover symptoms during the past decades (50). Instead, it is more likely that best results could be achieved by a **combination formula**, with several constituents targeting to the different arms contributing to the development of full-blown hangover state following heavy drinking.

The new formulation is designed to meet this need. The formulation consists of a principal active compound (slow-release L-cysteine) with established effect against the key alcohol metabolite (**acetaldehyde**) implicated by most authorities as **the main culprit** of alcohol hangover symptoms. To substantiate this main effect of acetaldehyde elimination by L-cysteine, the formulation contains the B-vitamin complex and vitamin-C. All these are interfered by alcohol intake, and many of the symptoms associated with depletion of these vitamins are shared by alcohol hangover state, as summarized in the text. Thus, an adequate supplementation of all these elements after heavy alcohol intake is the logical step to counteract these alcohol-associated effects, and has the potential to prevent or alleviate the hangover symptoms.

As an extra bonus additional to hangover prevention, this HDR-help formulation, through its main active

component (slow-release L-cysteine) contributes to the risk reduction of stomach and esophageal cancer (11-14). Together with *Helicobacter pylori* infection and atrophic gastritis, acetaldehyde (classified by IARC as group 1 carcinogen to humans) is an important risk factor of both esophageal and gastric cancer, and exposure to this carcinogen should be limited to the minimum (51).

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