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THERAPEUTIC EFFICACY OF *ALLIUM SATIVUM* AGAINST SLEEP DEPRIVATION-INDUCED DYSLIPIDEMIA IN ADULT FEMALE WISTAR RATS

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ABSTRACT

Sleep deprivation (SD) resulting from unhealthy lifestyle choices poses oxidative challenges and is strongly linked to an increased risk and prevalence of various metabolic disorders. While the negative effects of SD on mental health are well documented, its impact on lipid metabolism remains less explored. Allium sativum (Garlic) has been recognized for its cardioprotective properties in different preclinical models. This study aimed to investigate the cumulative effects of acute SD on lipid metabolism and assess the potential therapeutic benefits of aqueous A. sativum extract using female Wistar rats as the experimental model. The animals (N = 25) were categorized into five groups of five animals each (n = 5): Group I served as the normal control and was administered distilled water (1ml/kg), whereas Group II represented the negative control (sleep-deprived, untreated). Groups III, IV, and V received oral administration of A. sativum extract at doses of 100, 200, and 400 mg/kg, respectively, over three weeks. The study analyzed plasma lipid profiles following treatment and found significant reductions in total cholesterol (T. CHOL), triglycerides (TG), and low-density lipoprotein (LDL) across most treated groups. In contrast, high-density lipoprotein (HDL) levels increased, particularly in treated groups compared to Group II. The decrease in TG and LDL was statistically significant ($P \leqslant 0.05$), while HDL showed a notable rise. These results suggest that Allium sativum extract may help regulate lipid metabolism and offer protective benefits against cardiovascular diseases and atherosclerosis. However, the limitation of the study is that the acute SD model may not mimic chronic human SD.

Keywords: *Allium sativum*, Atherosclerosis, Lipid profile, Sleep deprivation

INTRODUCTION

Sleep plays a regulatory role in lipid metabolism, with disruptions in sleep patterns influencing lipid synthesis, mobilization, and storage (Broussard and Brady, 2010). Numerous studies have linked both short and long sleep durations to negative health outcomes, such as an increased risk of cardiovascular disease (Berentzen et al., 2014), diabetes mellitus (Chaput et al., 2009), hypertension (Fang et al., 2012), obesity (Wu et al., 2014), psychiatric disorders (Zitser et al., 2020), and overall mortality (Kong et al., 2011). Chronic sleep loss, often stemming from poor lifestyle habits, imposes significant oxidative stress on the body and has been increasingly linked to the onset and progression of various metabolic diseases. While its adverse effects on cognitive and psychological health are well documented, the specific impact of insufficient sleep on lipid regulation and metabolism remains relatively underexplored (Bajaj et al., 2024).

An abnormal circulating lipid profile characterized by hypertriglyceridemia, low serum High-Density Lipoprotein cholesterol (HDL-C), elevated serum total cholesterol (TC), and high serum Low-Density Lipoprotein cholesterol (LDL-C) is a key indicator of atherosclerosis and a well-established risk factor for cardiovascular disease (Bule et al., 2020; Nelson, 2013). Several cross-sectional studies have explored the relationship between sleep duration and lipid profile abnormalities, linking sleep patterns to conditions such as hypercholesterolemia (Berentzen et al., 2014), hyperlipidemia (Grandner et al., 2014), and imbalances in LDL-C and HDL-C levels (Zhan et al., 2014). However, some studies have reported no significant association (Modesti et al., 2016).

Currently, available drugs that lower LDL levels and triglycerides while increasing High-density lipoproteins (HDL) levels such as statins, fibrates, and bile acid sequestrants are associated with the risk of developing cardiovascular diseases, increased insulin resistance, and the onset of type 2 diabetes (Katsiki et al., 2013). Therefore, the presence of phytochemical compounds found in plants is now recognized as having important roles in disease prevention, possibly through their effects on oxidative damage (Baker et al., 2018).

The use of plants for treating and curing illnesses has been a fundamental part of human history since ancient times. However, the early 20th century saw the advent of hormones, chemotherapy, vitamins, antibodies, and, more recently, biotechnological advancements, leading to a significant decline in the role of herbal medicine in healthcare delivery (Patrick et al., 2015). Fortunately, the early 21st century has witnessed a resurgence in herbal medicine, driven in part by the rising cost of imported medications, which governments struggle to supply in sufficient quantities to meet public demand (Osai, 1998).

Garlic (Allium sativum L.) is widely used both as a spice and a medicinal herb. Recent studies on garlic have examined its various forms, including medicinal tablets, raw, boiled, cooked, and dried preparations (Gonona, 1997). Garlic is rich in bioactive compounds, containing 33 organosulfur compounds (OSC), several enzymes, 17 amino acids including all essential amino acids and vital minerals such as phosphorus, calcium, iron, potassium, magnesium, selenium, and zinc, along with vitamins A, B, C, and E (Metwally & Hashem, 2009).

Garlic has been a cornerstone of folk medicine since ancient times, used both as a preventive measure and as a treatment for various ailments (Lin et al., 2014). Extensive clinical and scientific studies support its efficacy in treating hypercholesterolemia, though the potency of garlic-based products varies significantly depending on the preparation

method (D'souza et al., 2012). The hypocholesterolemic effects of different garlic preparations are largely attributed to its water-soluble sulfur compounds, particularly S-allyl cysteine (SAC) and ajoene. Additionally, freshly crushed garlic bulbs have been found to contain high levels of allicin, with concentrations reaching 3.7 mg/dl (Gorinstein et al., 2006).

Garlic appears to promote the synthesis of nitric oxide, contributing to its antihypertensive and anticoagulant effects. Regarding its antioxidative properties, Rahman and Billington (2000) demonstrated that components of aged garlic extract inhibit the in vivo oxidation of LDL by chelating Cu^{2+} and scavenging superoxide ions, thereby preventing the oxidation of protein and lipid moieties in human LDL cholesterol (David et al., 2001).

Allicin (diallyl thiosulfinate), a volatile compound responsible for garlic's pungent odor, constitutes approximately 70% of the thiosulfinate content in a crushed clove (Rafieian-Kopaei et al., 2014). It is absent in intact garlic but forms when alliinase an enzyme derived from the non-proteinogenic amino acid S-allyl cysteine oxide (alliin) acts upon garlic upon crushing (Ried, 2016). The conversion of alliin to allicin begins when water is added to garlic powder, rapidly degrading into diallyl disulfide (DADS), vinyl dithiins, and ajoenes (Kang et al., 2008).

Several in vitro studies indicate that garlic and its components inhibit HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A), an enzyme associated with cholesterol and fatty acid synthesis (Gebhardt, 1993; Lau, 2001; Yeh and Liu, 2001; Guan et al., 2021). Additionally, allicin activates transient receptor potential channels (TRAAI and TRPV1), which are responsible for the characteristic burning sensation of raw garlic. The bioactive compounds in garlic vary depending on its cultivar, harvest, and storage conditions, while the mineral content in its bulb is influenced by the soil composition where it is grown (Ried, 2016).

Allicin is proposed as the primary compound responsible for garlic's hypocholesterolemic effects. However, commercially available garlic products contain negligible amounts of detectable allicin (<1 ppm) (Hosseini & Hosseinzadeh, 2015), and some bioactive compounds in garlic are susceptible to degradation when exposed to heat (Kay et al., 2010). This study aims to investigate the effects of garlic extract on lipid profile indices in adult female Wistar rats under sleep deprivation conditions.

MATERIALS AND METHODS

Materials

White transparent plastic cages, ethylenediaminetetraacetic acid (EDTA), plain sample bottles, syringes, oral cannulas, gloves, and a weighing machine (Model: XY100C, Serial Number: 1404273, Changzhou Xingyun). Spectronic 200 (Thermo Scientific, USA).

Plant Collection

Fresh garlic bulbs were collected from a local market in Zaria, Kaduna State, Nigeria. The bulbs were authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and assigned voucher number: V/No. 01249. Afterward, the bulbs were cut into small pieces, ground and sieved. It was weighed using a weighing scale.

Preparation of Extract

The Allium sativum bulb powder (100 g) was dissolved in 250 ml of distilled water and covered. After 48 hours, the mixture was filtered using a nylon sieve into a small container, and the residue was spread in a container and allowed to dry. Re-extraction with fresh 250 mL of distilled water was conducted for 24 hours. The percentage yield was 45 %. The extract was stored at standard room temperature with no direct exposure to light. The pooled and dried extract was used for this study. These aqueous extracts were administered to the respective animals daily by oral gavage for four weeks (El-Demerdash et al., 2005).

Experimental Animals

Twenty-five adult female Wistar rats (150-200 g body weight) were acquired from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. The rats were housed in transparent plastic cages and allowed a two-week acclimatization period with unrestricted access to commercial feed.

Experimental Induction of Paradoxical Sleep Deprivation

Rats were acclimated to the glass tank for one hour each day over three consecutive days before the addition of water. All experimental animals except those of the normal control group were exposed to the same regimen throughout the experimental period. The water level was maintained at 3 cm below the platforms (Rizk et al., 2020). Sleep deprivation was induced using the column-in-water method, in which rats were placed on platforms designed to prevent sleep, necessitating constant movement to avoid falling. This sleep deprivation protocol lasted for 20 hours [from 2 pm to 10 am daily] a day over 7 days. The choice of 7 days was based on the outcome of a pilot study, which showed significant disruption in animals' lipid profiles.

Experimental Design and Ethical Approval

The animals were weighed and randomly divided into five groups, each consisting of five animals (n = 5). The number of animals in a group was determined using the Effect size given as:

E (Effect size) = Total number of animals − Total number of groups (Charan and Kantharia, 2013). Group I was designated as the normal control and received distilled water, while Group II served as the negative control (SD-untreated). Groups III, IV, and V were treated with varying doses of the extract: 100, 200, and 400 mg/kg, respectively. The dosages of the extract used in this study were determined based on prior research (Njiga et al., 2020). The experimental procedures were conducted per the approved guidelines of the Ahmadu Bello University Ethical Committee on Animal Use and Care.

Animal Sacrifice

After 28 days of administration, the animals were sacrificed. They were anesthetized using sodium phenobarbital (60 mg/kg) (Tobar Leitão et al., 2021). Blood samples were collected via cardiac puncture and placed in a plain serum bottle. The serum was prepared by spinning blood samples for 20 minutes at a speed of 3500 rpm using a bench centrifuge. A clear supernatant was used.

Cholesterol Estimation

For cholesterol levels estimation, 1000 µL of working reagent (mixture of 200 IU/l of cholesterol esterase, 150 IU/l of cholesterol oxidase, 20 mmol/l of sodium phenolate, 2000 IU/l of HRP, 0.5 mmol/l of 4-aminoantipyrine, and 68 mmol/l of phosphate buffer) was mixed with 20 µL of blank, standard and test respectively in a separate vial. The mixture was then incubated at 37 °C for 10 min followed by measurement of

absorbance at 505 nm (Spectronic 200, Thermo Scientific, USA) (Singh et al., 2022).

Triglyceride Estimation

For estimation of triglycerides levels, 1000 µL of working reagent (mixture of 2.5 mmol/l of ATP, 0.8 mmol/l of 4-Aminoantipyrine, 2.5 mmol/l of Mg2+, 3,5 DHBS, 550 U/l of glycerol kinase, 2000 U/l of peroxidase, 8000 U/l of GPO, 3500 U/l of lipoprotein lipase and 53 mmol of buffer) was mixed with 10 µL of blank, standard and test respectively in a separate vial. The mixture was then incubated at 37 °C for 10 min followed by measurement of absorbance at 505 nm (Spectronic 200, Thermo Scientific, USA) (Singh et al., 2022).

Calculations of Risk Indices

Castelli's Risk Index (CRI): CRI is calculated from TC, LDL and HDL, and it is categorized into two: CRRI-I and CRRI-II.

$$\text{CRRI} - \text{I} = \text{TC/HDLc}$$

$$\text{CRRI} - \text{II} = \text{LDLc/HDLc}$$

The Atherogenic Index of Plasma (AIP) = $\text{Log } 10\ (\frac{\text{TG}}{\text{HDLc}})$ as described by Dharmaraj et al (2022).

Statistical Analysis

Data obtained from the study were analyzed and expressed as mean ± SEM. Statistical analysis was carried out using version 23 of the IBM Statistical Package for Social Sciences (SPSS). A one-way analysis of variance (ANOVA) was carried out, followed by Tukey's post hoc test (because of an equal number of samples per group), to determine the differences among the groups. Values with a P< 0.05 were considered statistically significant. GraphPad Prism 8 software (version 8.0.2; GraphPad Software, San Diego, California, USA) was used for charts.

RESULTS AND DISCUSSION

Results

Effect of Allium sativum Serum Total Cholesterol and Triglyceride

The level of serum cholesterol (Fig. 1a) was significantly (P = 0.010) higher in the SD-untreated group compared to the NC. However, in the SD group treated with Allium sativum, total cholesterol was significantly (P = 0.031) higher compared to the NC group and lower compared to the SD-untreated group. TRIG (Fig. 1b) in the SD-untreated group was significantly (P = 0.001) increased compared to NC. TRIG in all the other treated groups was significantly reduced (P = 0.042) compared to the SD-untreated group.

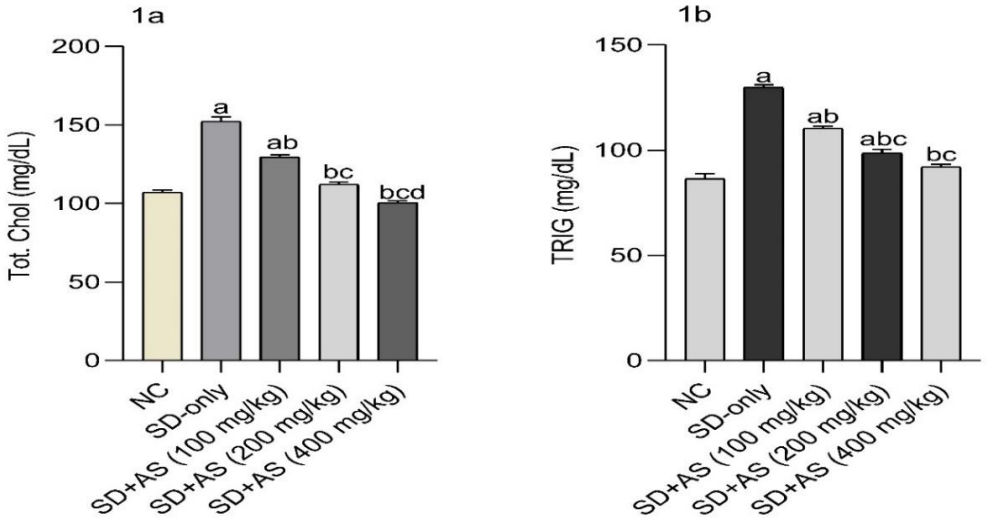

Figure 1: Results of AS-extract on serum Total cholesterol [Fig. 1a] and serum TRIG [Fig. 1b] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts: a= P<0.05 vs NC, b= P<0.05 vs SD-untreated, c= P<0.05 vs SD+AS (100 mg/kg), d= P<0.05 vs SD+AS (200 mg/kg).

Effect of Allium sativum Lipid Profile

High-density lipoprotein (Fig. 2a) was significantly (P = 0.0011) reduced in the SD-untreated group compared to the NC group. HDL was increased (P = 0.021) in the AS-treated groups compared to the SD-only group. Serum LDL (Fig. 2b) was higher (P = 0.004) in the SD-only group compared to NC. Administration of Allium S significantly lowered LDL (P = 0.001) compared to the SD-only group.

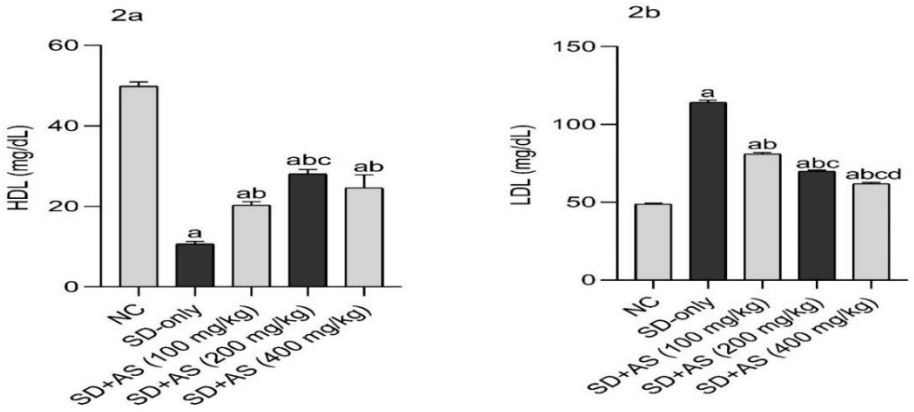

Figure 2: Results of AS-extract on serum HDL [Fig. 2a], serum LDL [Fig. 2b] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts: a= P<0.05 vs NC, b= P<0.05 vs SD-untreated, c= P<0.05 vs SD+AS (100 mg/kg), d= P<0.05 vs SD+AS (200 mg/kg)

Effect of Allium sativum Lipid Indices (Atherogenic index and Castelli Risk Ratio)

The atherogenic index (Fig. 3a) in the SD-only group was significantly higher (P = 0.001) compared to the NC. In the groups treated with AS at 100, 200 and 400 mg/kg, the AI was significantly decreased compared to the SD-only group; P = 0.001, 0.032, 0.001, respectively. The decrease observed in the AS-treated groups was in a dose-dependent fashion. In Fig. 3b, CRR-I was significantly higher in the SD-only group compared to the NC; P = 0.021. In all the groups treated with AS at 100, 200 and 400 mg/kg, the CRR-I was significantly reduced compared to the SD-only group; P = 0.004, 0.001, 0.001 in a dose-dependent fashion. In Fig. 3c CRR-II was significantly increased in the SD-only group (P = 0.013) compared to the NC. Treatment with AS significantly lowered CRR-II in a dose-dependent fashion compared to the SD-group; P = 0.001, 0.012, 0.022, respectively.

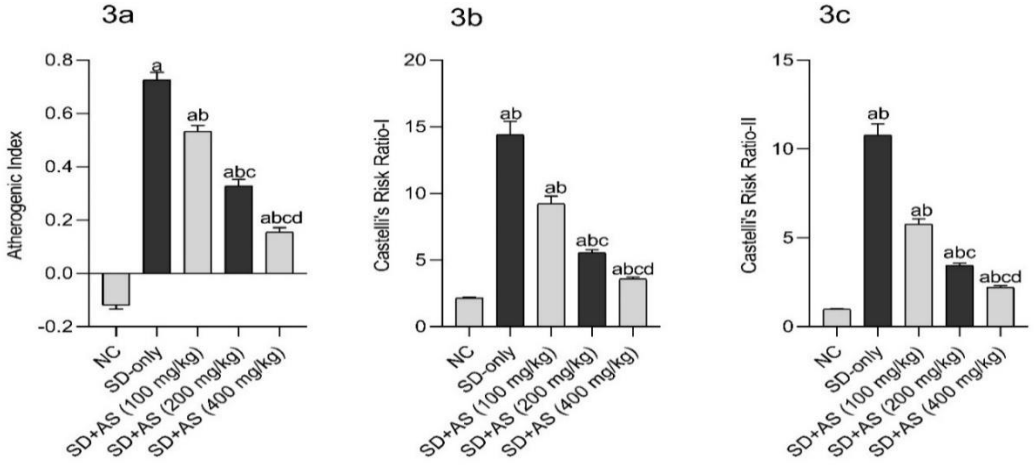

Figure 3: Results of AS-extract on atherogenic index [Fig. 3a], Castelli Risk Ratio-1 [Fig. 3b] and Castelli Risk Ratio-II [Fig. 3C] in adult female Wistar rats exposed to paradoxical sleep deprivation. NC = Normal control, SD = sleep deprivation, AS = *Allium sativum*. Superscripts: a= P<0.05 vs NC, b= P<0.05 vs SD-untreated, c= P<0.05 vs SD+AS (100 mg/kg), d= P<0.05 vs SD+AS (200 mg/kg)

Discussion

Sleep deprivation and sleep-related disorders are becoming increasingly prevalent in modern society, often intensified by stress, anxiety, financial difficulties, emotional instability, the demands of daily survival, physical discomfort, and substance use, among other challenges (Kim *et al*., 2015). In recent years, the impact of reduced sleep duration on dyslipidemia and metabolic syndrome has gained attention due to its effects on cardiovascular health. Elevated triglycerides (TG) and low levels of high-density lipoprotein (HDL) have been identified as risk factors for coronary heart disease, diabetes, and metabolic syndrome (Abdurahman *et al*., 2020). Additionally, sleep deprivation acts as a significant oxidative stressor, leading to increased production of reactive oxygen species (Pandey *et al*., 2018). Therefore, managing SD is a crucial strategy to mitigate these adverse effects.

In this study, we observed a significant increase in total cholesterol (Tot. Chol) and triglyceride (TRIG) levels (Figure 1a) in the SD-untreated group compared to the extract-treated groups, with a dose-dependent effect. The elevated Tot. Chol and TRIG levels may result from increased production, reduced clearance, or a combination of both mechanisms. Additionally, sleep deprivation can lead to adverse physiological conditions that contribute to TRIG accumulation (Gnocchi *et al*., 2015). However, treatment with *Allium sativum* (AS) extract mitigated the rise in Tot. Chol and TRIG levels, demonstrating their influence on lipid regulation and, consequently, cardiovascular health. Elevated

serum total cholesterol and its carrier proteins are strongly associated with an increased risk of coronary heart disease (Mahmood *et al*., 2009). Lipid management is well-established as an effective strategy for preventing and managing cardiovascular disease (Saba *et al*., 2011). In this study, the triglyceride-lowering effects of *Allium sativum* may be attributed to its ability to reduce hepatic TG content by inhibiting lipogenesis and promoting fatty acid oxidation (Xiong *et al*., 2019). Additionally, it may contribute to the downregulation of very low-density lipoprotein (VLDL) synthesis and secretion (Mulvihill *et al*., 2016).

Previously published epidemiological studies have reported inconsistent findings, and the relationship between sleep duration and abnormal lipid profiles remains debatable (Arora *et al*., 2011). Moreover, growing evidence suggests that reduced sleep duration is becoming an increasingly prevalent issue in modern society (Hasler *et al*., 2004). However, due to the cross-sectional nature of these data, they cannot be used to establish a definitive cause-and-effect relationship (Abreu *et al*. , 2015). In the present study, Fig. 2a shows that the sleep deprivation (SD)-untreated group reduces HDL. This may be attributed to impaired lipid metabolism in animals subjected to sleep deprivation (SD). The finding is consistent with previous studies, which shows improved HDL with improved sleep (Kaneita *et al*., 2008). Additionally, another investigation identified low HDL cholesterol, rather than triglycerides, as an independent marker of poor sleep quality (Abdurahman *et al*., 2020). However, Figure 2b revealed that the SD-untreated group was associated with elevated low-density lipoprotein (LDL) levels. Short sleep duration may contribute to an increased risk of high LDL by stimulating appetite, leading to greater consumption of saturated fats, and elevating stress levels, which in turn induce catecholamine-driven lipolysis (Spiegel *et al*., 2004).

Atherosclerosis is a chronic disease characterized by damage to the arterial wall due to inflammation and fibro-fatty deposits. The disease process involves several cell types, notably smooth muscle cells, monocyte-derived macrophages, T-lymphocytes, and platelets (El-Sabban, & Abouazra, 2008). It is widely recognized as one of the leading causes of morbidity and mortality worldwide (Bitzur *et al*., 2005). In our research, we observed a significant increase in atherogenic index levels among the sleep deprivation (SD) group without treatment (Figure 3a). This elevation in lipid levels (TRIG, HDL), an indicator of plasma atherogenicity, was strongly linked to poor sleep quality (Kumar *et al*., 2020). Furthermore, our findings demonstrated that administering AS-extract resulted in a notable reduction in circulating TG and LDL cholesterol levels in rats (Wu *et al*., 2015). Treatment with garlic extract inhibited lipid synthesis in both normal and SD-treated animals. Additionally, the extract suppressed the activity of acyl-CoA cholesterol acyltransferase, an enzyme involved in cholesteryl ester formation, while simultaneously stimulating cholesteryl ester hydrolase, which facilitates cholesteryl ester degradation. This mechanism may explain how garlic extract lowers lipid levels in SD-treated groups. The inhibition of modified LDL cholesterol uptake and the degradation of lipoprotein-derived cholesteryl esters suggest potential mechanisms for preventing lipid accumulation in aortic cells induced by SD (Wang *et al*., 2020; Orekhov & Tertov, 1997)

Castelli's Risk Index, a lipid ratio, serves as a crucial diagnostic marker for evaluating cardiovascular disease (CVD) risk, particularly when triglyceride (TG) and high-density lipoprotein (HDL) levels remain unchanged. It provides enhanced prognostic value compared to conventional LDL or HDL measurements (Bhardwaj *et al*., 2013; Shekhar *et al*., 2011).

In this study, lipid indices such as CRRI-I and CRRI-II (Fig. 3a & 3b) exhibited a significant positive correlation with total cholesterol and low-density lipoprotein (LDL), while maintaining a substantial negative correlation with HDL cholesterol. Notably, the sleep deprivation (SD)-untreated group demonstrated a marked increase in CRRI-I and CRRI-II lipid ratios relative to controls (Fig. 3a & 3b), in agreement with findings from Khan *et al*. (2014). The LDL/HDL ratio (CRRI-II) stands out as a more reliable predictor of heart disease risk than LDL alone. Numerous studies have emphasized the LDL/HDL ratio as an excellent metric for assessing the effectiveness of lipid-lowering treatments (Khan *et al*., 2013). A rising TC/HDL ratio signifies an elevated risk, with both TC/HDL and LDL/HDL ratios (CRRI-I and CRRI-II) being recognized as highly effective lipid-based predictive markers for future cardiovascular events. Furthermore, these index ratios are particularly adept at identifying atherogenic small LDL particles, making them sensitive biomarkers of atherosclerotic CVD (Adedokun *et al*., 2017). Elevated serum ratios of CRRI-I and CRRI-II, closely associated with poor sleep quality, have been identified as reliable indicators of atherosclerotic CVD (Sasikala & Kalyan, 2020). Interestingly, administration of AS extract resulted in a significant reduction in CRRI-I and CRRI-II levels among SD-treated groups. The polyphenolic compounds present in AS extract are known to enhance the serum TG/LDL ratio (David *et al*., 2001), potentially contributing to the favorable effects observed in this study. Additionally, allicin, a key component of AS, has been reported to influence these lipid ratios positively (Obisike *et al*., 2016).

CONCLUSION

Overall, the findings suggest that *Allium sativum* extract may offer beneficial effects on lipid metabolism under conditions of sleep deprivation. Its observed influence on cholesterol and triglyceride levels, as well as lipid indices, indicates potential for further exploration as a supportive agent in managing dyslipidemia. However, these results are preliminary with a small sample size; therefore, a need for additional clinical validation to confirm efficacy and safety in human populations.

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