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HEPATOPROTECTIVE ACTIVITY OF SINIGRIN AND CARVACROL AGAINST PARACETAMOL INDUCED LIVER INJURY IN WISTAR RATS

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ABSTRACT: Background: Sinigrin (allyl-glucosinolate or 2-propenyl-glucosinolate), an aliphatic glucosinolate, is naturally present in Brassicaceae plants, while carvacrol (2-methyl-5-isopropylphenol) is a monoterpene phenolic component of the essential oil generated by many aromatic plants. This study is aimed to determine the hepatoprotective potential of these phytoconstituents on Paracetamol-induced liver damage in Wistar rats. **Methods:** Seven groups of six Wistar rats, each weighing between 180 and 230 g, were selected. The animals' weight range was evenly divided throughout the groups. The control group, group I, got normal saline; the PCM group, group II, received paracetamol (3 g/kg, p.o.); groups III and IV, carvacrol (25, 50 mg/kg/p.o.); and the groups V and VI, sinigrin (25, 50 mg/kg/p.o.) once daily for 14 days. Silymarin was given daily to group VII-Standard at a dose of 25 mg/kg p.o. **Result:** Serum samples were used to determine levels of marker enzymes such as aspartate transaminases (AST), alanine transaminases, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and glutamyl transpeptidase (GGT). The proinflammatory cytokines interleukin-1beta (IL-1b) and interleukin-6 (IL-6) as well as nitrite levels and catalase activity all decreased in carvacrol and sinigrin-treated rats in a dose-dependent manner. **Conclusion:** The present study will provide scientific data and justify their traditional uses to treat various liver problems. This study confirmed that carvacrol and sinigrin have hepatoprotective potential against Drug Induced Liver Injury (DLI).

INTRODUCTION: The liver is a vital organ as numerous physiological systems depend heavily on the liver. These include regulating blood volume, maintaining the immune system, endocrine regulation of growth signalling pathways, and supporting various chemicals' metabolism [1].

Drug-induced liver injury (DILI) is a complex condition that can range in severity from subtle changes in the liver's biochemistry to jaundice, fulminant liver failure, or may even be fatal [2].

An adverse reaction to drugs or other xenobiotics causes drug-induced liver injury [3]. One of the most prevalent medications to induce acute liver damage is Paracetamol, which has emerged as a significant contributor to acute liver failure in industrialized nations like the United States and Europe. Many prescription and over-the-counter medications contain the antipyretic and analgesic paracetamol (acetaminophen), which is generally safe when

taken in therapeutic doses. However, continuous medication, inappropriate dosing, and inadvertent combination of more than one paracetamol-containing medication results in liver toxicity [4]. Metabolic activation of paracetamol results in the reactive metabolite N-acetyl-p- benzoquinone imine (NAPQI) and causes toxicity in both rats and humans, which is one of the main mechanisms of the toxicity. Hepatic GSH reserves effectively scavenge the poisonous metabolite N-acetyl-p-benzoquinone imine (NAPQI), preventing liver damage [5]. When taken in large amounts, paracetamol causes the glucuronidation and sulfation pathways to become saturated, which causes GSH to be depleted and the concentration of NAPQI to rise. These changes result in severe oxidative stress, mitochondrial dysfunction, an inflammatory reaction, and even cell death [6].

The covalent binding of PCM to mitochondrial proteins during an overdose causes mitochondrial oxidative stress and enhanced peroxy nitrate synthesis. This causes cell death through apoptosis or necrosis, primarily through the activation of caspase-3. Moreover, it causes hepatic damage by activating Kupffer's cells (phagocytic macrophages of the liver), which generate cytokines including IL-1, 6, and TNF-, chemokines, and neutrophil recruitment and accumulation [7]. Destruction of hepatocytes in turn results in the rise of serum levels of aminotransferases such as ALT and AST as well as ALP, and gamma-glutamyl transferases (γGT), which are most commonly used markers in hepatotoxic studies [8]. Hepatocyte damage eventually causes liver fibrosis and cirrhosis [9]. As a result, screening natural compounds is one of the primary methods for discovering new medicines. Plant-based antioxidants are remarkably risk-free and successful at preventing liver damage [10]. In the present study, carvacrol (2-methyl-5-isopropylphenol), a monoterpene phenolic constituent of the essential oil in the rhizome of family Poaceae and sinigrin (allyl-glucosinolate or 2 – propenyl - glucosinolate), an aliphatic glucosinolate present in the roots of the Brassicaceae plant family is compared to Silymarin for their potential to protect the liver against injury by Paracetamol. By assessing serum indicators (AST, ALP, GGT, and others), enzymatic markers (superoxide dismutase (SOD) and catalase (CAT), and non-enzymatic markers (GSH), as well as histological changes in the liver, their hepatoprotective effect was investigated.

MATERIAL AND METHODS: The present experimental study was conducted using Wistar rats with weights of about 220-250g procured from the central animal house of ISF College of Pharmacy, Moga, Punjab (India). All the animals were kept in clean polyacrylic cages and maintained in an air-conditioned animal house under standard laboratory conditions (room temperature 25 ± 3°C and relative humidity of 55-60 %) with a 12 h light/dark cycle. The water and food were offered *ad libitum*. The Institutional Animal Ethics Committee (IAEC) (ISFCP/IAEC/CPCSE/2020/P 52) gave its approval to the experimental protocol, and the experiments were carried out in accordance with regulations set forth for the use and treatment of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All experiments were performed using age-matched animals in an attempt to avoid variability between experimental groups.

Animal Grouping: The experimental Wistar rats were divided into seven groups of six animals each for the purpose of study.

TABLE 1: DIFFERENT GROUP OF WISTAR RATS TO STUDY HEPATOPROTECTIVE ACTIVITY

S. no.	Groups	Animal Species	No. of animals
1	Normal control	Wistar	6
2	Paracetamol control (3g/kg/p.o.)	Rats	6
3	PCM+Carvacrol (25mg/kg/p.o.)		6
4	PCM+Carvacrol (50mg/kg/ p.o.)		6
5	PCM+Sinigrin (25mg/kg/p.o.)		6
6	PCM+Sinigrin (50mg/kg/p.o.)		6
7	PCM+ Silymarin (25 mg/kg/ p.o.)		6

Hepatotoxicity was Induced by Paracetamol Induced Liver Damage Rat Model: A dose of 3 g/kg of paracetamol (Acetaminophen, Sigma Chemical Company, USA) was delivered orally

after being suspended in 0.5% Tween-80. Seven groups of six Wistar albino rats each, weighing between 180 and 230 g, were created. The animals' weight range was evenly divided throughout the groups. The control group, group I, got normal saline; the PCM group, group II, received paracetamol (3 g/kg, p.o.); groups III and IV, carvacrol (25, 50 mg/kg/p.o.); and the groups V and VI, sinigrin (25, 50 mg/kg/p.o.) once daily for 14 days. Silymarin was given daily to Group VII-Standard at 25 mg/kg p.o.

Parameters:

Measurement of Body Weight: The body weight of all the animals was measured during the treatment follow-up on day 0, day 7, day 14, day 21, and day 28 respectively, and marked changes in body weight were recorded.

Inflammatory Markers: In the present study, the quantitative estimation of inflammatory cytokines (IL-1β and IL-6) in plasma was done by using a rat immunoassay kit purchased from (KRISHGEN Biosystem, Ashley Ct, Whittier, CA). The quantizing IL-1β and IL-6 rat immunoassay is a solid phase ELISA designed to quantify rat IL-1β and IL-6 levels. It is a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using a microtiter plate reader (Bio-Rad Laboratories, USA). Concentrations of IL-1β and IL-6 were calculated from the standard curves. The levels of IL-1β and IL-6 were represented as pg/ml. Each procedure was performed three times.

Estimation of Interleukin-1β (IL-1β): IL-1β level was estimated by using a rat IL-1β kit (KRISHGEN Biosystem, Ashley Ct, Whittier, CA). It is a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA), which uses a microtiter plate reader read at 450nm. Concentrations of IL-1β were calculated from plotted standard curve.

Blood Collection and Excision of Liver: The research study's rats were weighed individually after 24 hours from the last dose was dispensed and blood samples were then taken by promptly puncturing each rat's retro-orbital plexus with a capillary tube. Clear serum samples were transferred into clean Eppendorf tubes and stored at -20°C for subsequent biochemical analysis after blood was collected into clean plain tubes and allowed to clot for 45 minutes. Liver tissues were swiftly taken from animals after the blood sample and cleaned with cold saline before specimens (small pieces from each liver) were transferred into 10% formalin for histological analysis.

Biochemical Parameters:

Estimation of LPO: The quantitative estimation of LPO content in the plasma was performed by the spectrophotometric method [11].

Estimation of Nitrite: The accumulation of nitrite (an indicator of nitric oxide production) in the plasma was determined by a colorimetric assay[12]. Gris's reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) was used. Equal volumes of plasma and Gris's reagent were mixed and incubated for 10 minutes at room temperature in the dark. Absorbance was measured at 540 nm with a spectrophotometer (UV-1700, Shimadzu, and Kyoto, Japan). Nitrite level was expressed in terms of nmol/ml sample. All assays were performed in triplicate.

Estimation of GSH: The GSH activity in plasma was estimated according to pre determined method. Each 3 ml reaction mixture consisted of 2.9 ml of 5, 5-dithiobis (2-nitrobenzoicacid) prepared in potassium phosphate buffer (0.1 M, pH 7.4) and 0.1 ml of plasma. The reaction mixture was incubated at 37 °C for 15 min and absorbance was recorded at 412 nm (spectrophotometer) and results were expressed as U/ml. All assays were performed in triplicate

Estimation of Catalase (CAT): CAT activity was assayed measuring the absorbance decrease at 240 nm in a reaction medium containing and 0.1–0.3 mg protein/mL[13].One unit of the enzyme is defined as 1µmol of H_2O_2 consumed per minute and the specific activity is reported as U/ml.

Statistical Interpretation: The results were expressed as mean ± SEM. The results like body weight, inflammatory markers, biochemical analysis were analyzed by repeated measure one way ANOVA followed by Bonferroni's post hoc test. Values with $P<0.05$ was statistically significant.

Histopathological Assessment: The fixed tissue samples in 10% formalin were dehydrated and cleared before being embedded in paraffin wax and sectioned into 5µm thick sections. Tissues were stained with hematoxylin and eosin (H&E) and viewed under a light microscope to detect any pathological signs.

RESULTS: Superoxide dismutase, catalase, glutathione, and other antioxidant enzymes as well as an increase in lipid peroxidation were found to be significantly reduced in the paracetamol-treated rats. However, compared to the control group, sinigrin and carvacrol treatment significantly improved the antioxidant status.

TABLE 2: EFFECTS OF VARIOUS INTERVENTIONS ON LPO, NITRITE, GSH, AND CATALASE IN PCM INDUCED HEPATOTOXICITY IN WISTAR RATS

Animalgroups	LPO (nmol/ml)	Nitrite (nmol/ml)	GSH (U/ml)	CAT (U/ml)
Normal control	22.85 ± 2.5	6.45 ± 0.93	5.75 ± 0.27	24.12 ±1.24
PCM	84.14 ± 6.4[a]	27.17 ± 1.6[a]	0.84 ± 0.32[a]	6.26 ±1.28[a]
PCM+CAR 25	56.21 ± 3.6[b]	22.26 ± 0.74[b]	2.85 ± 0.25[b]	13.16 ±1.32[b]
PCM+CAR 50	39.75 ± 2.1[bc]	16.21 ± 0.86[bc]	3.86 ±0.42[bc]	19.87 ±1.43[bc]
PCM+SIN25	62.4 ± 4.2[b]	21.4 ± 0.92[b]	1.92 ± 0.32[b]	12.54 ±1.76[b]
PCM+SIN 50	41.71 ± 3.4[bd]	15.83 ± 0.58[bd]	2.96 ± 0.38[bd]	19.42 ±1.68[bd]
PCM+SIL25	36.26 ± 2.8[be]	13.35 ± 0.78[be]	4.06 ± 0.72[be]	21.06 ±1.52[be]

Table 2 data is expressed as mean ± SD represented by columns and bars. ap <0.001 vs NC; bp < 0.001vs PCM; cdp < 0.05 vs SIL 25. Statistical analysis performed by one way ANOVA followed by Bonferroni's multiple comparison. NC, normal control; PCM, Paracetamol, CAR, Carvacrol; SIN, Sinigrin; SIL, Silymarin.

Hepatoprotective Activity:

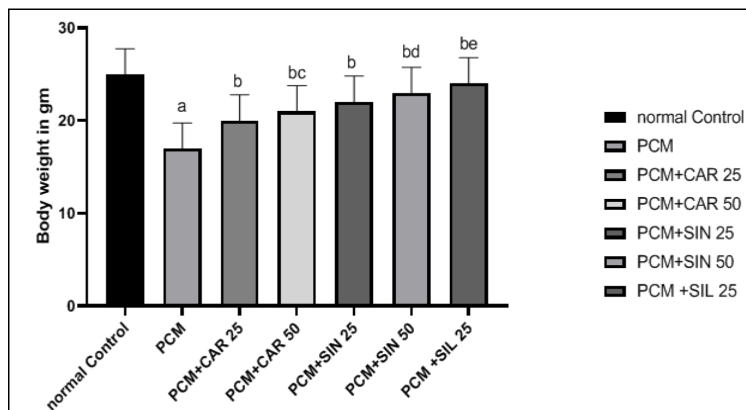

FIG. 1: EFFECTS OF VARIOUS INTERVENTIONS ON BODY WEIGHT IN PCM INDUCED HEPATOTOXICITY IN RATS. Data is expressed as mean ± SD represented by columns and bars. [a]$p < 0.001$ vs NC; [b]$p < 0.001$vs PCM; [cd]$p < 0.05$ vs SIL 25. Statistical analysis performed by one way ANOVA followed by Bonferroni's multiple comparison. NC, normal control; PCM, Paracetamol, CAR, Carvacrol; SIN, Sinigrin; SIL, Silymarin.

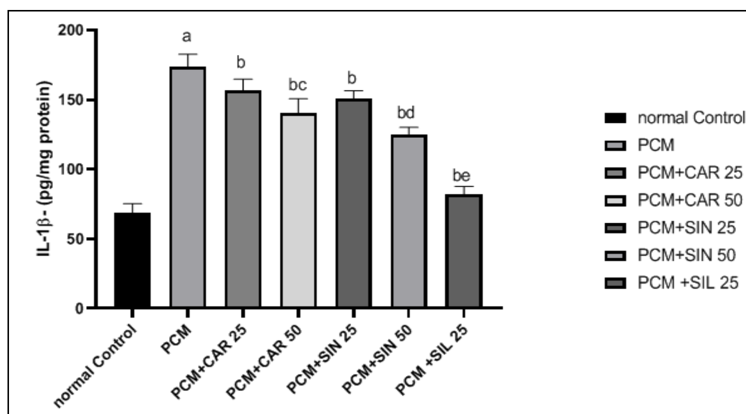

FIG. 2: EFFECTS OF VARIOUS INTERVENTIONS ON IL-1B IN PCM INDUCED HEPATOTOXICITY IN RATS. Data is expressed as mean ± SD represented by columns and bars. [a]$p < 0.001$ vs NC; [b]$p < 0.001$vs PCM; [cd]$p < 0.05$ vs SIL 25. Statistical analysis performed by one way ANOVA followed by Bonferroni's multiple comparison. NC, normal control; PCM, Paracetamol, CAR, Carvacrol; SIN, Sinigrin; SIL, Silymarin.

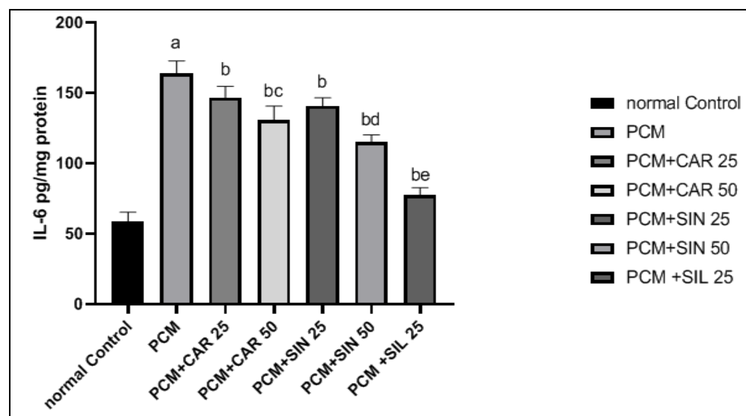

FIG. 3: EFFECTS OF VARIOUS INTERVENTIONS ON IL-6 IN PCM INDUCED HEPATOTOXICITY IN RATS. Data is expressed as mean ± SD represented by columns and bars. $^{a}p < 0.001$ vs NC; $^{b}p < 0.001$vs PCM; $^{cd}p < 0.05$ vs SIL 25. Statistical analysis performed by one way ANOVA followed by Bonferroni's multiple comparison. NC, normal control; PCM, Paracetamol, CAR, Carvacrol; SIN, Sinigrin; SIL, Silymarin.

Histopathology of Liver:

Normal control **Paracetamol control (3g/kg/p.o.)** **PCM + Carvacrol (25mg/kg/p.o.)**

PCM + Carvacrol (50 mg/kg/ p.o.) **PCM + Sinigrin (25 mg/kg/p.o)** **PCM + Sinigrin (50 mg/kg/p.o.)**

PCM + Silymarin (25 mg/kg/p.o.)

FIG. 4: HISTOPATHOLOGY OF LIVER SHOWING NORMAL, PCM, SINIGRIN, CARVACROL AND SILYMARIN TREATED HEPATOCYTES

In the control group, normal liver morphology was observed. By contrast, livers of PCM-administered rats resulted in marked histopathological changes such as infiltration of large number of lymphocytes, inflammation of sinusoids, hepatocyte necrosis and fatty vacuoles.

DISCUSSION: Paracetamol induced hepato-toxicity is a well-known model for studying drug-induced liver damage in experimental animals. In this study, we investigated the potential protective effects of carvacrol and sinigrin against paracetamol-induced hepatotoxicity in rats. The results obtained shed light on the hepatoprotective properties of these natural compounds and their potential application in liver protection. The evaluation of liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, is crucial for assessing liver damage. In our study, we observed that treatment with carvacrol and sinigrin significantly attenuated the elevation of serum ALT and AST levels caused by paracetamol administration.

These findings indicate that carvacrol and sinigrin effectively prevented liver cell injury and preserved liver function. Oxidative stress is a key mechanism involved in paracetamol-induced hepatotoxicity. Excessive production of reactive oxygen species (ROS) can lead to lipid peroxidation and subsequent liver damage. Our study demonstrated that carvacrol and sinigrin treatment significantly reduced the levels of Paracetamol-induced hepatotoxicity and is a well-known model for studying drug-induced liver damage in experimental animals. In this study, we investigated the potential protective effects of carvacrol and sinigrin against paracetamol-induced hepatotoxicity in rats. The results obtained shed light on the hepatoprotective properties of these natural compounds and their potential application in liver protection. The evaluation of liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, is crucial for assessing liver damage. Our study demonstrated that carvacrol and sinigrin treatment significantly reduced the levels of malondialdehyde (MDA), a marker of lipid peroxidation, and increased the levels of reduced glutathione (GSH), an important endogenous antioxidant, in the liver tissue of rats. Moreover, the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, were restored to near-normal levels. These findings suggest that carvacrol and sinigrin possess potent antioxidant properties, which may contribute to their hepatoprotective effects by reducing oxidative stress. Histopathological examination of liver tissue is essential for assessing the extent of liver damage and the efficacy of therapeutic interventions. In our study, paracetamol administration caused severe hepatocellular necrosis, inflammation, and congestion. However, treatment with carvacrol and sinigrin ameliorated inflammation, and congestion observed in the liver tissue. These results indicate that carvacrol and sinigrin have the potential to protect liver cells from paracetamol-induced damage and maintain tissue integrity. Inflammatory responses play a significant role in the progression of hepatotoxicity. Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-I beta are known to be elevated in liver injury.

In our study, we observed that carvacrol and sinigrin treatment significantly reduced the levels of IL-6 and interleukin-I beta in the liver tissue of rats exposed to paracetamol. This anti-inflammatory effect suggests that carvacrol and sinigrin may modulate the immune response and attenuate liver inflammation during hepatotoxicity. The exact mechanisms underlying the hepatoprotective effects of carvacrol and sinigrin in paracetamol-induced hepatotoxicity remain to be fully elucidated. However, it is plausible that their antioxidant and anti-inflammatory properties contribute to their protective effects. Carvacrol has been reported to scavenge free radicals, inhibit lipid peroxidation, and enhance antioxidant enzyme activities. Sinigrin, on the other hand, has been shown to possess anti-inflammatory properties and modulate various signalling pathways involved in liver injury.

CONCLUSION: Our research supports the anti-hepatotoxic properties of carvacrol and sinigrin in rats exposed to paracetamol. By maintaining liver function, lowering oxidative stress, improving histopathological alterations, and controlling inflammatory responses, these natural substances demonstrated hepatoprotective benefits. The results of this study add to the body of knowledge on

natural substances that may have therapeutic advantages for liver protection. With their anti-inflammatory and antioxidant capabilities, carvacrol and sinigrin have demonstrated promising results in reducing the hepatotoxicity caused by paracetamol.

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CONFLICTS OF INTEREST: None.

REFERENCES:

1. Trefts E, Gannon M and Wasserman DH: The liver. Curr Biol 2017; 27(21): 1147-51.
2. Sandhu N and Navarro V: Drug-induced liver injury in gi practice. Hepatology Communications 2020; 4(5): 631-45.
3. Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, Devarbhavi H, Merz M, Lucena MI, Kaplowitz N and Aithal GP: Drug-induced liver injury. Nature Reviews Disease Prime 2019; 5(1): 58.
4. Chowdhury A, Nabila J, Adelusi Temitope I and Wang S: Current etiological comprehension and therapeutic targets of acetaminophen-induced hepatotoxicity. Pharma Res 2020; 161: 1051029.
5. Liao J, Lu Q, Li Z, Li J, Zhao Q and Li J: Acetaminophen-induced liver injury: Molecular mechanism and treatments from natural products. Frontiers in Pharmacology 2023; 14: 1122632.
6. Ramachandran A and Jaeschke H: Oxidant stress and acetaminophen hepatotoxicity: mechanism-based drug development. Antioxidants & Redox Signaling 2021; 35(9): 718-33.
7. Kourkoumpetis T & Sood G: Pathogenesis of alcoholic liver disease: an update. Clinics in Liver Disease 2019; 23(1): 71-80.
8. Kalas MA, Chavez L, Leon M, Taweesedt PT and Surani S: Abnormal liver enzymes: A review for clinicians. World Journal of Hepatology 2021; 13(11): 1688.
9. Rockey DC: Hepatic fibrosis and cirrhosis. Yamada's Textbook of Gastroenterology 2022; 2000-23.
10. Akbari B, Baghaei-Yazdi N, Bahmaie M and Mahdavi Abhari F: The role of plant-derived natural antioxidants in reduction of oxidative stress. Bio Factors 2022; 48(3): 611-33.
11. Guendouz A, Belguet A and Dahamna S: The Estimation of Lipid Peroxidation and Osmotic Fragility of Human Red Blood Cell Induced by Pesticides Residues Detected in Some Vegetables Crops. Asian Journal of Dairy & Food Research 2023; 42(3).
12. Suárez-Cuenca JA, Domínguez-Pérez GA, Hernández-Muñóz RE, Hernández-Patricio A, Vera-Gómez E, Gutiérrez-Buendía JA, Salamanca-García M, Montoya-Ramírez J, Gaytán-Fuentes OF, Aranda-Rodríguez C and Rojas-Noverón AR: Non-alcoholic fatty liver disease severity is related to plasma pro-oxidative biomarkers rather than liver tissue-measured nitrogen metabolism biomarkers in population with obesity and metabolic syndrome. Metabolic Syndrome and Related Disorders 2023; 21(2): 115-21.
13. Szczepanczyk M, Paul L, Ruzgas T and Björklund S: Comparison of oxygen electrode chronoamperometry and spectrophotometry for determination of catalase activity. Oxygen 2023; 3(1): 77-89.

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