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Effect of Green Tea, Coffee, and Cocoa Powder Administration on Antioxidant Enzymes of Liver in Experimental Rat Model

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ABSTRACT

Green tea, coffee, and coca powder are natural products known for their potent antioxidant, chemoprotective, anti-inflammatory, and anti-tumorigenic properties. This study aimed to investigate the effects of green tea, coffee, and coca powder on liver antioxidant enzymes in an experimental rat model using biochemical parameters. Twenty-four male albino Wistar rats were randomly divided into four groups (n=10 per group). Group I (control) received a standard diet and distilled water; Group II was orally administered 1% coffee (1 g/100 mL); Group III received 5% green tea (5 g/100 mL) orally; and Group IV was treated with coca powder at a dose of 1 g/kg body weight orally. The daily intake volumes of green tea, coffee, and coca powder were recorded for Groups II, III, and IV, respectively. The impact of these substances on liver antioxidant enzymes was assessed by measuring oxidative stress markers (tissue malondialdehyde, MDA) and antioxidant enzyme activity (catalase and superoxide dismutase, SOD). The results demonstrated that green tea, coffee, and coca powder effectively restored antioxidant enzyme levels. The study highlights the strong antioxidant properties of these compounds in liver tissue. Furthermore, the findings suggest that the administration of green tea, coffee, and coca powder may offer protective benefits against pathological conditions and mitigate the harmful effects of various hepatotoxic agents.

INTRODUCTION

Plant extracts have been used by traditional medical practioners for the treatment of liver disorders for centuries (Mohan, Maurya, & Singh, 2025). It is being acknowledged that plants contain non-nutritional constituents with beneficial health effects, such as anti-inflammatory and anti-carcinogenic properties (Kang et al., 2024). Previous studies showed that oxidative stress and DNA damage can initiate the tumor formation and the normal process of oxidation produces highly reactive free radicals (Kang et al., 2024). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. These free radicals readily react and damage biomolecules and DNA (Maćków et al., 2024). Antioxidants "mop up" these free radicals and eliminate them before they can damage healthy tissue and lead to tumor formation. As antioxidants inhibit free radical damage, they also may block tumor formation(Russo, Spagnuolo, & Russo, 2024). Beneficial effects can be achieved by increased antioxidant capacity in the body may be the reduction of oxidative damage to important molecules (Russo et al., 2024). Antioxidants are present in variety of foods (fruits, vegetables, green tea, coffee, chocolate and soya) and also present in body (glutathione, melatonin, SOD, catalase enzyme). There are three richest antioxidant regimes green tea, Coffee and coca powder.

Components of green tea include Epigallocatechingallate (EGCG) Polyphenols, Catechin and Caffeine. Mechanisms of action of green tea may be inhibition of cancer cell proliferation and induction of apoptosis (Randisi, Perletti, Marras, & Gariboldi, 2025). Green tea polyphenols are also antimutagenic and is effective in reducing the formation of carcinogens in the body and reducing chromosomal damage during mutagen exposure (Li et al., 2024). Green tea catechins, act as an antioxidant scavenger of reactive oxygen species as superoxide, hydroxyl and peroxyl radicals, inhibition of lipid peroxidation and inhibition of 2'-deoxyguanosine oxidation in DNA to 8-hydroxy-2'-deoxyguanosine (Custodio-Mendoza, Ares-Fuentes, & Carro, 2023). Coca (*Theobroma cacao* L.) and its related products are rich sources of antioxidant flavonoids, containing higher amounts per serving than otherpolyphenol rich foods such

as red wine or tea (Soumahoro, Konan, Benjamin, & Soro, 2023). Flavanols are the most abundant flavonoids in coca and comprise monomeric flavanols [(+) catechin and (-)-epicatechin], oligomeric (procyanidins B1, B2 and C1) and polymeric forms (procyanidins) (Laveriano-Santos et al., 2022). The scientist suggested that green tea, coffee and coca powder help the liver health in 2 ways. By protecting liver cells and triggering immune system (Yang, Zhang, & Yang, 2025). By considering the multifunctions of green tea, cofee and coca powder the aim of present study was to investigate the antioxidant enzymes activity and oxidative status of green tea, cofee and coca powder in experimental rat model.

MATERIALS AND METHODS

Animals and Diet

Wistar albino rats of male sex (200-250g b.w.), purchased from the animal house of ICCBS, (Karachi, Pakistan), and were taken for the study. Animals were acclimatized to the laboratory conditions 1 week before the start of experiment and caged in a quite temperature-controlled room (23±4 °C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

Experimental Design

Animals were allocated in to six experimental groups (n=4):

Group I: These animals were designed as controls and given standard diet and water.

Group II: Received 5% (5 gm/100 mL) green tea extracts (Tapal Gulbahar green tea) prepared in distilled water, orally on daily basis and the volume of green tea consumed by each rat was measured on 11:30 am every morning.

Mean intake of green tea extract in these rats was 48.7±10.58 mL on the first day which was increased to 120.3±8.86 mL on 45th day of treatment.

Group III: Received 1% coffee extract (Nestle, Nescafe) prepared by taken 1gm of coffee dissolved in 100 mL of distilled water and the volume of coffee consumed by each rat was measured on 11:30 am every morning. Mean intake of coffee in these rats was 30.40±8.43 mL on the first day which was increased to 98.9±13.55 mL on 45th day of treatment.

Group IV: received coca powder extract (Rasmoor) 1g/kg b.w. orally for 45 consecutive days, at 11:30 am every morning.

Sample Collection

After 48 hours of last dose of treated groups, animals were decapitated and blood was sample collected from head wound in the lithium heparin coated tubes. A portion of blood was taken in the separate tube to collect the plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70°C until analysis.

Preparation of Liver Homogenate

A portion of liver was weighed, perfused with saline and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 min at 4°C to get the post mitochondrial supernatant which was used to assay SOD (super oxide dismutase), CAT (catalase) and MDA (malonyldialdehyde) activities.

Analytical Methods

Assessment of Oxidative Status

The Malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of Thiobarbituric Acid Reacting Substances (TBARS)(Mas-Bargues, Escriva, Dromant, Borras, & Vina, 2021). Briefly, the reaction mixture consisted of 0.2 mL of 8.1% sodium dodecyle sulphate, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 mL of 10% (w/v) of PMS. The mixture was brought up to 4.0 mL with distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Assessment of Antioxidant Status

Catalase activity was assayed by the method of mani *et* al (Mani, Iyyanar, & Rajendran, 2024). Briefly, the assay mixture consisted of 1.96 mL phosphate buffer (0.01 M, pH 7.0), 1.0 mL hydrogen peroxide (0.2 M) and 0.04 mL PMS (10%) in a final volume of 3.0 mL. About 2 mL dichromate acetic acid reagent was added in 1 mL of reaction mixture, boiled for 10 min, cooled. Changes in absorbance were 20 mM recorded at 570 nm.

Levels of SOD in the cell free supernatant were measured by the method of Kono [15]. Briefly, Solution A: 1.3 mL of solution A (0.1 mM EDTA containing 50 mM Na CO, pH 10.5). Solution B: 0.5 mL of solution B (90 mm NBT-nitro blue tetrazolium dye). Solution C: 0.1 mL of solution C (0.6% TritonX-100 in solution A). Solution D: 0.1 mL of solution D Hydroxylamine hydrochloride, pH 6.0) was mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 mL of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) required inhibiting the reduction rate by 50% in one minute.

Statistical Analysis

Results are presented as mean ± SE. Statistical significance and differences from control and test values were evaluated by Student's t-test. Statistical probability of $p<0.01$, <0.05 were considered to be significant.

RESULTS

Table 1

Effects of Green Tea, Coffee, and Coca Powder on Liver Oxidative Stress Markers (CAT, SOD, and MDA Levels)

S.No	Parameter (Units)	Control	Green Tea	Coffee	Coca Powder
1	CAT (µgm/gm	1.915 ± 0.464	3.2 ± 0.47	0.774* ± 0.063	5.75* ± 0.12

	of liver tissue)				
2	SOD (mm/ gm of liver tissue)	21.753 ± 2.72	14.57* ± 1.10	11.39* ± 0.48	54.12* ± 10.48
3	MDA (nm/gm of liver tissue)	2.264 ± 0.823	0.844 ± 0.117	0.699 ± 0.149	28.73* ± 7.16

n = 6, Values are Mean ± SD Significant differences between Control, green tea, cofee and coca powder–treated rats by Student's t-test *P<0.01, **P<0.05

The table compares the effects of green tea, coffee, and coca powder on liver tissue parameters—CAT (catalase), SOD (superoxide dismutase), and MDA (malondialdehyde)—against a control group. CAT levels were highest in coca powder (5.75 µgm/gm) and lowest in coffee (0.774 µgm/gm), while green tea showed a moderate increase (3.2 µgm/gm). SOD activity was significantly elevated in coca powder (54.12 mm/gm) but reduced in green tea (14.57 mm/gm) and coffee (11.39 mm/gm) compared to the control (21.753 mm/gm). MDA levels, a marker of oxidative stress, spiked dramatically with coca powder (28.73 nm/gm) but decreased with green tea (0.844 nm/gm) and coffee (0.699 nm/gm). The results suggest coca powder may induce oxidative stress despite high antioxidant enzyme activity, while green tea and coffee exhibit protective effects.

Effect of Green tea, Coffee and Coca powder on Catalase activity (CAT)

Catalase activity slightly increased in green tea treated rats but results were not significant. Coffee treated rats showed significant decreased catalase activity as compared to control (p<0.01) while the increased activity of catalase was observed in coca powder treated rats (p<0.01).

Effect of Green tea, Coffee and Coca powder on Superoxide dismutase activity (SOD)

SOD activity in green tea treated rats was significantly decreased (p<0.01) as compare to control. Coffee treated rats showed significant decreased SOD activity as compared to control (p<0.01) while the increased activity of SOD was observed in coca powder treated rats (p<0.01).

Effect of Green tea, Coffee and Coca powder on (MDA

Liver MDA level was decreased in green tea and coffee treated rats as compared to control but results were not significant while level of MDA was found to be significantly increased in cooca powder treated rats (p<0.01) as compared to control.

DISCUSSION

Formation of reactive oxygen species is a normal consequence of a variety of essential biochemical reactions (Idoko, Bonomi, & Eneji, 2023). It is also known that oxygen radicals could be formed in excess in chronic diseases (L. Lei, Yang, Zhang, & Zhang, 2021). Therefore, an adequate range of antioxidative defences within and outside the cells has also been considered to be very important to offer protection against oxidative damages of cell components including membrane phospholipids (Roy, Bansal, Siddiqui, & Chaudhary, 2023).

The effect of green tea, coffee and coca powder on antioxidants enzymes of liver was estimated. Oxidative status was measured by tissue MDA and antioxidant enzymes activity was measured by Catalase and SOD levels. Green tea, Coffee and coca powder restored antioxidant enzymes. The study showed the potent antioxidant properties of green tea, coffee and coca powder antioxidant activity in liver tissue. This study also indicated that administration of green tea, coffee and coca powder may be beneficial for pathological effects and also counteracted on deleterious effects of different chemotoxic materials which induced liver injury.

Herbal polyphenolic compounds in the cell can function as an antioxidant and prooxidant by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions (Sunday et al., 2024). Coffee components cafestol, kahweol, cholinergic acid and caffeine have an antioxidant (Elkamhawy et al., 2023), chemoprotective and anti-inflammatory property (Robichaud et al., 2025) reverse lipid peroxidation, enzymatic leakage and enhance cellular antioxidant defense mechanism, reported by (Fujii & Yamada, 2023) . Similarly, Green tea polyphenol EGCG (epigallocatechingallate) and catechin are promising anticancer potential (Djordjević et al., 2022). However, prolonged administration of green tea and coffee may cause liver enzymes alterations reported by Noori et al. (2012) (Idoko et al., 2023) administration of green tea, coffee and coca powder increase the antioxidant enzymes.

Our results preclude the antioxidant effects of green tea, coffee and coca powder in liver. Green tea, coffee and coca powder has potent potential to decreased lipidperoxidation, restored antioxidant enzymes. Green tea, coffee and coca powder are useful supplements which improves the liver health.

Antioxidant with good bioavalibility or molecules that have antioxidant enzyme activity may not only protect cells against the direct injurious effects of oxidants, but may alter the inflamatory events that play an imortant part in the pathogenesis of various diseases. We aimed to review highlights the role of antioxidant and antioxidant brevages in liver health (T. Lei et al., 2022).

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