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Synergistic Effects of *Sabicea calycina* and *Carpolobia lutea* on Biochemical Indices in Crude Oil-Exposed Wistar Rats

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Abstract

The environmental contamination caused by crude oil has been linked to significant biochemical and physiological disturbances in living organisms. This study investigates the ameliorative potential of the synergistic potentials of *Sabicea calycina* and *Carpolobia lutea* extracts on the biochemical indices of male Wistar rats challenged with crude oil exposure. Twenty-five male Wistar rats averagely weighing 155.26 ± 5.58 g were randomly divided into five groups of five rats per group. Group A; non-crude oil-challenged rats without treatment, group B, the crude oil-challenged group without treatment, group C, the crude oil-challenged group treated with 50 mg/kg of the composite extract, group D, the crude oil-challenged group treated with 100 mg/kg of the composite extract and group E, the crude oil-challenged group treated with 150 mg/kg of the composite extract. Biochemical indices, including liver function (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, globulin and albumin/globulin ratio), oxidative stress biomarkers/antioxidant status (malondialdehyde, catalase, superoxide dismutase and Glutathione-S-transferase), and serum electrolytes (sodium and potassium) were analyzed. Results showed that crude oil exposure significantly ($p < 0.05$) disrupted these biochemical indices, evidenced by elevated liver enzymes, elevated serum electrolytes and increased oxidative stress with a marked reduction in antioxidant status. Treatment with the composite of *S. calycina* and *C. lutea* extracts significantly ($p < 0.05$) ameliorated these effects, as indicated by the improved liver function serum electrolytes, reduced malondialdehydes and increased antioxidants in the composite extract treated groups.

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Introduction

The Niger Delta of Nigeria is distinguished by its extensive oil and gas reserves alongside its rich biodiversity. (Abubakar *et al.*, 2016). This region is a major contributor to national revenue, with petroleum accounting for over 80% of government income, more than 95% of export earnings, and exceeding 40% of Nigeria's Gross Domestic Product (GDP). Despite large untapped reserves, current production averages 2.2 million barrels of oil per day, with an estimated 240,000 barrels spilt into the environment (UNDP Project, 2012). Furthermore, research indicates that 11–54 mg/L of oil dissolves into the coastal waters (Ordinioha and Brisibe, 2013). These oil spills and associated gas flaring activities lead to the contamination of surface and groundwater, atmospheric air, and agricultural produce with hazardous hydrocarbons, including carcinogenic polycyclic aromatic hydrocarbons (PAH), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), brominated flame-retardants (BFRs) and chlorinated flame retardants (CFRs), naturally occurring radioactive materials such as uranium and thorium, and trace metals that bioaccumulate in food crops (Abosede and Kpomah, 2024). The individual and combined toxic effects of these pollutants are linked to both acute and chronic health issues in the region, including haemotoxicity (Igwe *et al.*, 2016), hepatotoxicity, carcinogenesis via chromatin DNA damage, reduced fertility and sexual dysfunction, as well as systemic diseases that adversely affect male reproductive function (Adeyemi and Adeyemi, 2021; Iniaghe and Kpomah, 2022; Iniaghe and Kpomah, 2023).

Despite notable progress in medical therapies and the expansion of healthcare infrastructure in Nigeria, the use of herbal medicine remains prevalent among the population. This persistence is largely attributed to its year-round availability, relative affordability, and the commonly held belief that, as natural products, herbal remedies are associated with fewer adverse effects compared to conventional pharmaceuticals (Kpomah and Arhoghro, 2012; Kpomah *et al.*, 2024). The individual and composite ethanol extract of *Carpolobia lutea* and *Sabicea calycina* are two local

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plants commonly used for medicinal purposes. *C. lutea* G. Don (Polygalaceae) is a small tree that grows to about 15 feet high, and it is extensively distributed across the rainforest of tropical Africa. It is commonly called a 'cattle stick'. From an ethnopharmacological perspective, various parts of the plant have been traditionally utilized for the treatment and management of a wide range of health conditions. The leaves, for instance, are employed as an antipyretic and are used in the treatment of ulcers, malaria, skin infections, venereal diseases, sterility, and gastrointestinal issues such as stomach discomfort and diarrhoea. They are also applied in cases of headache, leprosy, snakebite, and wounds, and serve as vermifuge and taenifuge agents. Additionally, the leaves are used to facilitate childbirth. The root bark is traditionally used for alleviating rheumatism, general body pain, and mental health disorders such as insanity. Meanwhile, the dried stem bark is inhaled as snuff to relieve migraines. Furthermore, in Southern Nigeria, a decoction of the root is reputed to act as a sexual stimulant Kpomah *et al*., 2024). *S. calycina* belongs to the Rubiceae family.

Plant Source and Authentication

The stems of both *C. lutea* and *S. calycina* were collected from Odi in the Kolokuma/Opokuma area of Bayelsa State, Nigeria. The plant species were identified and authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State. Voucher specimens were assigned the numbers UHAE-2019808 and UHAE-2019809, respectively, and deposited in the university herbarium for future reference

Preparation of Composite of *C. lutea S. calycina* Extract

The plant samples (*S. calycina* and *C. lutea*) were thoroughly washed with distilled water to remove debris and impurities. They were then air-dried in a shaded area until a constant weight was achieved. The dried samples were subsequently pulverised using an electric blender (Blender 462, Nakai, Japan). A total of 200 g of the powdered material (comprising 100 g each of *C. lutea* and *S. calycina*) was extracted with 600 mL of absolute ethanol at room temperature for 24 hours, with continuous agitation using a flask shaker (Model Denly A-500). The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated to dryness using a rotary evaporator at 40°C, yielding 6.39 g of extract. The concentrated extract was reconstituted in distilled water to obtain the required doses for the study.

Animals Used for the Study

Twenty-five healthy male Wistar rats, with an average weight of 160 ± 5.79 g, were procured from the Animal House Unit of the Department of Biochemistry, Federal University Otuoke. The animals were housed in a clean, well-ventilated room maintained at a temperature of 28–30°C, under a natural light/dark cycle. They had free access to standard rat pellets and water *ad-libitum* throughout the one-week acclimatization period, the two-week exposure to a crude oil-contaminated environment, and the 21-day ameliorative treatment phase. All procedures involving the animals were conducted following the guidelines of the Institutional Animal Ethics Committee (IAEC). The protocols for the use of these animals were approved by the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State, under approval number DRQA/FUO/0100/13/12/23

Experimental Design

The twenty-five male Wistar rats were grouped into five divisions of five per group and exposed to the following treatment regimen.

Group A: Positive control: received 50 mL of distilled water only

Group B: Negative control: exposed to Bonny light crude oil and administered 50 mL of distilled water

Group C: exposed to Bonny light crude oil and administered 50 mg/kg of composite extract)

Group D: exposed to Bonny light crude oil and administered 100 mg/kg of composite extract

Group E: exposed to Bonny light crude oil and administered 200 mg/kg of composite extract

Simulation of Crude Oil-Contaminated Environment

A crude oil-contaminated environment was simulated in a designated section of the Animal House. To achieve this, 20 mL of Bonny Light Crude Oil (BLCO) was uniformly applied to the bedding material within the metabolic cages designated for the environmentally challenged groups. The animals in these groups were housed in the contaminated environment for two weeks.

Method Extract Administration

The composite plant extracts were administered orally using an oropharyngeal cannula. The doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight were administered once daily in the morning (between 08:00 and 09:00 hours) for 21 days. The extracts were reconstituted in distilled water, which served as the vehicle, and were delivered under controlled conditions to ensure precise dosing and minimize animal stress.

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Method of Blood and Tissue Collection

On the 22nd day of the experiment, the animals were anaesthetized in a closed chamber containing chloroform-soaked cotton wool to induce deep anaesthesia. Following complete anaesthesia, blood samples were obtained via cardiac puncture using sterile syringes and transferred into plain, sterile sample tubes. The collected blood was allowed to clot at room temperature for 15 minutes and subsequently centrifuged at 2,000 revolutions per minute (rpm) for 10 minutes using a bench-top centrifuge (Model CE-800). The resulting sera were carefully harvested and stored appropriately for subsequent biochemical assays. Immediately after blood collection, the liver was excised, rinsed in ice-cold saline, and homogenized in phosphate buffer using a homogenizer to prepare tissue homogenates for biochemical analyses.

Assay Kits

Commercial assay kits were employed for the biochemical analyses conducted in this study. Kits for the determination of plasma proteins specifically total protein and albumin as well as liver enzyme activities including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were obtained from Teco Diagnostics Ltd. (Anaheim, CA, USA). Assays for oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and malondialdehyde (MDA), were carried out using kits supplied by Randox Laboratories Ltd. (Crumlin, United Kingdom). All procedures were performed following the manufacturer's protocols and quality control standards.

Biochemical Assays

Liver function tests were conducted to assess hepatic integrity and potential biochemical alterations induced by experimental treatments. The activities of serum aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the colourimetric method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was measured following the colourimetric protocol of the Rec. GSCC (1972). Serum total protein concentration was estimated using the Biuret method, while albumin was quantified using the Bromocresol Green (BCG) dye-binding method. Globulin concentration was calculated by subtracting albumin from total protein values, and the albumin/globulin (A/G) ratio was derived by dividing albumin concentration by globulin concentration. Hepatic antioxidant and oxidative stress biomarkers were assessed using standard spectrophotometric methods. Superoxide dismutase (SOD) activity in liver homogenates was evaluated using the method of Misra and Fridovich (1972). Catalase (CAT) activity was determined according to the procedure of Kaplan and Groves (1972). Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured using the thiobarbituric acid reactive substances (TBARS) method described by Buege and Aust (1978). The activity of glutathione S-transferase (GST) was assayed according to the method of Habig *et al*. (1974).

Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation (SD) for five replicates per group. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between group means. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 17.0.

Results

Effect of 50, 100 and 200mg/kg body weight of a composite mixture of *S. calycina* and *C. lutea* on antioxidant enzyme system after 21 Days in Wistar Rats Exposed to Bonny Light Crude Oil

The effect of graded doses (50, 100 and 200 mg/kg body weight) of composite mixture of *S. calycina* and *C. lutea* extract on antioxidant enzymes of male Wistar rats in unit/mg tissue is presented in Table. 1.0 Findings from the result indicated a significant increase ($p<0.05$) in concentrations of antioxidant parameters of superoxide dismutase (SOD), Glutathione-S-transferases (GST), and catalase (CAT) by the 200 mg/kg extract and a concomitant decrease in MDA concentration by the 200 mg/kg extract

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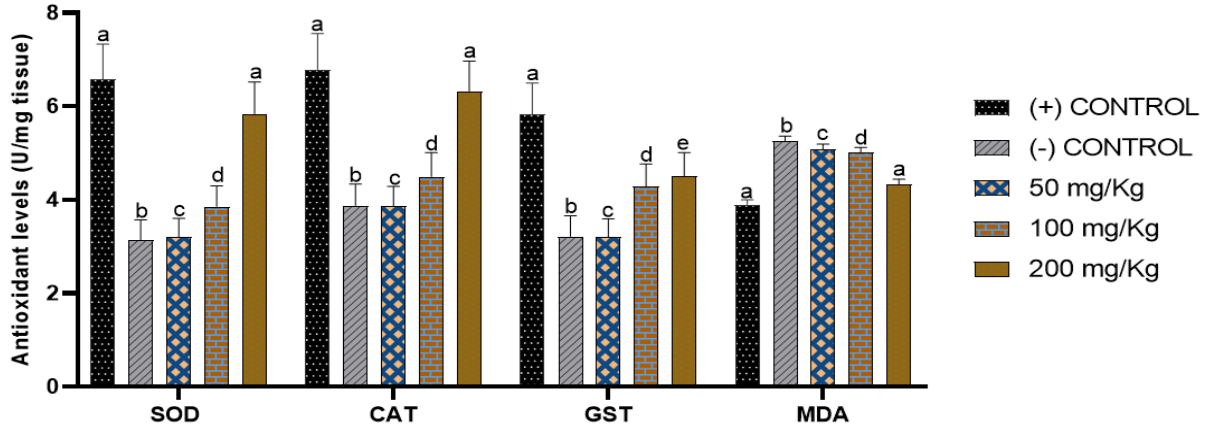

Figure 1.0: Antioxidant enzyme system of male wistar rats after 21 days of composite mixture of *S. calycina* and *C. lutea* extract dosing are means of five replicate determinations ± standard deviation, values on the same column with same superscript letters 'a' are not significantly different ($p > 0.05$) from the (+) control

Key: SOD = superoxide dismutase; CAT = catalase; GST = Glutathione-S- transferase; MDA = malondiadehyde

Effects of 50, 100 and 200 mg/kg of Combined Extract of *C. lutea* and *S. calycina* on Plasma Enzyme Activity in Wistar Rats Exposed to Bonny Light Crude Oil

The values of total protein (TP), albumin, globulin and A/G ratio for male Wistar rats after 21 days of treatment with ethanolic extract of a composite mixture *S. calycina* and *C. lutea* are presented in Figure 2.0. The results obtained indicated that only the 200 mg/kg of the extract had a positive significant change ($p < 0.05$) in the concentrations of total protein, albumin, globulin and A/G ratio

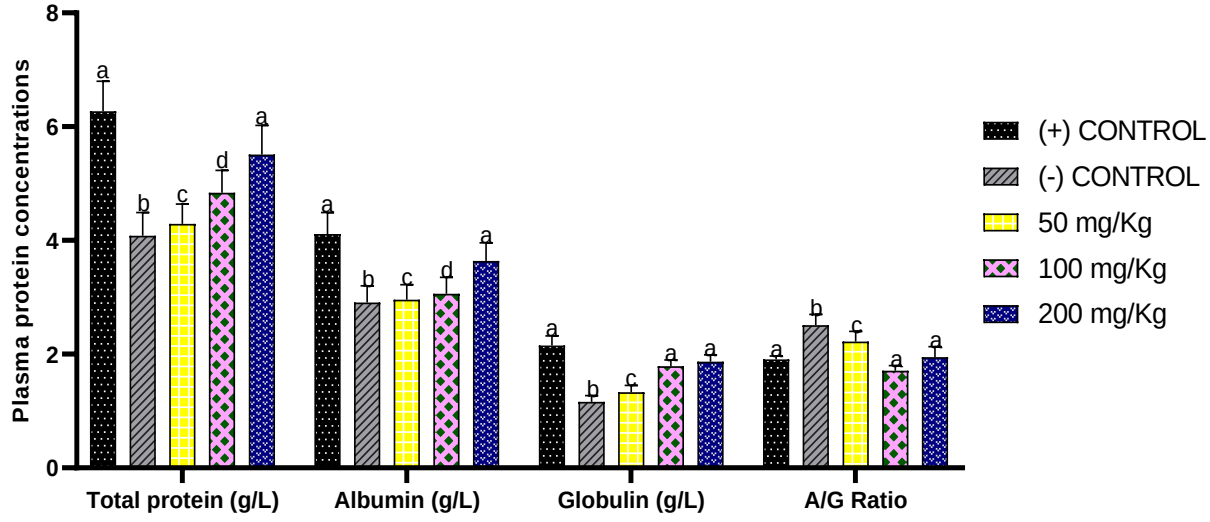

Figure 2.0: Concentrations of total protein, albumin, globulin and A/G ratio for male wistar rats after 21 days of a composite mixture of *S. calycina* and *C. lutea* extract dosing are means of five replicate determinations ± standard deviation, values on the same row with the same superscript letters 'a' are not significantly different ($p > 0.05$). One-way analysis of variance (ANOVA) followed by posthoc tuckey.

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Effects of 50, 100 and 200 mg/kg of Combined Extract of *C. lutea* and *S. calycina* on Plasma Enzyme Activity in Wistar Rats Exposed to Bonny Light Crude Oil

The concentrations of plasma enzyme activities of male Wistar rats treated with graded doses of a composite mixture of *C. lutea* and *S. calycina* (50, 100 and 200 mg/kg body weight) are presented in Figure 3.0

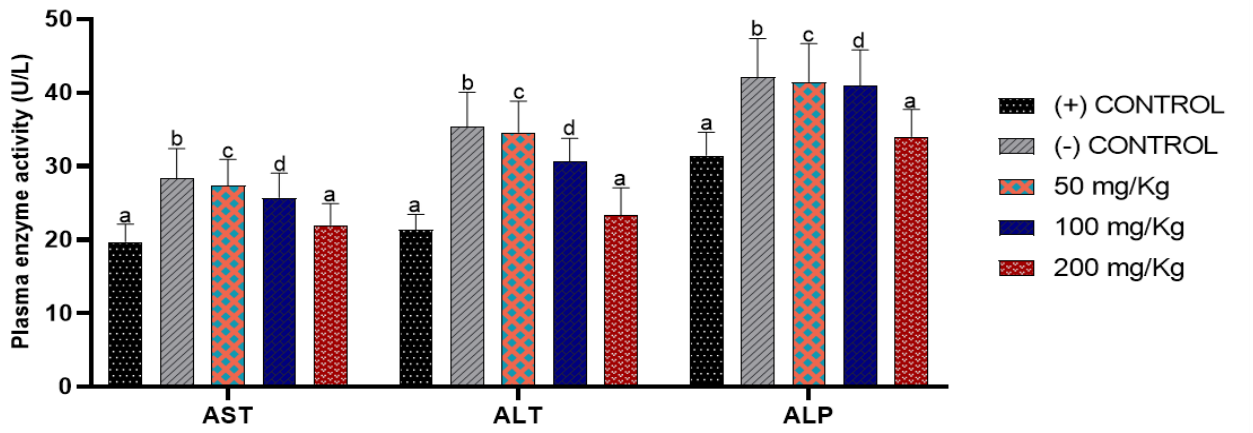

Figure 3.0: Concentrations of aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) for male wistar rats after 21 days of a composite mixture of *S. calycina* and *C. lutea* extract dosing are means of five replicate determinations ± standard deviation, values on the same row with same superscript letters are not significantly different ($p > 0.05$); while those with different superscript letters are significantly different ($p < 0.05$). One-way analysis of variance (ANOVA) followed by posthoc tuckey.

Discussion

The present study assessed the impact of a composite mixture of *S. calycina* and *C. lutea* extracts on the antioxidant defence system in male Wistar rats. Antioxidant biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and malondialdehyde (MDA), were evaluated after 21 days of oral administration at varying doses (50, 100, and 200 mg/kg). The (+) control group exhibited the highest levels of SOD and CAT, which were not significantly different ($p > 0.05$) from those in the 200 mg/kg treatment group, suggesting that high-dose extract administration preserved antioxidant enzyme activities at levels comparable to the untreated healthy group. SOD and CAT are critical enzymes involved in the detoxification of reactive oxygen species (ROS) (Evuen and Kpomah, 2023). The reduction in SOD and CAT activities observed in the (-) control group indicates oxidative stress, which was significantly ameliorated in the 200 mg/kg group, suggesting the efficacy of the extract in restoring redox balance. Similarly, GST levels showed a dose-dependent increase, with the 200 mg/kg group recording significantly elevated activity compared to both controls. GST plays a vital role in phase II detoxification by conjugating reduced glutathione to electrophilic compounds, thus facilitating their excretion (Kpomah and Arhoghro, 2023). The enhanced GST activity in the treated groups implies that the composite extract may augment endogenous detoxification capacity. MDA, a lipid peroxidation product and a reliable marker of oxidative damage (Kpomah *et al.*, 2024), was highest in the (-) control and 50 mg/kg groups, but significantly lower in the 200 mg/kg treatment group. This inverse relationship between MDA levels and antioxidant enzyme activities reinforces the protective role of the extract against lipid peroxidation and oxidative injury. Overall, the results suggest that the composite extract of *S. calycina* and *C. lutea* possesses potent antioxidant properties, especially at higher doses. This may be attributed to the presence of phytochemicals such as flavonoids and polyphenols reported in both plants (Osadebe *et al.*, 2014; Kpomah *et al.*, 2018; Kpomah *et al.*, 2020), which are known to modulate oxidative stress pathways.

The effects of a composite mixture of *S. calycina* and *C. lutea* extracts on plasma protein concentrations were evaluated in male Wistar rats. The results demonstrated a dose-dependent improvement in total protein, albumin, and globulin levels after 21 days of

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treatment, particularly at the highest dose (200 mg/kg), suggesting potential hepatoprotective and protein-sparing properties of the plant mixture. In the untreated negative control group, a significant reduction in total protein and albumin levels was observed compared to the positive control ($p < 0.05$), indicating possible hepatic dysfunction or protein catabolism. This is consistent with reports that hepatic damage or systemic inflammation often leads to a decrease in plasma protein synthesis (Kpomah *et al.*, 2017). Administration of the extract mixture at 200 mg/kg restored total protein and albumin concentrations close to those of the positive control group, with no significant differences ($p > 0.05$). This suggests that the phytochemical constituents of *S. calycina* and *C. lutea* may enhance protein synthesis or prevent protein degradation. Bioactive compounds such as flavonoids, saponins, and polyphenols present in these plants are known to exert antioxidant and hepatoprotective effects, which may account for this observation (Ajiboye *et al.*, 2010; Kpomah *et al.*, 2018; Owo *et al.*, 2025). Globulin concentrations were significantly increased in all treatment groups compared to the negative control, with no significant differences from the positive control group. This increase might reflect enhanced immunoglobulin production, as globulins play a key role in immune response (Kaneko *et al.*, 2008). The A/G ratio, while variable, remained within normal physiological limits across all groups, and the values observed in treated animals did not significantly deviate from controls. This suggests a balanced synthesis of albumin and globulin fractions, a useful marker of hepatic and renal function (Kumar and Clark, 2016). Overall, the data indicate that the combined extract of *S. calycina* and *C. lutea* at higher doses can ameliorate protein deficits in plasma, likely through hepatoprotective and antioxidant mechanisms. These findings align with previous studies demonstrating the therapeutic potential of plant-based bioactive compounds in maintaining plasma protein homeostasis (Salih *et al.*, 2021).

The effects of a composite mixture of *S. calycina* and *C. lutea* on hepatic enzyme activity in male Wistar rats were assessed through quantification of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) following 21 days of oral administration at varying doses (50, 100, and 200 mg/kg). These enzymes are routinely employed as biomarkers for hepatic function, with elevated plasma levels typically indicating hepatocellular injury or altered membrane permeability. The results revealed dose-dependent changes in hepatic enzyme activities, with significant reductions observed at higher doses. Notably, AST levels were significantly elevated in both control groups compared to the treated groups, with the highest dose group (200 mg/kg) showing the most substantial reduction ($p < 0.05$). This suggests a potential hepatoprotective effect of the extract mixture at higher concentrations. Similarly, ALT levels, which are more specific indicators of hepatocellular damage, followed a comparable trend. The 200 mg/kg group exhibited a significantly lower ALT activity relative to both controls and the 50 mg/kg group, indicating that the composite extract may mitigate hepatocellular membrane disruption. Interestingly, ALP levels showed a less pronounced response across treatment groups, with only the control groups exhibiting significantly higher enzyme activity. The 200 mg/kg dose group had significantly lower ALP levels compared to both controls and the lower dose groups, suggesting a possible ameliorative effect on biliary function or hepatobiliary stress. These findings collectively imply that the composite mixture of *S. calycina* and *C. lutea* may exert a dose-dependent hepatoprotective effect, likely through antioxidative or anti-inflammatory mechanisms, though further mechanistic studies are warranted. The decreased plasma levels of AST, ALT, and ALP in treated rats underscore the potential therapeutic utility of this phytomedicinal combination in protecting hepatic integrity under stress or toxicity conditions.

Conclusion

The present study demonstrates that the composite extract of *S. calycina* and *C. lutea* exerts potent antioxidant and hepatoprotective effects in male Wistar rats. Administration of the extract at higher doses (particularly 200 mg/kg) significantly enhanced the activities of key antioxidant enzymes (SOD, CAT, GST), reduced lipid peroxidation (MDA levels), and improved plasma protein profiles suggesting attenuation of oxidative stress and restoration of hepatic function. Moreover, the extract markedly reduced plasma levels of hepatic enzymes (AST, ALT, ALP), indicative of hepatocellular protection. These effects are likely mediated by phytochemicals such as flavonoids and polyphenols, known for their antioxidative and anti-inflammatory properties. Collectively, these findings support the therapeutic potential of *S. calycina* and *C. lutea* as a natural antioxidant and hepatoprotective agent, warranting further investigation into their mechanisms of action and possible clinical applications.

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