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Synergetic efficacy of Combined Garlic and Clove's Aqueous Extract on Trona-induced Osteopontin inflammation

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ABSTRACT

The synergistic efficacy of the aqueous extract of combined garlic and clove (CGC) was studied against a single garlic aqueous extract (GE) administration in trona-induced osteopontin inflammation using male Wistar rats (180-200 g) within 35 days. Group 1 received standard animal chow and water ad libitum, and group 2 received 200 mg/kg body weight of trona (TRN). Group 3 was given 200 mg/kg body weight of trona but co-treated with 100 mg/kg body weight of CGC; group 4 was co-administered with 200 mg/kg body weight of trona and 100 mg/kg body weight of garlic extract; group 5: 200 mg/kg body weight of trona and 150 mg/kg body weight of CGC. In contrast, 6 was given 200 mg/kg body weight of trona and 150 mg/kg body weight of garlic extract. The assault revealed a significant ($p < 0.05$) increase in the plasma levels of osteopontin (15-fold), alanine transaminase (ALT) (2.6-fold), and a corresponding significant ($p < 0.05$) decrease in the concentration of reduced glutathione (GSH) (2.7-fold) in the agonist assaulted group when compared with the animal on standard chow. However, the disorders were ameliorated by the synergetic potential of CGC more than the single application of GE in a dose-dependent manner, revealing a significant and more synergetic recuperation in the co-treated animals. The study further revealed significant histo-hepatic remediation by CGC more than GE, as microscopically depicted in the photomicrographs of the fibrotic lesion induced by TRN on the parenchymal tissue of

the treated rats' hepatocytes. More so, the result was able to affirm the folkloric use and direct simultaneous increase/great correlation of the novel osteopontin, a proteinous cytokine, and the inflammatory hepatic bio-indicator, ALT. Hence, it's established that CGC could be considered a good herbal therapeutic candidate for hematological and hepatic-embedded osteopontin regeneration.
Keywords: Osteopontin, Glutathione, Garlic, Clove, Synergy, Trona.

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INTRODUCTION

Osteopontin (OPN) is a novel hematological, pro-inflammatory, proteinous, and hepatic synthesizing chemokine (Cabiati et al., 2020) that is primitively known to mediate in the parathyroid hormone, related to calcification and phosphorylation of bone (Ihara et al., 2001), wound healing, carcinogenesis, and mostly infiltrated into the plasma via secretion by osteoblasts, activated Thymus (T) immunological lymphocytes, endothelial (Cho et al., 2009), and Kupffer cells (Chiba et al., 2002). It is required for normal neuronal development along with diverse biological functions. Recent studies have also established the implication in the pathogenesis of a variety of diseased states, such as chronic immunological disturbances, hepatic fibrosis (George et al., 2022), atherosclerosis, oxidant cascade, glomerulus toxicity, and several inflammatory unhealthiness (Uenoyama et al., 2008). Structurally, osteopontin is an acidic biomolecule of arginine-glycine-aspartate with a molecular mass of approximately 44 kilodaltons (kDa), composed of about 300 amino acids and accounted to regulate cell trafficking, playing also a signal role in the response of various organs to xenobiotics. This protein contributes mostly as a great reliable cytokine, though novel, and is coherently established to be integrated with Nitric oxide (NO), interleukin-1 beta (IL-1β), and tumor necrosis factor (TNF) trafficking during pathological disturbances; hence, its roles in immunological-related modulation, liver sinusoidal inflammation, blood integrity, and cell apoptosis can't be undermined. While Matsui et al. (2003) established a direct influence of OPN proliferation on the pathogenesis of atherosclerosis, signaling tunica intima fibrosis in mice after 36 weeks, other studies had also established the challenges along the plasma integrity of assaulted animal models (Giachelli et al., 1993). Hence, osteopontin (OPN), a proteinous biomolecule, is investigated as a chemotactic bio-indicator along with other established markers, alanine transaminase (ALT) and reduced glutathione (GSH), in this trona-induced assault in Wistar rats.

Trona (TRN), sometimes called "potash," is commonly used in many homes in African countries, e.g., Nigeria, Mali, Burkina Faso, Niger, Chad, and Ghana, and is a mineral of hydrated tri-sodium bicarbonate [Na3H(CO3)2·2H2O], occasionally exploited as a saline lake deposit (Evbu et al., 2016). It is called 'Kaun' in the Yoruba speaking region and 'kanwa' in Hausa and coexists with other minerals such as thermonatrite, halite, and gypsum but is commonly consumed as a food additive in many homes, where it is mostly applied as a tenderizer to soften foods like corn, meat, and beans (Nielsen, 1999). The mineral further gained popularity in its importance as next-to-table salt (Sodipo, 1993), as it is applied

to prepare a delicacy called ‘owo’ in Edo and Delta States of Nigeria. However, relevant studies conducted in vivo by Evbu et al. (2016) and Imafidon et al. (2016) established that certain doses of trona in meals may compromise the health status of individuals. While Ajayi & Akhigbe (2015) reported the metabolic derangement and fetal loss of 83.33% and 100% at 250 mg/kg and 500 mg/kg body weight, respectively, of trona administered to pregnant rats within 4 weeks, Ajayi et al.'s pronounced dosages at 200 and 400 mg/kg body weight could significantly escalate a series of radical cascades in experimental rodents within 28 days (Ajayi & Akhigbe, 2017). Although many enthusiastic researchers have recorded the biochemical and intrinsic toxicity of trona, there is still a paucity of data concerning the ability of conventional medicine to cushion the health hazards (McGill & Jaeschke, 2019).

The liver is a very predominant organ of all animals, mostly interferes with all xenobiotics, naturals, and trona inclusive, is physiologically inclined to detoxify, digest, and store glycogen and low-density lipoprotein, and promotes hormonal, enzymatic, and non-enzymatic biochemical modulators necessary for digestion and maintaining metabolic homeostasis. Nonetheless, necrosis of the hepatocytes embedding Kupffer cells could be triggered by many factors, more precisely the xenobiotic-induced intrinsic injury (XIIJ), and this still accounts for more than 10 percent per 10,000 persons of metabolic hypertrophy, intracellular fibrosis, and eventual cardio-hepatic failure in most cases (Jersey, 2016). More so, other research-based investigators have suggested the possible and improper moderation of some hepatic enzymes and bio-proteinous indicators associated with XIIJ. Such include modulations in the concentration of OPN (Cabiati et al., 2020) and activities of some hepatic enzymatic and non-enzymatic activities (ALT, GSH) (Gupta et al., 2013), derailing homeostasis, signaling transduction responses, and eventual mortality. The understanding and interwoven relationship of OPN, ALT, and GSH’s role in XIIJ development and the eventual immunological storm is not yet known, talk less of perhaps hepatic regeneration by the components of the aqueous extract of combined garlic and clove (CGC). The in-vivo disturbances amidst pathologies also necessitate a series of atherogenic morphologies involving highly oxidant species and hepatic ALT enzyme imbalances (Moriles & Azer, 2022), with a great association as sometimes demonstrated by the blunted incretin effect, de-mobilizing glucagon (Tanaka et al., 2011), and degrading plasma integrity (Geula & Darvesh, 2004). Hence, ALT, a well-known haem-based hepatic enzyme, was considered in this study to ascertain the instinctive correlations with OPN in trona-induced pathology and to evaluate and clarify the potency of CGC over garlic extract (GE) as used in concoctions in folkloric belief (Clemens et al., 2019).

Recently, increasing interest in natural medicine and indigenous health care has been tremendously observed worldwide, with about 80% of the population relying on biodiversity within their cultural heritage to survive and overcome their health challenges (Izuchukwu, 2018). This is precipitated by the conceptual failure of allopathic medicine to down-regulate the prevalence of deleterious human diseases in our society. With this great concern, an unprecedented investigation of the synergetic efficacy of CGC over the singular medicinal importance of GE was exemplified in this study, to possibly complement the shortfall in conventional therapy and to attenuate the deleterious prevalence triggered by continual home usage of trona.

Garlic and clove are well-known natural products commonly used as spices and

condiments in soup and as prophylactics in day-to-day household usage. Garlic (Allium sativum) (Linn), which belongs to the family Amaryllidaceae, has been recorded to express variant efficacies against some pathological conditions, which include immunological storm, cancer (Das & Saha, 2009), hepatotoxicity (Jiang et al., 2022), atherosclerosis (Shouk et al., 2014), diabetes, microbial infection (Garba et al., 2022), ischemic stroke (Zhu et al., 2018), and more so, significantly inhibiting angiotensin-converting enzyme (Singh & Kumar, 2017) in spontaneous hypertensive models. The phytochemical and sole lead compounds established in these regards are allicin (Siddique et al., 2015), 5-hydroxymethylfurfural, organosulfur, polyphenol, and some volatile essential compounds (Ahmed & Wang, 2021). Cloves (Syzygium aromaticum) (L.) are the aromatic flower buds of a tree in the family Myrtaceae, which has been used through folkloric belief for ages to treat various health concerns, with Eugenol (Elbestawy et al., 2023), β-caryophyllene, α.-humulene, CIS-calamenene, syringic acid, caffeic acid, and protocatechuic acid (Jimoh et al., 2017) established as the lead phytochemicals responsible for the pharmacological efficacies. Such aftermath research-based potencies of cloves are antimicrobial (Kačániová et al., 2021), anticarcinogenic, anti-hyperglycemic (Taher et al., 2015), immunomodulatory, anti-obesity, antiulcer (Xue et al., 2022), insecticidal (Haro-González et al., 2021), anti-vomiting (Mohammad Nazrul Islam Bhuiyan, 2012), and also predominantly used in the fragrance, flavoring, and cosmetic industries (Nurdjannah & Bermawie, 2012). However, the synergistic efficacy of combined garlic and clove's aqueous extract and single application of garlic extract in trona-induced osteopontin inflammation remains unexplored.

MATERIALS AND METHODS

Source of materials

Garlic, cloves, and trona were purchased from the traditional practitioners in Oshodi market, Lagos State, Nigeria, and identified and authenticated by Dr. Famuwagun (FUTA) (Federal University of Technology, Akure) and Dr. Oche, Herbarium Unit, Nigeria Natural Medicine Development Agency, Federal Ministry of Innovation, Science, and Technology, Nigeria.

Chemicals

All chemicals used were of the analytical grade from BDH Chemicals Limited, Poole, England.

Plant pulverization

Garlic and cloves were sorted from dirt, but garlic was minced and air dried for 50 days at 26–30 °C until a constant weight was obtained, and eventually pulverized using an industrial fine grinder at the production laboratory, product development department, Nigerian Natural Medicine Development Agency, Federal Ministry of Innovation, Science, and Technology, Nigeria. The resulting samples were thereafter stored in an airtight amber container and kept in the refrigerator for future extraction.

Preparation of Aqueous Extracts

Decoction (by cooking) of the air-dried samples (750 g) each (cloves, garlic) was prepared using 2000 ml of drinking water for 2 hours. Thereafter, the resulting solutions were allowed to cool, filtered using a clean muslin cloth and a Whatman No. 1 filter, evaporated to dry di-herbal extracts using a freeze drier, and later stored in an airtight amber bottle until further use (Eyo, 2016).

Acute Lethal toxicity evaluation

The CGC was prepared by mixing an equal weight of aqueous extracts of clove

and garlic and the acute lethal toxicity (LD50) determined by using up and down techniques, with little modification as described by (Rispin et al., 2002).

Phytochemical Analysis

The qualitative and quantitative analysis of CGC and GE were carried out at ISO registered laboratory (ISO-17025:2017) of Nigeria Natural Medicine Development Agency, Federal Ministry of Innovation, Science and Technology, Nigeria using standard procedures as described by (Lone & Jain, 2022), (Oboho et al., 2023) and (Mahmood et al., 2019).

Anti-oxidative potency of CGC and GE

The anti-oxidative ability of the two extracts was carried out using the method described by (Rasul Suleria et al., 2012) and (Jimoh et al., 2017).

Experimental Design

Wistar male rodents were housed at the animal house of Nigeria Natural Medicine Development Agency, Federal Ministry of Innovation, Science and Technology, Lagos, with approved Laboratory number ISO-17025:2017, under the supervision of Nigeria College of Natural Medicine Technology (NICONMTECH) (accredited) and Department of Biochemistry, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Ogbomosho, Oyo State. Rats (n=30) of 180-200g were equally divided into six groups and housed under an illumination of 12:12h light/dark cycle and the animals were acclimated for two weeks at the early stage of the study before the intrinsic assault.

Group 1 received standard animal chow, water ad libitum

Group 2 received 200 mg/kg body weight of trona

Group 3 was given 200 mg/kg bodyweight of trona and 100 mg/kg body weight of CGC

Group 4 was placed on 200 mg/kg body weight of trona and 100 mg/kg body weight of garlic extract (GE)

Group 5 was co-exposed to 200mg/kg body weight of trona and 150mg/kg body weight of CGC

Group 6 was co-exposed to 200mg/kg body weight of trona and 150mg/kg body weight of garlic extract (GE).

Animal sacrifice

On the 36th day (24hour-fasting), all rodents were anesthetized using minimal concentration of chloroform vapour, as Inhalation anesthetics for an average of 1-2 minutes (Moke et al., 2015) in a mini fume hood and thereafter sacrificed, blood collected through cardiac puncture, plasma OPN evaluated, and liver histology examined.

Liver histology

The excised liver of all the euthanized rats were rinsed with phosphate-buffered saline (PBS) solution, dried with paper towel, fixed using 10% formaldehyde for 48 hours. The liver organ was later rinsed with PBS to remove excess fixative, stained with hematoxylin and eosin (H&E staining), photomicrographs recorded at X200 magnifications to assess overall tissue morphology and hepatic details using a microscope (Olorunnisola et al., 2021).

Biochemical serum analysis

Osteopontin was analyzed using the quantitative measurement technique of enzyme-linked immunosorbent assay (ELISA) kit method postulated by (Peng et al., 2023), while Reduced glutathione was estimated according to the method described by (Olabisi & Bamikole, 2015) and Plasma alanine aminotransferase (ALT) determined colorimetrically using the standard kits of Randox laboratories, as described by (Imafidon et al., 2016).

Statistical analysis

Mean ± Standard deviation data sheets from this study were subjected to GraphPad software, with a significant value set at $p<0.05$ under a one-way analysis of variance and Tukey's Multiple Comparison Test. Mean values with different superscript letters are significantly different at $p<0.05$.

RESULTS

The bioactivities of plant secondary metabolites in healthcare have been recorded through research-based aggregates since ancient times. Such anti-oxidative classes of compounds qualitatively detected in CGC, as listed in Table 1, are alkaloids, phenolics, flavonoids, tannins, glycosides, steroids, terpenoids, saponins, volatile essentials, citral, coumarins, phenolatanins, anthocyanins, anthraquinones and carotenoids, while quinines weren't detected. More so, the quantitative analysis of CGC with an extraction yield of 23.35% revealed the compositions of alkaloid (8.30 ±0.04 mg/100g), tannin (5.10 ± 0.05 mg/100g), saponin (4.700 ± 0.03 mg/100g), flavonoid (2.55 ±0.05 mg/100g), with no significant difference in the number of phenols (1.12 ± 0.01 mg/100g) and anthraquinone (1.10 ± 0.02 mg/100g) ($p > 0.05$), while others are out of scope or negligible. This quantitative analysis was achieved using Thin-Layer Chromatography (TLC) Densitometry and Ultraviolet-Visible (UV-Vis) Spectrophotometry and the final results obtained from the two analytical procedures were compared. While the result of the phytochemical screening of GE discovered the presence of alkaloids, phenolics, flavonoids, tannins, glycosides, steroids, terpenoids, saponins, anthraquinones, citrals, phenols, carotenoids, and volatile essentials without any indicator of anthocyanins, coumarins, or quinines, as shown in Table 3, In addition, the antioxidative potencies of CGC and GE using the 1,1-diphenyl-2-picrylhydrazyl assay (DPPH) disclosed a closed value of 76.30 ± 1.69% and 70.81 ± 2.47%, respectively, measured spectrophotometrically against a reference quercetin antioxidant.

The administration of trona at 200mg/kg/bodyweight into group 2 rodents as depicted in figure 1 after 35 days, stimulated a significant metabolic influence ($p > 0.05$) resulting in an infiltration of plasma OPN by 15fold when compared to group of animals on normal chow, signaling an inflammation on hepatic cells. However, co-treatment with CGC and GE in the other groups significantly ameliorated the OPN outrage in a dose-dependent manner, with the former at 150mg/kg/bodyweight showing no significant difference after co-treatment to the animals on normal chow but more prominent than the latter amidst the same doses of 100 (group 4) and 150mg/kg/bodyweight (group 6). It's of interest to also pronounce that group 3 at 100 mg/kg body weight of CGC established low significant difference in their pharmacological actions ($p < 0.05$) against OPN when compared to group 6 co-treated with 150mg/kg body weight of GE.

More so, the stringent assault triggered by trona on the activities of ALT by 2.6fold ($p > 0.05$), further collaborated the hepatic necrosis. This predictably promoted oxidative stress via an imbalanced redox reaction which coherently depleted the metabolic level of antioxidant glutathione by 2.7fold as seen in figure 3. Be as it may, CGC and GE adducts revealed a shred of significant dose-dependent evidence in the scavenging potential and recuperative role on GSH and ALT related lesion of co-treated groups when compared with others on normal chow. The result represented in Figure 3 further indicated the significant deleterious effect of TRN on non-enzymatic GSH antioxidant of group 2 ravaged with the trona when

compared to animals on normal chow. Withal, co-administration in the other groups with CGC and GE, ameliorated the activity of ALT and GSH depletion in a dose-dependent manner. It's worthy to note that CGC exhibited a more ameliorating tendency to near normal than GE over the same doses, while group 4 and 6 co-treated with 100 mg/kg body weight and 150mg/kg body weight of GE respectively revealed no significant difference ($p < 0.05$) amidst GSH level recuperating potential.

Histologically, sections of the liver tissue further revealed normal physiological hepatocytes, central vein (CV), portal vein (PV), and basophilic portion, with no pathologic changes in group 1 fed with normal chow as depicted in Figure 1 above (G1 refers), while assaulted group 2 (G 2) revealed a photomicrograph of the significant necrotic effect of TRN on parenchymal tissue of the hepatocyte signifying the intrinsic cirrhosis on the Kupffer cells and the entire architecture, leading to congestive hepatopathy, with group 3 co-administered with 100mg/kg body weight of CGC, though ranked runner-up after 150mg/kg body weight of CGC (group 5), however showed accomplished dose-dependent regeneration/efficacy on the fibrotic lesion instigated by the agonist. Plate 4 (G 4) further established the radical scavenging efficacy and recuperating potential of garlic extract at 100 mg/kg body weight on the hepatocyte histology, though not significant when compared to G3 treated with CGC of the same dose. Group 5 co-treated with 150 mg/kg body weight of CGC revealed the most significant dose-dependent efficacy of all, attenuating and reversing the deleterious and necrotic effect of TRN on the hepatic intracellulars and the central vein when compared with all other plates of interest. Conclusively, the liver micrograph established the intrinsic injuries, pro-inflammatory effect and severe alteration by TRN on the hepatic cytoarchitecture of assaulted animals.

Table 1: The result of the qualitative screening of CGC.

Alkaloids	+
Phenolics	+
Flavonoids	+
Tannins	+
Glycosides	+
Steroids	+
Terpenoids	+
Saponins	+
Anthraquinones	+
Citral	+
Coumarins	+
Phlobatanins	+
Carotenoids	+
Anthocyanins	+
Volatile essentials	+
Quinine	-

Keys: + Presence, - Absence

Table 2: Quantitative Composition of CGC

Composition	**Quantitative amount**
Alkaloid	8.30 ±0.04mg/100g^{A}
Tannin	5.10 ± 0.05mg/100g^{B}
Saponin	4.70 ± 0.03 mg/100g^{C}
Flavonoid	2.55 ±0.05 mg/100g^{D}
Phenols	1.12 ± 0.01 mg/100g^{E}
Anthraquinone	1.10 ± 0.02 mg/100g^{E}
Others	Negligible

Table 3: Qualitative screening of GE.

Alkaloids	+
Phenolics	+
Flavonoids	+
Tannins	+
Glycosides	+
Steroids	+
Terpenoids	+
Saponins	+
Anthraquinones	+
Citral	+
Phlobatanins	+
Carotenoids	+
Volatile essentials	+
Anthocyanins	-
Quinine	-
Coumarins	-

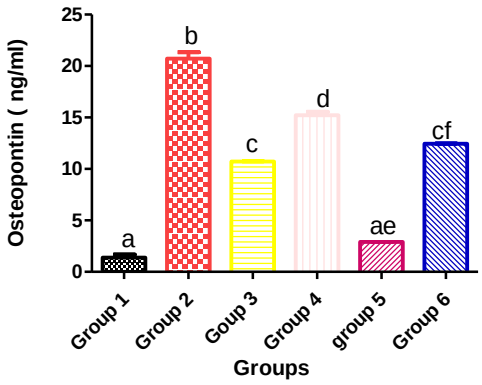

Figure 1: Effect of CGC and GE on plasma Osteopontin parameter (ng/ml) ($p<0.05$) of trona-assaulted rats.
KEYS:
Group1 - Control group administered normal chow.
Group2 - Group assaulted with 200mg/kg body weight of trona.
Group3 - Group assaulted and co-treated with 100 mg/kg body weight of CGC.
Group4 - Group assaulted and co-treated with 100 mg/kg body weight of GE.
Group5 - Group assaulted and co-treated with 150mg/kg body weight of CGC.
Group6 - Group assaulted and co-treated with 150mg/kg body weight of GE.

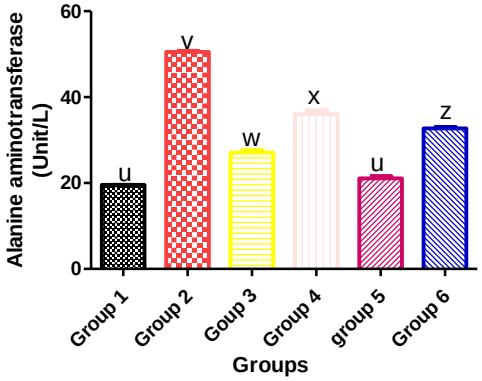

Figure 2: Effect of CGC and GE on plasma ALT parameter ($p<0.05$) of trona-assaulted rats.
KEYS:
Group1 - Control group administered normal chow.
Group2 - Group assaulted with 200mg/kg body weight of trona.
Group3 - Group assaulted and co-treated with 100 mg/kg body weight of CGC.
Group4 - Group assaulted and co-treated with 100 mg/kg body weight of GE.
Group5 - Group assaulted and co-treated with 150mg/kg body weight of CGC.
Group6 - Group assaulted and co-treated with 150mg/kg body weight of GE.

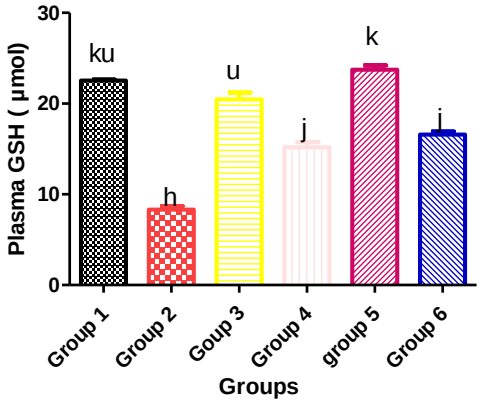

Figure 3: Effect of CGC and GE on plasma GSH parameter ($p<0.05$) of trona-assaulted rats.
KEYS:
Group1-Control group administered normal chow.
Group2 - Group assaulted with 200mg/kg body weight of trona.
Group3 - Group assaulted and co-treated with 100 mg/kg body weight of CGC.
Group4 - Group assaulted and co-treated with 100 mg/kg body weight of GE.
Group5 - Group assaulted and co-treated with 150mg/kg body weight of CGC.
Group6 - Group assaulted and co-treated with 150mg/kg body weight of GE.

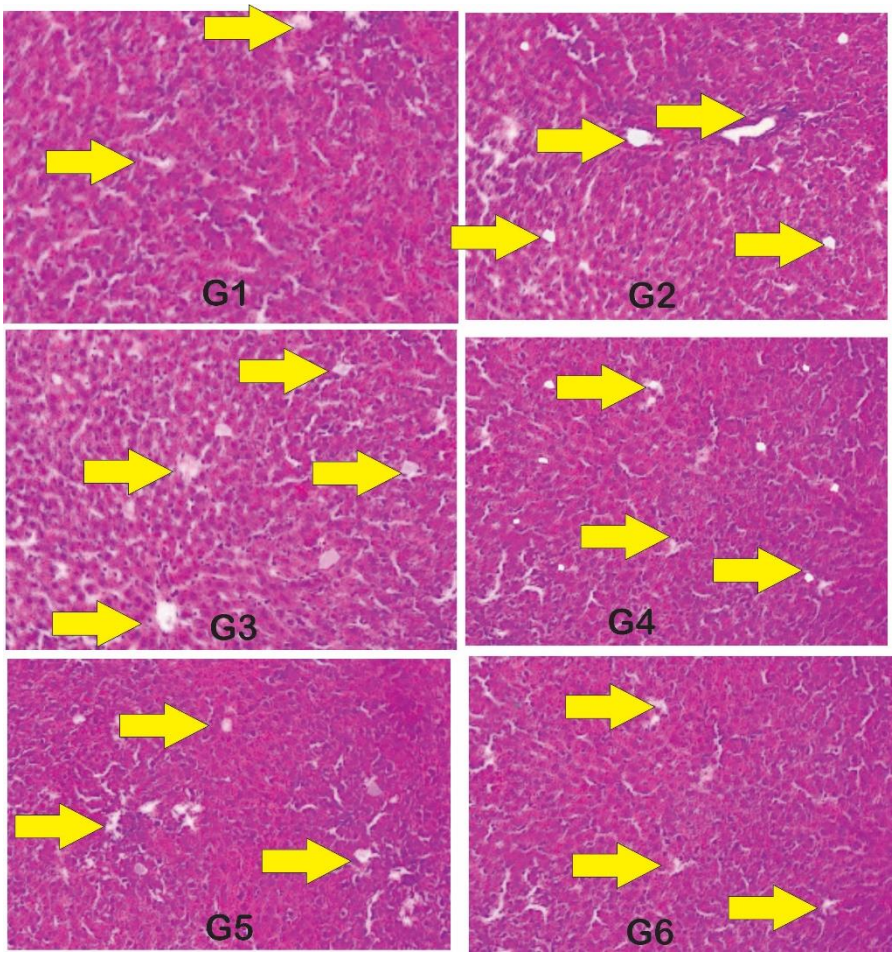

Figure 4: Sectional photomicrograph (X200 H&E) of rats' hepatocytes subjected to trona and co-treated. G1- Normal group. G2- Trona-assaulted group with intrinsic cirrhosis on the Kupffer cells (yellow arrow refers). G3- assaulted and treated with 100 mg/kg body weight of CGC. G4- -assaulted and treated with 100 mg/kg body weight of GE. G5- assaulted and treated with 150 mg/kg body weight of CGC. G6- assaulted and treated with 150 mg/kg body weight of GE. *Yellow arrows indicate intrinsic hepato-toxic effect of trona on the Kupffer cells and central vein.*

DISCUSSION

The investigation established the lethal dose (LD50) of CGC above 3000 mg/kg/bodyweight, but with depression, obtuseness (lack of quickness), glossy responses, and dull eyes recorded after 7–48 hours of the administration, following the protocol stated by Rispin et al. (2002); however, gradual appetite was regained thereafter. More so, the reductant class of compounds qualitatively detected in CGC with an extraction yield of 23.35% are alkaloids, phenolics, flavonoids, Tannins, Glycosides, Steroids, Terpenoids, Saponin, Volatile essentials, citral, coumarins, phlobatanins, anthocyanins, anthraquinones and Carotenoids with an absence of quinines. While the phytochemicals detected in CGC but not in GE are coumarins and anthocyanins, which could have potentiated the therapeutic synergism of the herbal extract over the single therapeutic action of GE. Further quantitative analysis of CGC (100 g) also revealed the following compositional order: alkaloid at 8.30 ±0.04 mg, tannin at 5.10 ± 0.05 mg, saponin at 4.700 ± 0.03 mg, flavonoid at 2.55 ±0.05 mg, phenols at 1.12 ± 0.01 mg and anthraquinone at 1.10 ± 0.02 mg.

It is also worthwhile to report that CGC exhibited more reductant ability than GE, precisely as evaluated and recorded by their radical scavenging abilities with reference to 1,1-diphenyl-2-picrylhydrazyl (DPPH+), connoting their intricacies to neutralize intracellular damaging free radicals. This implies that CGC could be used as fortified functional food supplements and therapeutic agents for the prevention and management of oxidant-related diseases such as endothelial radical cascades, cancer, and hepatic diseases. Moreover, the intricacy of the volatile metabolites mostly integrated by cloves (El-Saber Batiha et al., 2020) in CGC is also of important consideration, as accounted for by DeCarlo et al. (2019).

More importantly, all bio-molecules found in various plants that demonstrated invitro modulations on DPPH are what give them their divine therapeutic and antiradical worth. Phenolics, Terpenoids, and alkaloids, though present in the di-herbal extract are the main classes of plant intermediates also exhibiting unequivocal anti-radical efficacies and phagocytosis both for plants themselves and animals consuming them as foods, hence assisting in the prevention against reactive species, inflammation, hepatic infections, and hematological disturbances as accomplished.

Though, the significant necrosis on the entire histological architecture of the hepatocytes, up-regulation of plasma ALT which are collaborated by the report of (Imafidon et al., 2016) and (Ajiboye et al., 2015), with eventual decrease in the concentration of reduced GSH have been established in previous diseased states of various animal models. However, the result in this study was one of its kind to accomplish the interwoven disturbances of plasma OPN, a novel, and necrotic osteoblast proteinous cytokine with ALT and GSH in the serum.

Osteopontin has been recorded along with normal neuronal development and various bone-rebuilding activities. In addition, George et al. (2022 further reported OPN abnormal infiltration during pathological state in animal models, which is collaborative as it signaled the response of hepatic organ and hematological parameters to the assault in this study. The TRN intrinsic injury is coherently stimulated with osteopontin (OPN) integration via the protein kinase C-modulating cycle, hexosamine influences in rat aortic smooth vascular muscle, activation in the carotid arteries, and also mediated by bradykinin in the renal cortex, hence indicating the eventual increase in the proteinous hematological parameters as accounted for by Takemoto et al. (2000). This is evidence and perhaps represents the basic modalities or undertone by which the established significant increase of OPN could have been recorded in the group of rodents ravished with the agonist, though further consistent with the record of Shirakawa & Sano (2021). More so, Takemoto et al. (2000) further reported the direct interaction of the DNA sequence of a specific gene from smooth vascular muscle cells as it is transcribed and transported into a molecule called messenger RNA (mRNA), a template for the significant novel transcription to proteinous OPN within the integral intracellular components of the hepatic cells. Hence, the significant synthesis of OPN from the template in response to the intrinsic injury by the agonist could have been dose-dependently mitigated (Yue et al., 1994) in the co-treated animals to a near-normal level. The dose-dependent and significant amelioration accomplished in the groups co-treated with CGC at 100 and 150 mg/kg b/dw, though with more pharmarcological action than GE at the same doses, could have been triggered by the phytochemicals detected in the herbal mixture and eventually regenerated the haem-based parameters, deactivating the continual release of OPN. As it is

also accomplished, group 3 at 100 mg /kg body weight of CGC established a low significant difference in their pharmacological actions ($p < 0.05$) against OPN when compared to group 6 co-treated with 150 mg/kg body weight of GE. The inference thus denotes that the synergetic efficacy of plants at lower doses is more beneficial than a single application of a plant at a higher dosage.

Hepatocyte regeneration is very crucial for salubrious health after intrinsic injury, in which the interwoven mediation of exogenous or endogenous antioxidants is very important. On the other hand, ALT activity increases in the plasma during the acute trona-induced hepatic fibrogenesis in the assaulted group, suggesting notable degradation in the histologically revealed Kupffer cells of the hepatocytes (Chiba et al., 2002). Thus, trona at the tested doses was hereby established to play a distinct role as a potential hepatic toxicant for those using it as a tenderizer at home. This intrinsic assault connotes the unfavorable synthesis of mRNA-coding ALT from the traumatized hepatocyte and hence facilitates its perpetual and significant synthesis and trafficking via the intracellular ribosomes from the nucleus to the endogenous tissues and the bloodline, as reported by Ndrepepa (2021). Nevertheless, the phytochemical metabolites detected in the di-herbal CGC, as depicted in Table 1, may have predominantly subjugated the trona-induced perpetual synthesis of ALT from the radicalized hepatic cells, as also predicted by Garba et al., 2022) and CGC thereby acting as toxicant chelator/scavenging intermediates in the hepatic nomenclature and its eventual rejuvenation. The evidence thus established the synergetic efficacy of CGC as a dependable therapeutic for the management of trona-induced hepatotoxicity over GE. This is perhaps subjugated by the intent potency of phytochemicals, Eugenol, and other aromatic oils reported by El-Saber Batiha et al. (2020). Though the degree of intrinsic injury to hepatic integrity triggered by trona and compromising the Kupffer cells and its related GSH biomarker (Chiba et al., 2002) was established, the historical usage of trona as a tenderizer continues to date.

Considerably, a recent study of TRN by Imafidon et al. (2016) revealed the presence of sodium, iron, magnesium, calcium, zinc, and lead, which were reported to be injurious to health, in rodent hepatocytes and intraglomerular mesangial cells, the main initiators of inflammations embedded turbulent interleukins and other cytokines (Iwano et al., 1992). Hence, toxicological assessment of rats in group 2 administered 200 mg/kg body weight of TRN triggered the significant denaturing of the hepatocytes, as revealed in the histology, due to the culpable metabolic etiologies of possible heavy metals in the agonist. However, CGC, which is affirmed to contain multiple compounds, exhibited their potential synergistically by possibly mopping up the effect of the heavy metals (Kuok et al., 2017), hence dose-dependently recuperating the hepatic cells and boosting the GSH concentration in the co-treated groups to near normal.

Moreover, the liver is potentially susceptible to intrinsic injury, as it receives its blood supply (approximately 80%) from hepatic portal veins. The hepatocytes thus eventually received the trona agonist, hence resulting into severe alteration and vascular congestion (Mobisson Phd et al., 2023) in the cytoarchitecture of assaulted groups' hepatocytes, as supported by (Imafidon et al., 2016), (Evbu et al., 2016), and (Ajiboye et al., 2015). Kupffer cells seem to be the target in the liver toxicants, which in turn induced the expression of ALT and Interleukins 6 (IL-6) (Moris et al., 2016). Withal, the fibrotic lesion was

also dose-dependently attenuated by CGC in the co-treated groups as revealed in the histology.

Clove, which is known to fast-track the homeostatic flow of nutrients throughout the body by increasing blood circulation and mopping up plagues along tunica intima at the recommended dose, was inherently mediated by the presence of detected alkaloids, flavonoids, saponins, tannins, anthraquinones, and flavonoids as nature's biological modifiers in hepatocytes, as reported by Giachelli et al. (1993). The essential oil of Syzyginum aromaticum (Clove) made up of 75–85% eugenol, 15% eugenyl acetate and 51.2% β-caryophylline, with others viz-a-viz Eugenol (Elbestawy et al., 2023), β-caryophyllene, α.-humulene, CIS-calamenene, syringic acid, caffeic acid and protocatechuic acid (Jimoh et al., 2017), could also have synergistically rejuvenated the hepatic nomenclature and dose-dependently recuperated the hepatocytes architecture (Garba et al., 2022), in conjunction with allicin (Siddique et al., 2015), 5-hydroxymethylfurfural, organosulfur, Ajoene, allyl polysulfides, vinyldithiins, S-allylcysteine polyphenol, and some volatile essential compounds (Ahmed & Wang, 2021) from garlic. This is very well possible, as the liver has phenomenal capacity for repair and regeneration after injury (Clemens et al., 2019).

All these compounds have been established to remediate various metabolic diseases, including intracellular disorders, pathogenic infiltrations, skin infections, wounds, and symptoms of toxicity (Arreola et al., 2015). Compromised liver architecture brings about variation in the expression of bio-indicators, which to a certain extent can infer the extent of hepato-histological damage or as clinical diagnoses of hepatocellular injury. More emphatically, valuable information accomplished in cognizance of the integrity of CGC over GE, however, supported the traditional use of decocted concoction, as it mitigated the hepatocytes hypersensitivity and remediated its haem-based related biomarkers (OPN and ALT), which ought to signal the hepatic damage and necrotic dilemma (Garba et al., 2022) induced by trona.

CONCLUSION

The results showed that CGC contains synergetic secondary metabolites which could be used as prophylaxis and therapeutic agent in pharmaceutical and functional food industries, to remediate trona induced OPN related intrinsic morbidities, which is also consistent with the in-vitro antioxidative potencies accounted for CGC over GE, using 1,1-diphenyl-2-picrylhydrazyl assay (DPPH) in the study. It is of interest to also note that the result further revealed accomplished in-vivo therapeutic dominance of CGC over GE and moreover, it is the first of its kind to establish the interwoven association of plasma OPN levels (a novel marker), GSH and ALT in trona-assaulted Wistar rats. While further research is needed to investigate the mode of actions responsible for the aforementioned potentials in the combined galenicals.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this manuscript.

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AUTHOR CONTRIBUTION

All authors were really informed about the final manuscript before submission. Specifically, OSO and TIF provided the concept and design, AAA and ACA supervised the desk analysis, GE and KOO engaged in data acquisition, data analysis, experimental studies and manuscript review; ATO authenticated the galenicals and helped in sourcing and drying; JPA cared for and sacrificed the experimental rodents and assayed all biomarkers of interest; ABF did the phytochemical analysis of the CGC and GE; and TIF proceeded with the literature search, data validation, analysis and manuscript preparation/submission.

ETHICAL PROTOCOL

The experimental protocol was approved by the Institutional Animal Care Committee of Nigeria College of Natural Medicine Technology (NICONMTECH) (accredited), Federal Ministry of Innovation, Science, and Technology; co-supervised by the Department of Biochemistry, Faculty of Basic Medical Sience, Ladoke Akintola University of Technology, Ogbomosho, Oyo state, and guided by the laydown rules and regulations of the National Institute of Health (NIH), (1985) and Institute of Laboratory Animal Resources, National Institutes of Health, USA, 1996.

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