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Original Research Article

Effects of Caffeine-Artemisinin Combination on Liver Function and Oxidative Stress in Selected Organs in 7,12-Dimethylbenzanthracene-Treated Rats

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ABSTRACT

Oxidative stress plays significant role in inflammation and diseases like cancer, chemo-preventive compounds that have protective properties such as antioxidants, may mitigate disease process. The study investigated the effects of caffeine and artemisinin on 7,12-dimethylbenzanthracene (DMBA)-induced injury in liver, kidney and lung of Wistar rats. Animals were administered DMBA only (negative control), no treatment (normal control), 25 mg/kg caffeine (Caff), 4mg/kg artemisinin (Art), 25 mg caffeine + 4 mg/kg artemisinin (Caff+Art1), or 50 mg caffeine + 8 mg/kg b.w artemisinin (Caff+Art2) for 2weeks. Liver function tests were carried out, and oxidative stress markers assessed in liver, kidney and lungs. There was overall liver protection and antioxidant effects in groups administered Caff and Caff+Art1 compared to Art and negative control ($p<0.05$), while high dose Caff+Art2 significantly increased MDA, GSH, compared to normal control. There was a significant decrease in aspartate aminotransferase, alanine aminotransferase, total protein and albumin in groups administered with Caff, Caff+Art1 compared to negative control ($p<0.05$) while direct bilirubin, creatinine and ALP levels were similar in both treatment groups. The findings suggest that there is higher antioxidant effects with low dose caffeine alone or in combination with low dose artemisinin against damages induced by DMBA, hence caffeine plus artemisinin may offer prevention against diseases relating to oxidative damage.

Keywords: Antioxidant, Oxidative stress, Caffeine, Artemisinin, Liver function**.**

Introduction

Oxidative stress is implicated in several diseases including cancer where it triggers epigenetic or genetic changes that promotes tumour development.[1,2] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play significant roles in damages to the DNA which results in cancer and other diseases due to imbalanced oxidant levels which impact on cell growth, survival, development, and tumorigenesis. Oxidants are also implicated in inflammation and lipid peroxidation, [1,2] and major organs affected are crucial for maintaining the body's homeostasis and overall health. With the increasing incidence of cancers,[3] non-communicable diseases and associated deaths globally,[4,5] strategies to prevent unwarranted deaths are important. Studies that evaluate the efficiency of natural bioactive compounds for their potentials to prevent oxidative damage and improve bodily functions are very crucial. Antioxidants including glutathione, superoxide dismutase (SOD), transferases, and catalase (CAT) enzymes function as defense mechanism in humans by preventing formation of free radicals, to inhibit redox reactions or repairing tissue damage by free radicals.[1,2,6] Naturally occurring compounds, especially those commonly consumed in meals or beverages, have potent antioxidative effects and can reduce the risk of development of cancers in persons who at higher risk.

Several studies have reviewed the antioxidant effects of plants including caffeine and their beneficial effects in human diseases and health conditions.[7-11] Caffeine is a plant derived alkaloid, that is found in beverages such as coffee, and cocoa drink; which has been reported to have antioxidant effects. It also shows *in vivo* anticancer properties, enhanced cytotoxic effects with anticancer drugs, by abolishing DNA damage checkpoint.[12] Artemisinin is a bioactive compound also contained in beverages and is thought to have many health benefits due to its anticancer, antimalarial, antimicrobial, antioxidant, and immune benefits.[13-16] Formation of oxidative species in the body involves several endogenous and exogenous mechanisms, hence compounds that inhibit the formation of these species are of great importance. The study evaluated the protective effects of caffeine and artemisinin on oxidative stress markers: malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) in liver, kidney and lungs and on activities of Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), total protein, albumin, direct bilirubin, and creatinine after DMBA treatment of rats.

Materials and Methods

Thirty healthy Wistar rats, 4-6 weeks old (100 g to 180 g) were purchased from Federal University of Agriculture, Abeokuta, Nigeria. The experimental procedures followed standard guidelines for use of experimental animals and was approved by Covenant University Health Research Ethics Committee (CHREC/46/2020). The animals were housed in separate cages, fed with animal feed and water *ad libitum.* They were allowed to acclimatize for two weeks, weighed and grouped randomly in into 6 groups of five animals each.

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Drug preparation and treatment

Pure caffeine and artemisinin were purchased from Sigma Aldrich, England. Stock solution of artemisinin and caffeine were prepared by dissolving 1 mg in 1 mL distilled water, while working solutions were prepared on a daily basis based on the weight of each rat. Animals were administered oral doses, with first dose of 40 mg/kg DMBA in olive oil and a second dose of 20 mg/kg at 4 weeks; a modified method of Lai and Singh.[17] Animals were dosed once daily for 2 weeks with 25 mg/kg caffeine (Caff), 4 mg/kg artemisinin (Art), 25 mg Caff + 4 mg/kg art (Caff+Art1) or 50mg Caff + 8mg/kg art (Caff+Art2). Normal group received no treatment all through. After completion of treatment, animals were sacrificed using mild euthanasia with diethyl ether, thereafter blood was obtained by cardiac puncture. Liver, kidney and lung tissues were obtained and stored for analysis as well as whole blood in EDTA bottles, 0.2 g tissue was homogenized in 1.8 mL of homogenizing buffer and centrifuged at 5000 rpm for 10 mins. The supernatant was stored at -20°C freezer until it was ready to be used for further assays.

Antioxidant assays

Thiobarbituric acid-reactive substances (TBARS): Malondialdehyde (MDA) a marker of lipid peroxidation was determined using the protocol reported by Ohkawa *et al.*[18] Briefly, 0.5 mL of supernatant was added to 1 mL of tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent (thibarbituric acid 0.37%, 0.24 N HCl and 15% TCA), was heated at 100°C for 30 minutes and allowed to cool, centrifuged at 2000 rpm for 15mins, and clear supernatant was collected. Blank was prepared from 1.14 ml concentrated HCl added to 50 ml distilled water. Absorbance was read at 532 nm against blank, MDA was calculated using the molar extinction coefficient for MDA-TBA complex of $1.56 \times 10^5 M^{-1}CM^{-1}$.

Superoxide Dismutase (SOD): was determined according to the method described by Misra and Fridovich.[19] To 50µl of the homogenate, 1 mL each of 75 mM Tris-HCL buffer (pH 8.2), 30 mM EDTA and 2 mM pyrogallol was added. Change in absorbance at 420nm was recorded for 3 minutes.

Reduced Glutathione (GSH): GSH was estimated according to Sedlak and Lindsay.[20] Briefly, 300ul of 10% TCA was added to tissue homogenate, the mixture was centrifuged at 5000 rpm for 10 minutes 500ul supernatant was collected. Supernatant was treated with 250 µL of Ellman's reagent (19.8 mg of DTNB in 100 mL of 0.1% sodium nitrate) and 1500 µL of phosphate buffer (0.2 M, pH 8.0) and absorbance was read at 412 nm.

Liver function tests

Alanine Aminotransferase (ALT)[21,22], *Aspartate Aminotransferase (AST)*[21,22], *Alkaline Phosphatase (ALP)*[23] tests were done using previously described methods with specific Randox® test kit and following the manufacturer's guide.

Bilirubin (BIL): Direct (conjugated) bilirubin forms blue coloured complex when reacted with diazotized sulphanilic acid in alkaline medium. Total bilirubin was measured according to the colorimetric method described previously.[24] Absorbance was read at 546nm using distilled water as blank.

Total protein (TP): This assay is based on detection of cupric ions reaction with the peptide bonds of proteins and polypeptides in an alkaline solution to produce a purple coloured complex. Absorbance was read at 546 nm and it is directly proportional to the concentration of protein in the sample.

Albumin: At pH4.2, albumin bind with bromocresol green to produce a blue-green complex. The change in absorbance at 628 nm correlates with the concentration of albumin. Absorbance was read at 628nm after mixing and incubating for five minutes at room temperature.

Creatinine: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration. Absorbance was read at 492nm after thirty seconds and two minutes respectively.[25]

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM), and analysed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test. Statistical analysis was done using the (SPSS) Statistical Package for Social Sciences (Version 20.0, IBM, NY, USA), p-value <0.05 was considered statistically significant.

Results and Discussion

Antioxidant profiles

Table 1 shows the MDA (TBARS) activities in the liver, kidney and lungs. In the liver, DMBA significantly increased MDA levels compared to normal control while treatment with Caff, Art and Caff +Art1 reversed this to levels comparable to that of control, but not with Art and Caff+Art2. Liver mean MDA values in normal, Caff, Art and Caff+Art1 treated groups were 4.25 ± 0.09, 5.13 ± 0.36, 4.84 ± 0.33 and 4.42 ± 0.09 µM, respectively, compared to 7.01 ± 0.25, and 7.46 ± 0.4 µM, respectively ($p<0.05$) in Caff+Art2 and DMBA negative control groups. In the kidney, mean MDA value in normal, Caff, Art and Caff+Art1 treated groups were 1.16 ± 0.21, 1.51 ± 0.13, 1.73 ± 0.12, and 1.3 ± 0.12 µM, respectively, compared to 2.79 ± 0.23, and 3.32 ± 0.3 µM, respectively ($p<0.05$) in Caff+Art2 and DMBA negative control groups. Similarly, in the lungs, DMBA significantly increased MDA levels, but Caff and Caff+Art1 significantly reduced this compared to Art and Caff+Art2. In all 3 organs, MDA level was higher in liver compared to kidney and lungs. Table 2 shows SOD activities in the liver, kidney and lungs. In the liver, DMBA significantly decreased SOD level compared to normal control while treatment with Caff and Caff +Art1 reversed this to levels comparable to that of control, but not Art and Caff+Art2. The corresponding mean values are 10.86 ± 0.3, 9.8 ± 0.38 and 10.67 ± 0.48 µM, in normal, Caff and Caff +Art1 compared to 4.68 ± 0.52, 3.49 ± 0.52 and 3.84 ± 0.53 µM in Art, Caff+Art2 and DMBA negative control groups. This follows the same pattern as MDA activity. SOD activity was relatively similar in all 3 organs except for groups treated with artemisinin alone. Table 3 shows GSH activities in the liver, kidney and lungs. DMBA significantly reduced GSH level when compared to normal control while treatment with Caff, Caff +Art1 and Art reversed this decrease to levels comparable to that of control, but not in the kidney. GSH activity was relatively similar in liver and lungs except in kidney. Table 4 shows the catalase activities in the liver, kidney and lungs. DMBA significantly reduced catalase level when compared to normal control while treatment with Caff, Caff +Art1 reversed this decrease to levels comparable to that of control. GSH activity was relatively similar in liver, lung and kidney.

Increasing burdens of non-communicable diseases are reported globally, particularly in the past decade, and are attributable to life-style changes, alcohol, oxidative stress and industrialization.[26] Over 41 million deaths occur annually due to NCDs accounting for 71% of global deaths and more than 40% of these deaths are premature and preventable.[4,5] Oxidative stress is an underlying cause of several diseases including cancers, atherosclerosis, diabetes, cardiovascular diseases etc, in the presence of other risk factors.[27-29] We evaluated biomarkers of oxidative stress including malondialdehyde, reduced glutathione, superoxide dismutase, and liver function enzymes; alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, albumin, total protein, creatinine and bilirubin after DMBA exposed animals were treated with caffeine and/or artemisinin. The justification for combining these two naturally occurring compounds was to evaluate synergy or potentiation of the effects of the individual compounds; as both have been reported to have potential antioxidant effects and other health benefits when used alone or combined with other drugs.[6-7,9,11,13,16,30-32] Consistently in the liver and lungs, remarkable antioxidant effects and protection from liver damage by caffeine were observed showing wide range modulation of the biochemical markers. Overall, better organ protection was observed with the 25mg dose of caffeine and its combination with 4mg/kg artemisinin, compared to DMBA-treated animals. Although, the difference between the antioxidant effects of 25mg caffeine and its combination with 4mg/kg artemisinin was not significant, we observed marked opposite effects in groups treated with artemisinin alone or higher dose of caffeine plus artemisinin, with similar patterns on liver function enzymes. Caffeine has been shown in previous studies where doses of 25-40 mg were used to have potent antioxidant

Table 1: Effects of caffeine and artemisinin on malondialdehyde activity levels

Treatment	Concentration (mg/kg b.w)	Liver (µM)	Kidney (µM)	Lungs (µM)
DMBA (-ve ctrl)	40mg/kg + 20mg/kg	7.01 ± 0.25^{b}	3.32 ± 0.30^{b}	4.26 ± 0.12^{b}
Normal	-	4.25 ± 0.09^{a}	1.16 ± 0.21^{a}	2.11 ± 0.54^{a}
Caffeine (Caff.)	25mg/kg	5.13 ± 0.36^{a}	1.51 ± 0.13^{a}	2.61 ± 0.21^{a}
Artemisinin (Art.)	4mg/kg	4.84 ± 0.33^{ab}	1.73 ± 0.12^{a}	2.65 ± 0.12^{b}
Caff + Art1	25mg/kg + 4mg/kg	4.42 ± 0.09^{a}	1.3 ± 0.12^{a}	2.3 ± 0.24^{a}
Caff + Art2	50mg/kg + 8mg/kg	7.46 ± 0.40^{b}	2.79 ± 0.23^{b}	4.01 ± 0.15^{b}

Values are expressed as mean ± SEM, different superscripts are significantly different at $p < 0.05$

Table 2: Effects of caffeine and artemisinin on superoxide dismutase activity levels

Treatment	Concentration (mg/kg b.w)	Liver (µM)	Kidney (µM)	Lungs (µM)
DMBA (-ve ctrl)	40mg/kg + 20mg/kg	3.84 ± 0.53^{b}	3.37 ± 0.31^{b}	4.26 ± 0.19^{b}
Normal	-	10.86 ± 0.34^{a}	9.91 ± 0.24^{a}	10.57 ± 0.20^{a}
Caffeine (Caff.)	25mg/kg	9.8 ± 0.38^{a}	8.75 ± 0.31^{a}	9.58 ± 0.40^{a}
Artemisinin (Art.)	4mg/kg	4.68 ± 0.52^{b}	4.09 ± 0.36^{b}	4.66 ± 0.40^{b}
Caff + Art1	25mg/kg + 4mg/kg	10.67 ± 0.48^{a}	9.04 ± 0.4^{a}	10.91 ± 0.47^{a}
Caff + Art2	50mg/kg+8mg/kg	3.49 ± 0.52^{b}	3.78 ± 0.11^{b}	4.87 ± 0.44^{b}

Values are expressed as mean ± SEM, different superscripts are significantly different at $p < 0.05$

Table 3: Effects of caffeine and artemisinin on glutathione activity levels

Treatment	Concentration (mg/kg b.w)	Liver (µM)	Kidney (µM)	Lungs (µM)
DMBA (-ve ctrl)	40mg/kg+ 20mg/kg	17.93 ± 0.92^{b}	1.367 ± 0.03^{b}	13.68 ± 0.08^{b}
Normal	-	9.916 ± 0.75^{a}	1.047 ± 0.02^{a}	8.37 ± 0.77^{a}
Caffeine (Caff.)	25mg/kg	11.17 ± 0.66^{a}	1.131 ± 0.04^{a}	8.945 ± 0.60^{a}
Artemisinin (Art.)	4mg/kg	13.38 ± 2.03^{ab}	1.140 ± 0.04^{a}	9.651 ± 0.27^{a}
Caff + Art1	25mg/kg+4mg/kg	10.996 ± 0.26^{a}	1.131 ± 0.01^{a}	10.29 ± 0.26^{a}
Caff + Art2	50mg/kg+8mg/kg	16.69 ± 1.84^{b}	1.288 ± 0.02^{b}	15.61 ± 0.40^{b}

Values are expressed as mean ± SEM, different superscripts are significantly different at $p < 0.05$

Table 4: Effects of caffeine and artemisinin on catalase activity levels

Treatment	Concentration (mg/kg b.w)	Liver (µM)	Kidney (µM)	Lungs (µM)
DMBA (-ve ctrl)	40mg/kg + 20mg/kg	3.84 ± 0.53^{b}	8.73 ± 0.77^{b}	9.91 ± 0.74^{b}
Normal	-	10.86 ± 0.34^{a}	13.68 ± 0.84^{a}	17.93 ± 0.9^{a}
Caffeine (Caff.)	25mg/kg	9.80 ± 0.38^{a}	11.77 ± 1.02^{a}	15.37 ± 0.66^{a}
Artemisinin (Art.)	4mg/kg	4.68 ± 0.52^{ab}	8.94 ± 0.60^{a}	11.171 ± 0.66^{a}
Caff + Art1	25mg/kg + 4mg/kg	10.67 ± 0.48^{a}	15.61 ± 0.40^{a}	16.699 ± 1.85^{a}
Caff + Art2	50mg/kg + 8mg/kg	3.49 ± 0.52^{b}	10.29 ± 0.26^{b}	10.99 ± 0.26^{b}

Values are expressed as mean ± SEM, different superscripts are significantly different at $p < 0.05$

properties and the findings corroborate these reports.[6-7,9,11,30] Reactive oxygen species (ROS) induces oxidative stress which causes damages to cells; this is implicated in DMBA metabolism, via cytochrome P450 liver system forming epoxides and other toxic reactive species, that can lead to cell death. Reactive oxygen species bind irreversibly to DNA, thus forming DNA adducts that can initiate carcinogenesis.[33] ROS induced autophagy is implicated in cancers, ischemic damages in cardiovascular,[12,34-35] as well causing oxidative stress in diseases like diabetes and other diseases;[36] hence these combinations may serve to prevent different disease mechanisms. DMBA is a known carcinogen,[10,17,32] in the groups treated with Caffeine and/or artemisinin combinations, no tumor developed. Anti-oxidative enzymes – SOD and catalase activities were lowered in DMBA group, this was similar to other studies in humans and animal models.[7,11,30,37] The ability of caffeine to significantly impact levels of these antioxidant biomarkers suggests its antioxidative mechanisms includes activation of the redox-sensitive pathway with responses like radical scavenging by SOD and catalase, that functions to dismutate superoxide radicals to hydrogen peroxide (H_2O_2) and further convert to water and oxygen (O_2). Up-regulation of detoxification via glutathione-dependent pathways and other antioxidant enzymes are also important as they serve as first-line antioxidant defense against ROS; these were enhanced by low dose caffeine alone or its combination but not low dose artemisinin alone. Studies have reported

free-radical scavenging effects of artemisinin on 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH)[13] as well as reducing nitric oxide (NO), prostaglandin E_2 (PGE_2), and proinflammatory cytokine (IL-1β, IL-6, and IL-10) production.[13] The study indicates artemisinin is active via a different antioxidant pathway since it counters the antioxidant effects of caffeine; shown by higher oxidative stress markers. Artemisinin generates ROS and increases cellular oxidative stress as a mechanism of its effects on malaria parasites,[38] or alkylating carbon-centered radicals in cancer.[14] The consistently negative effects of high dose caffeine (50mg/kg) plus high dose artemisinin (8mg/kg) in Caff+Art2 suggests two possible mechanisms: (1) a masking effect by caffeine, of artemisinin's poor protective activity; which becomes apparent at a higher dose of both drugs or (2) both drugs induced further oxidative stress in the animals. The latter is not surprising and may be the likely cause of the adverse effects seen consistently in Caff+Art2 group. Artemisinin's mechanism of antimalarial/anticancer effects is via generation of free radicals and reactive carbon species;[14,38] and at higher dose (100-150mg/kg), caffeine has been shown to increase lipid peroxidation and oxidative damage in brain of animals.[39-40] These together with toxic species generated by DMBA will have significant damage to the liver and other organs as shown with the results obtained with Caff+Art2 results in our study.

Liver function tests

Figures 1-6 shows summary of biochemical markers of liver function, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin levels in the liver of animals administered Caff and Caff+Art1 was similar to normal control, but their levels were significantly lower compared to negative control. Albumin level in animals treated with Art was also similar to normal control. However, ALT, AST and TP levels were significantly higher in Art and Caff+Art2 which was similar to negative control.

There was no significant difference in ALP, Creatinine (CRT) and Direct bilirubin (DBIL) compared to controls in all treatment groups.

Caffeine alone significantly reduced levels of AST and ALT compared to DMBA group and Art. This is similar to another study.[9] Artemisinin alone (at 4mg/kg) or combined with caffeine at 8mg/kg showed poor protective effects on liver enzymes and proteins depletion induced by DMBA. Significantly higher biochemical enzyme activities – AST, ALP and ALT, were observed in Art unlike Caff or Caff+Art1; previous studies have shown reduced enzyme activities with caffeine,[9] thus implicating artemisinin in elevating the biochemical enzymes in Caff+Art2 group.

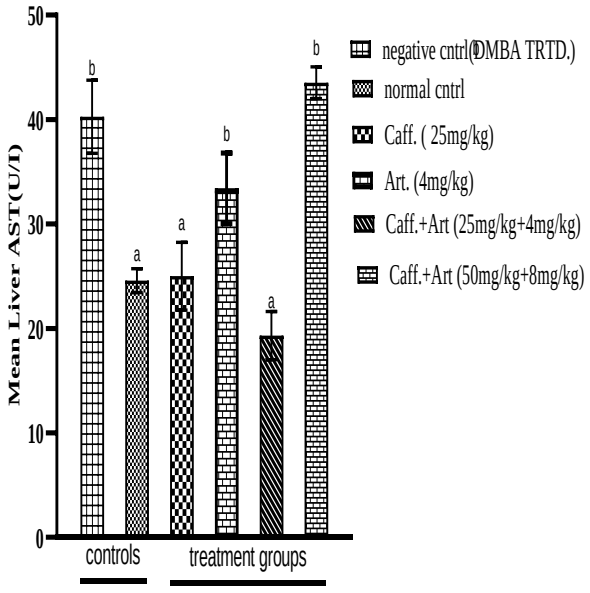

Figure 1: Effects of Caffeine and artemisinin on Aspartate aminotransferase (AST) levels in liver

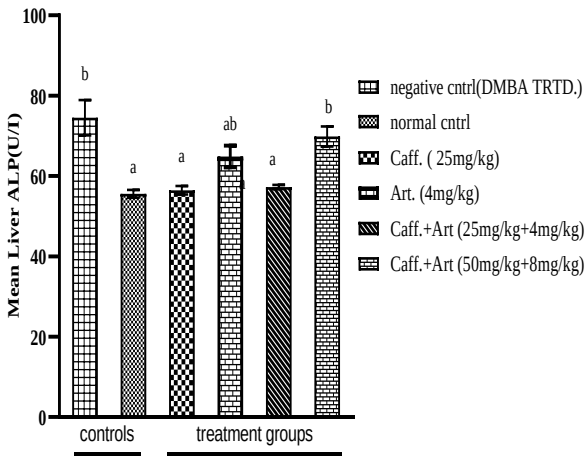

Figure 2: Effects of Caffeine and artemisinin on Alkaline phosphatase (ALP) levels in liver.

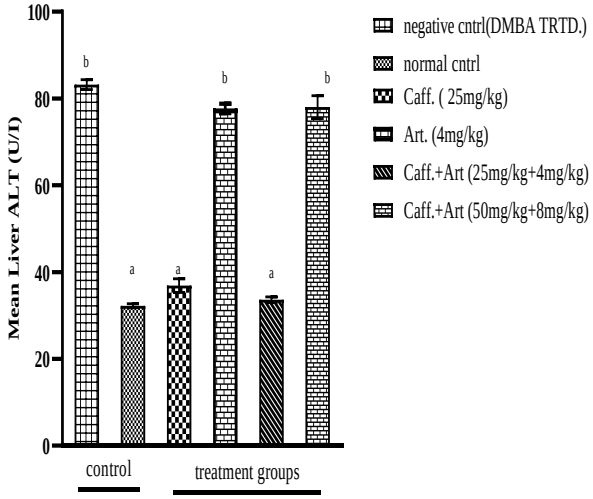

Figure 3: Effects of Caffeine and artemisinin on Alanine aminotransferase (ALT) levels in liver

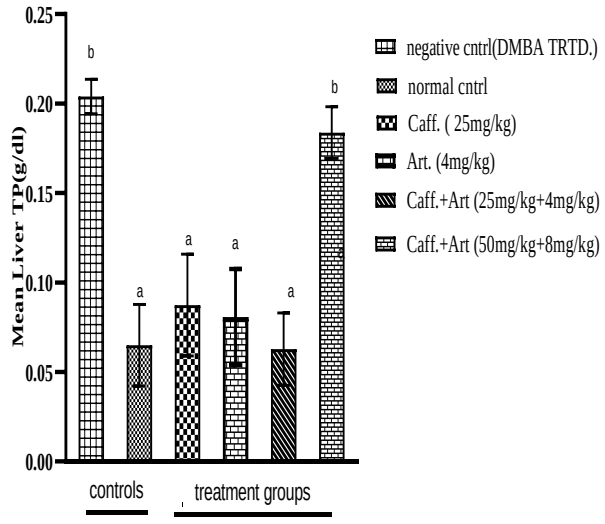

Figure 4: Effects of Caffeine and artemisinin on Total Protein activity levels in liver

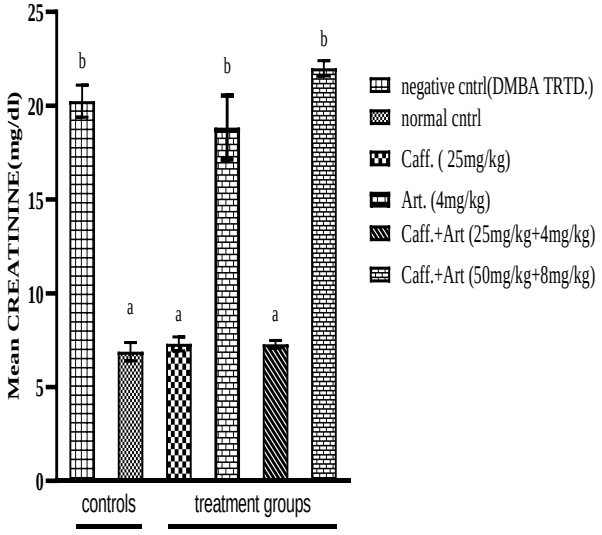

Figure 5: Effects of Caffeine and artemisinin on Creatinine levels in kidney

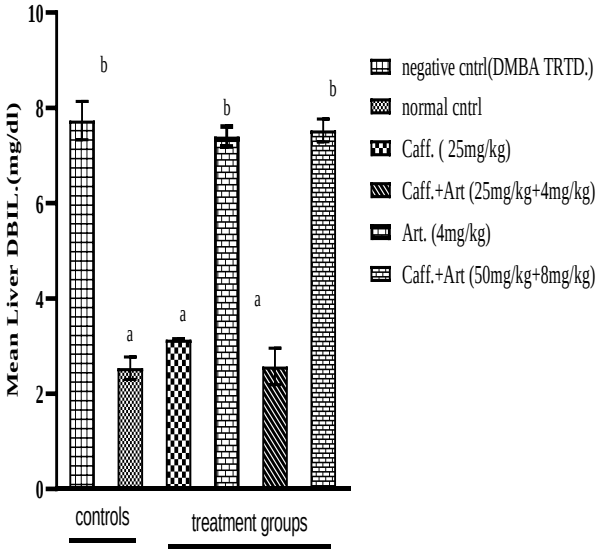

Figure 6: Effects of Caffeine and artemisinin on direct bilirubin (DBIL) levels in liver

Artemisinin has been previously reported to induce oxidative stress,[41,42] immunomodulation and neurotoxicity,[43-46] or moderate elevations of liver transaminases,[47,48] but no significant effect in serum/plasma ALT, AST, ALP were reported in other studies.[49]
This finding agrees with a report of moderate ALP levels after artemether treatment in CCl_4-liver damage in rats.[48] Total protein, direct bilirubin and albumin levels were adversely increased by DMBA treatment, indicating protein depletion with high dose of the combination. Artemether has been previously reported to deplete total protein and albumin in experimental animal after repeated doses,[49] but this was reversed by Caff, Caff+Art1 but not Art or Caff+Art2. This further emphasizes the unfavourable effects of combined or high dose Caff+Art2 as seen with most biochemical markers assessed in liver, lungs and kidney in this study. In another study, sub-chronic administration of 8mg/kg artemisinin showed no deleterious effects on serum albumin, total protein, creatinine and urea but reported significant increase in ALP, total cholesterol, triglycerides, total bilirubin and glucose levels.[50]

Conclusion

Combination of artemisinin with caffeine showed antioxidant and liver protective properties however caution is required to determine safe dose that has beneficial antioxidant effects since both caffeine and artemisinin are regularly consumed in form of tea and beverages, it is noteworthy that at higher dose, may be deleterious to liver, lungs and kidney. The study shows the potential benefits of a daily regular intake in the experimental animals clearly demonstrates their lone or combined low dose beneficial effects with no adverse effects.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state Nutri J. 2016; 15:71.
2. Murata M, Thanan R, Ma N, Kawanishi S. Role of nitrative and oxidative damage in inflammation-related carcinogenesis. J Biomed Biotech. 2012; 623019.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68:394-424.
4. World Health Organisation. Non-communicable diseases country profile 2018. World Health Organisation. Geneva, Switzerland, 2018, ISBN 9789241514620.
5. GDB Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388(10053):1659-1724.
6. Mut-Salud N, Álvarez PJ, Garrido JM, Carrasco E, Aránega A, Rodríguez-Serrano F. Antioxidant Intake and Antitumor Therapy: Toward Nutritional Recommendations for Optimal Results. Oxid Med Cell Longev. 2016; Article ID 6719534.
7. Paşaoğlu H, Ofluoğlu Demir FE, Yilmaz Demirtaş C, Hussein A, Paşaoğlu OT. The effect of caffeine on oxidative stress in liver and heart tissues of rats. Turk J Med Sci. 2011; 41:665-671.
8. Wang G, Bhoopalan V, Wang D, Wang L, Xu X. The effect of caffeine on cisplatin-induced apoptosis of lung cancer cells. Exp Hematol Oncol. 2015; 4:5.
9. Amer MG, Mazen NF, Mohamed AM. Caffeine intake decreases oxidative stress and inflammatory biomarkers in experimental liver diseases induced by thioacetamide: Biochemical and histological study. Int J Immunopath Pharmacol. 2017; 30:13-24.
10. Liu H, Zhou Y, Tang L. Caffeine induces sustained apoptosis of human gastric cancer cells by activating the

caspase-9/caspase-3 signalling pathway. Mol Med Rep. 2017; 16:2445-2454.
11. Baldissera MD, Souza CF, Descovi SN, Petrolli TG, da Silva AS, Baldisserotto B. A caffeine-supplemented diet modulates oxidative stress markers and prevents oxidative damage in the livers of Nile tilapia (Oreochromis niloticus) exposed to hypoxia. Fish Physiol Biochem. 2019; 45:1041-1049.
12. Zhou B-BS, Chaturvedi P, Spring K, Scott SP, Johanson RA, Mishra R, Mattern MR, Winkler JD, Khanna KK. Caffeine abolishes the mammalian G2/M DNA damage checkpoint by inhibiting ataxia-telangiectasia-mutated kinase activity. J Biol Chem. 2000; 27:10342-10348.
13. Kim WS, Choi WJ, Lee S, Kim WJ, Lee DC, Sohn UD, Shin HS, Kim W. Anti-inflammatory, Antioxidant and Antimicrobial Effects of Artemisinin Extracts from Artemisia annua L. Korean J Physiol Pharmacol. 2015; 19(1):21-27.
14. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev. 1996; 60:301-315.
15. Abba ML, Patil N, Leupold JH, Saeed MEM, Efferth T, Allgayer H. Prevention of carcinogenesis and metastasis by Artemisinin-type drugs. Cancer Letters. 2018; 429:11-18.
16. Zhang T, Zhang Y, Jiang N, Zhao X, Sang X, Yang N, Feng Y, Chen R, Chen Q. Dihydroartemisinin regulates the immune system by promotion of $CD8^+$ T lymphocytes and suppression of B cell responses. Sci China Life Sci. 2020; 63(5):737-749.
17. Lai H and Singh NP. Oral artemisinin prevents and delays the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer in the rat. Cancer Letters. 2006; 231:43-48.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351-358.
19. Misra HP and Fridovich I. Inhibition of superoxide dismutases by azide. Arch Biochem Biophy. 1978; 189:317-322.
20. Sedlak J and Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192-205.
21. International Federation of Clinical Chemistry. Physicochemical Quantities and Units in Clinical Chemistry. J Clin Chem Clin Biochem. 1980; 18:829-854.
22. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28(1):56-63.
23. Wright PJ, Leatherwood PD, Plummer DT. Enzymes in Rats: Alkaline Phosphatase. Enzymologia. 1972; 42:317-327.
24. Jendrassik L and Grof P. Colorimetric method of determination of bilirubin. Biochem Z. 1938; 297:81-82.
25. Bartels H and Bohmer M. Kinetic determination of creatinine concentration. Clin Chem Acta. 1972; 37:193-197.
26. Fernández-Navarro P, García-Pérez J, Ramis R, Boldo E, López-Abente G. Industrial pollution and cancer in Spain: An important public health issue. Environ Res. 2017; 159:555-563.
27. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018; 13:757-772.
28. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, D'Arcangelo D, Norelli S, Valle G, Nisini R, Beninati S, Tabolacci C, Jadeja RN. Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases. Biomed Res Int. 2019; 7:8748253.
29. National Cancer Institute, 2017. Antioxidant and Cancer prevention. Available at https://www.cancer.gov/about-cancer/causes-prevention/risk/diet/antioxidants-fact-sheet.
30. Metro D, Cernaro V, Santoro D, Papa M, Buemi M, Benvenga S, Manasseri L. Beneficial effects of oral pure caffeine on oxidative stress. J Clin Transl Endocrin. 2017; 10:22-27.
31. Higuchi T, Kawaguchi K, Miyake K, Han Q, Tan Y, Oshiro H, Sugisawa N, Zhang Z, Razmjooei S, Yamamoto N. Oral recombinant methioninase combined with caffeine and doxorubicin induced regression of a doxorubicin-resistant synovial sarcoma in a PDOX mouse model. Anticancer Res. 2018; 38(10):5639-5644.
32. Efferth T. Cancer combination therapies with artemisinin-type drugs. Biochem Pharmacol. 2017; 139:56-70.
33. Koul A, Arora N, Tanwar L. Lycopene mediated modulation of 7, 12 dimethlybenz (A) anthracene induced hepatic clastogenicity in male Balb/c mice. Nutricion Hospitalaria. 2010; 25(2):304-310.
34. Hayat MA. Autophagy: In Cancer, other pathologies, inflammation, immunity, infection and aging: Role in human diseases. Academic Press, MA, USA, 2015; 5:48.
35. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nature Rev Drug Dis. 2009; 8:579-591.
36. Kaczmarczyk-Sedlak I, Folwarczna J, Sedlak L, Zych M, Wojnar W, Szumińska I, Wyględowska-Promieńska D, Mrukwa-Kominek E. Effect of caffeine on biomarkers of oxidative stress in lenses of rats with streptozotocin-induced diabetes. Arch Med Sci 2019; 15(4):1073-1080.
37. Endesfelder S, Strauß E, Scheuer T, Schmitz T, Bührer C. Antioxidative effects of caffeine in a hyperoxia-based rat model of bronchopulmonary dysplasia. Resp Res. 2019; 20:88.
38. Kavishe RA, Koenderink JB, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: Pros and Cons. FEBS J 2017; 284:2579-2591.
39. Karas M and Chakrabarti SK. Influence of caffeine on allyl alcohol-induced hepatotoxicity in rats. *In vivo* study. J Environ Path Toxicol Oncol. 2001; 20:141-154.
40. Al Moutaery K, Al Deeb S, Ahmad Khan H, Tariq M. Caffeine impairs short-term neurological outcome after concussive head injury in rats. Neurosurgery 2003; 53:704-711.
41. Mota TC, Garcia TB, Bonfim LT, Portilho AJS, Pinto CA, Burbano RMR, Bahia MO. Markers of oxidative-nitrosative stress induced by artesunate are followed by clastogenic and aneugenic effects and apoptosis in human lymphocytes. J Appl Toxicol. 2019; 39:1405-1412.
42. Li Q, Ni W, Deng Z, Liu M, She L, Xie Q. Targeting nasopharyngeal carcinoma by artesunate through inhibiting Akt/mTOR and inducing oxidative stress. Fundam Clin Pharmacol. 2017; 31(3):301-310.
43. Schmuck G, Roehrdanz E, Haynes RK, Kahl R. Neurotoxic mode of action of artemisinin. Antimicrob Ag Chemother. 2002; 46:821-827.
44. Nontprasert A, Pukrittayakamee S, Dondorp AM, Clemens R, Looareesuwan S, White NJ. Neuropathologic toxicity of artemisinin derivatives in a mouse model. Am J Trop Med Hyg. 2002; 67:423-429.
45. Hou L, Huang H. Immune suppressive properties of artemisinin family drugs. Pharmacol Ther. 2016; 166:123-127.
46. Xia M, Liu D, Liu Y, Liu H. The Therapeutic Effect of Artemisinin and Its Derivatives in Kidney Disease. Front Pharmacol. 2020; 11:380.
47. Nwanjo HU, Iroagba II, Nnatuanya IN, Eze NA. Antifertility activity of dihydroartemisinin in male albino rats. Int J Endocr. 2007; 4:35-42.
48. Oguntibeju OO, Akinola FF, Okonkwo KG. Effect of artemether on rat hepatocytes during acute damage. Afr J

Biotech. 2011; 10:13238-13243.

49. Okunlola AI, Okunlola CK, Okani CO, Adewole OS, Ojo SK, Abiodun AA, Bejide RA, Ojewole AO. Histological and biochemical effects of arteether on the liver of wistar rats. Afr J Tradit Complement Altern. Med. 2013; 10:155-160.
50. Bigoniyaa P, Sahua T, Tiwari V. Hematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicol Rep. 2015; 2:280-288.