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Research Article

# Caffeine-Containing Local Products and Their Effects on Liver and Kidney Histopathology: A Comparative Study

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**Abstract:** Caffeine has several modified natural and synthetic forms and it is the most abundantly consumed drug around the globe. It is present in fruits, seeds and leaves of many plants but coffee contains highest content of caffeine and is considered its richest source. It is also found in much high ratio in non-alcoholic beverages, i.e., tea and carbonated drinks like coke. The aim of current mammalian model based investigation was to evaluate the possible histopathological variations influencing the function of kidney and liver due to the intake of selective caffeinated products: Arabica and Robusta beans' blend based coffee and coke. The 30 albino mice of male gender and 1.5 month age were acclimatized for ten days and later on, categorized as group I (control group which was treated with standard mice feed), experimental group II (treated with coffee blend) and experimental group III (treated with coke). The renal and hepatic histological sections were cut and stained for weekly observations. Hematoxyline and eosin staining was used and microscopic observations were recorded at 40X. The ANOVA based statistical analyses showed that significant variations occurred in body weight, diameter of renal blood vessels, glomeruli and of necrotic areas of both experimental groups ($p \leq 5\%$). Moreover in hepatic tissues, vein wall thickness, diameter of bile ducts and liver lobules also indicated significant variations. The conclusion is excessive and regular intake of above mentioned composition having coffee and coke may result in diverse physiological disturbances and a programmed general public awareness is required regarding their limited intake.

**Keywords:** Caffeine, Coffee, Coke, Liver, Kidney.

## 1. INTRODUCTION

In daily life, exposure to caffeine products is quite common for people. Among these products, coffee (family Rubiaceae) has its 2 major species: *Coffea arabica* and *Coffea robusta* [1]. Though coffee is consumed round the globe but it also causes serious side effects on vital organs like in liver, disturbances in serum enzymatic activities, hepatic cirrhosis, hepatocellular carcinoma and other liver injuries and extent of damage varies from type of coffee and frequency of its consumption [2]. Similarly, excessive use of coffee causes nephrotoxicity, reduction of kidney volume and of glomerular diameter along with disturbances in blood serum urea and nitrogen levels and body weight loss [3]. Moreover, a soft drink usually consists of many ingredients, for example, a sweetener, carbonated water and either oragnic or synthetic additive. Additionally, caffeine, food colours and stabilizers like constituents may be included in soft drinks. Similarly, a worldwide frequently consumed and much demanding soft drink is Coca-Cola (Coke). It metabollically boosts the ecstatic effect but its regular in take may results in metabolic disturbances espacially related to renal and hepatic finstions [4]. The effects of carbonated soft drinks on health are not clear; however, they are considered as a link with obesity, osteoporosis, renal and hepatic disorders along with other severe health problems which are linked and directly proportional to the usage of these drinks. Numerous compounds are present in carbonated soft drinks which consist of caffeine that is behaviorally active substance and has the highest consumption rate throughout the world [5].

In this regard, recent data suggests that

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excessive intake of coke may cause metabolic syndrome, hyperuremia, atherosclerosis, elevated level of uric acid and creatinine in blood and chronic kidney disease [6]. However, the average lethal dose of caffinated products is 200 mg/kg body weight and casue hepatic and renal toxicity [14, 15], like swelling of hepatocytes, renal vascular congestion, focal autotophy, epithelial degeneration [16]. In addition to this, liver toxicity was also reported but its comparative severity is not known yet in selected caffinated products [4]. That is why, coke and blended composition having coffee were selected in current research design to know the range of its possible renal and hepatic side effects spectrum.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

The 30 albino mice (*Mus musculus*) of male gender and 1.5 months age (body weight around 28 g/ mouse) were bought from UVAS, Lahore.

### 2.2. Dose Preparation and Optimization

For dose preparation and optimization, 19.95 ml of water + 0.05 g of coffee (*C. arabica* and *C. robusta* blend) and its 0.1 ml was orally administrated as dose during experimentation, whereas 0.1 ml of coke was given without any dilution. Moreover, for control group 0.1 ml distilled water was given.

### 2.3. Study Design

Mice were acclimatized for 10 days in the animal house of Minhaj University Lahore at room temperature (25 °C ± 2 °C) and were fed locally available standard food and clean water. Then mice were grouped and tagged in control group labeled as (Control) while experimental group was distributed into experimental group A (treated with coffee: *C. arabica* and *C. robusta* blend) and experimental group B (treated with coke). The body weights of all mice were measured at the start and end of the experimentation [13].

### 2.4. Histopathological Observations

The renal and hepatic tissues of mice of both experimental groups were collected on weekly basis and for their fixation 10% formalin solution was used. Histological sections of liver and kidney were prepared and Haematoxyline and Eosin (H&E) staining was done. The microscopic readings were noted at 40X while microphotographs were captured at 10X with PixelPro software.

### 2.5 Statistics

The data was statistically anlyzed through single factor analysis of variance using SPSS (Statistical Package for the Social Sciences) version 20 at $p \leq 5\%$ [7].

## 3. RESULTS AND DISCUSSION

The tabulated data is presenting the ANOVA based results of variations after intake of selective caffeinated products in mice body weight (Table 1). The mice body weight was significantly increased due to intake of coke than coffee (*C. arabica* and *C. robusta*) blend on completion 2nd week and a pattern of gradual body weight decline was noticed till 4th week, 15.33 g, 15 g and 14 g, respectively. Because presence of high sugar content in composition of coke results in body weight gain but in coffee the concentration of caffeine is high which boosts up body weight loss [8].

Whereas, variations based on vasodilation and vasoconstriction were noticed in histopathological studies of renal blood vessels diameter and wall thickness (Figure 1) and in this regard, significant rise was observed at the end of 2nd week in diameter

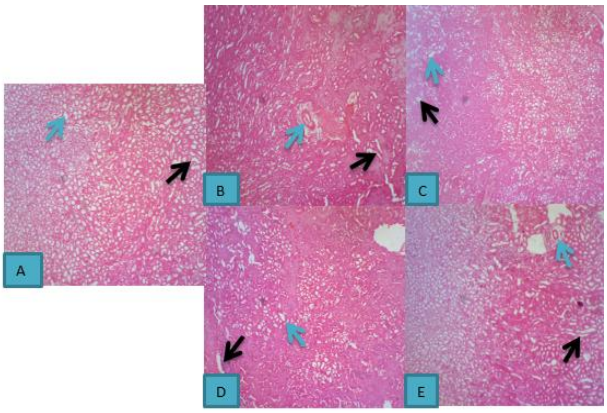


**Fig. 1.** Diameter of blood vessels of kidney, A = Control group, B = group A at the end of 1st week, C = group B at the end of 1st week, D = group A at the end of 4th week, E = group B at the end of 4th week presenting the diameter of vein (µm) with black arrows while blue arrows are indicating diameter of artery (µm). All microphotographs are taken at 100X magnification, H & E staining was used.

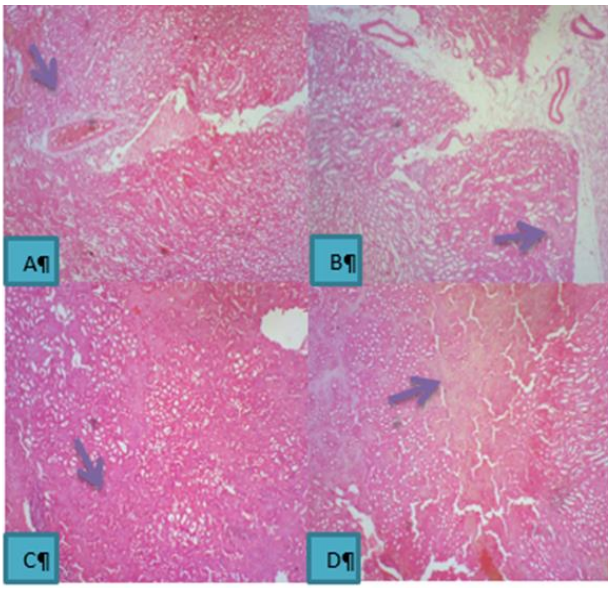


**Fig. 2.** Diameter of necrotic area of kidney, A = group A at the end of 1st week, B = group B at the end of 1st week, C = group A at the end of 4th week, D = group B at the end of 4th week presenting the diameter of necrotic area (µm) with purple arrows. All microphotographs are taken at 100X magnification, H & E staining was used.

of veins with 176.5 µm (Table 2) after exposure to coke (group B) and it happened due to biochemical and genetic alterations [9]. However, impact of coffee (*C. arabica* and *C. robusta* blend) intake compared to coke was found more influential on glomerular diameter, and maximum increase was of 120.46 µm on completion of 3rd week. Similarly, the diameter of kidney damaged area (Table 2) due to necrosis was significant (111 µm) in histological sections of group A at 2nd week (Figure 2) which might be due to over stressed blood filtration in glomeruli because of vasodilatory influence leading to bursting effect of selected caffeinated products [10].

**Table 1.** Effect of caffeinated products on body weight (g).

| Weeks | Mean (µm) ± S.EM(n) | | |
|---|---|---|---|
| | Control | Group A | Group B |
| 1 | 1.33 ± 0.33 (3) | 12 ± 1 (3) | 12.67 ± 0.33 (3) |
| 2 | | 8 ± 3 (3) | *15.33 ± 0.33 (3) |
| 3 | | 6.67 ± 0.882 (3) | 15 ± 1.155 (3) |
| 4 | | 7 ± 1 (3) | *14 ± 2.082 (3) |

*Values of Mean ± SEM (n). Data of respective columns were compared by employing single factor analysis of

Moreover, the histopathological observations for blood vessels in liver tissues indicated variations but a prominent increase in arterial diameter of 198.7 µm in group A than group B (120.5 µm) at 3rd week stage (Table 4). But in case of veins the pattern of gradual vasoconstriction was noted in group A, while in group B at 3rd week stage (Figure 3) maximum diameter of 159.6 µm was recorded due to biochemical alterations at cell and molecular levels [9]. Table 5 is presenting significant results of diameter of bile duct till 28th day (83.47 µm) for group A compared to group B (70.8 µm) but overall considerable variations in bile duct diameter were observed in group B treated with coke. For the diameter of liver lobule significant results of 384.63 µm in group A and 151 µm in group B at 1st week, and 286.35 µm in group B (treated with coke) and 165.89 µm in group A (treated with selected coffee) at 4th week [11]. Another histopathological change was due to necrosis in hepatic tissue (Figure 4) which was noticed in both experimental groups but comparatively more tissue (164.84 µm) was damaged after intake of coke than coffee treated group of 142.65 µm [12] and and such effects of tissue hemorrage and necrosis have also been repoted in other recently examined caffiniated products [17].

**Table 2.** Effect of caffeinated products on renal artery and vein diameter (µm).

| Weeks | Mean (µm) ± S.EM(n) | | | | | |
|---|---|---|---|---|---|---|
| | Renal Artery Diameter | | | Renal Vein Diameter | | |
| | Control | Group A | Group B | Control | Group A | Group B |
| 1 | 126.8 ± 63.51 (3) | 110.95 ± 12.003 (3) | 238.81 ± 26.48 (3) | 117.29 ± 13.11 (3) | 94.0433 ± 33.9 (3) | 89.82 ± 17.4 (3) |
| 2 | | 102.5 ± 20.16 (3) | 127.86 ± 5.88 (3) | | 90.87 ± 22.9 (3) | *176.5 ± 15.6 (3) |
| 3 | | 97.213 ± 5.9 (3) | 225.41 ± 62.31 (3) | | 126.8 ± 6.6 (3) | 130 ± 19.02 (3) |
| 4 | | 116.23 ± 5.6 (3) | 90.63 ± 9.62 (3) | | 164.84 ± 16.8 (3) | 146.88 ± 6.93 (3) |

*Values of Mean ± SEM (n). Data of respective columns were compared by employing single factor analysis of variance and results were found significant at 5% (*).

**Table 3.** Effects of caffeinated products on glomerular and renal necrotic area diameter (µm).

| Weeks | Mean (µm) ± S.EM (n) | | | | |
|---|---|---|---|---|---|
| | Diameter of Glomeruli | | | Diameter of Renal Necrotic Area | |
| | Control | Group A | Group B | Group A | Group B |
| 1 | 57.06 ± 5 (3) | 66.57 ± 3.7 (3) | 61.3 ± 14.22 (3) | 152.16 ± 6.6 (3) | 150.05 ± 46.7 (3) |
| 2 | | 78.2 ± 4.3 (3) | 75.023 ± 1.06 (3) | * 111 ± 24.21 (3) | 181.8 ± 5.6 (3) |
| 3 | | *120.46 ± 12.68 (3) | 106.72 ± 6.43 (3) | 209.7 ± 6.33 (3) | 209.22 ± 6.34 (3) |
| 4 | | 104.61 ± 3.17 (3) | 103.6 ± 4.61 (3) | 207.73 ± 8.8 (3) | 206.05 ± 8 (3) |

*Values of Mean ± SEM (n). Data of respective columns were compared by employing single factor analysis of variance and significant results were found at 1% (*).

**Table 4.** Effect of caffeinated products on diameter of liver artery and vein (µm**).**

| Weeks | Mean (µm) ± S.EM(n) | | | | | |
|---|---|---|---|---|---|---|
| | Hepatic Artery Diameter | | | Hepatic Vein Diameter | | |
| | Control | Group A | Group B | Control | Group A | Group B |
| 1 | 95.1 ± 11.43 (3) | 155.33 ± 47 (3) | 144.8 ± 26.54 (3) | 110 ± 35.5 (3) | 262.0533 ± 178.45 (3) | 108.834 ± 50.6 (3) |
| 2 | | 134.2 ± 33.4 (3) | 82.42 ± 5 (3) | | 223 ± 62 (3) | 105.7 ± 11.032 (3) |
| 3 | | 198.7 ± 72 (3) | 120.5 ± 11.43 (3) | | 172.24 ± 9.4 (3) | 159.6 ± 34.84 (3) |
| 4 | | 90.9 ± 16.61 (3) | 137.7 ± 25.53 (3) | | 183 ± 34.4 (3) | 108.84 ± 18.42 (3) |

*Values of Mean ± SEM (n). Data of respective columns were compared by employing single factor analysis of variance and no significant results were found.

**Table 5.** Effect of caffeinated products on bile duct, liver lobule and necrotic area diameter (µm).

| Weeks | Mean (µm) ± S.EM(n) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver Bile Duct Diameter | | | Liver Lobule Diameter | | | Liver Necrotic Area | |
| | Control | Group A | Group B | Control | Group A | Group B | Group A | Group B |
| 1 | 63.4 ± 3.7 (3) | 69.74 ± 5.5 (3) | 58.12 ± 2.8 (3) | 85.6 ± 3.17 (3) | *384.63 ± 77 (3) | 151.003 ± 76.8 (3) | 117.3 ± 22.3 (3) | 108.84 ± 8.3 (3) |
| 2 | | 65.51 ± 2.8 (3) | 62.34 ± 2.8 (3) | | 150.05 ± 28.5 (3) | 99.33 ± 19.05 (3) | 137.4 ± 9.4 (3) | 124.7 ± 11.032 (3) |
| 3 | | 74.5 ± 20 (3) | 73.44 ± 8 (3) | | 153.22 ± 13.77 (3) | 158.5 ± 41.6 (3) | 106.33 ± 8.84 (3) | 105.7 ± 8.66 (3) |
| 4 | | *83.5 ± 2.8 (3) | 70.8± 4.23 (3) | | 166 ± 23 (3) | **286.4 ± 8.3 (3) | 142.65 ± 13 (3) | 164.84 ± 40 (3) |

*Values of Mean ± SEM (n). Data of respective columns were compared by employing single factor analysis of variance and results were found at 5% (*) and 1% (**) significant for liver bile duct and lobule diameter. Whereas liver tissue necrosis based results were not found significant.

## 4. CONCLUSIONS

In the present study, hepatic and renal histopathological effects due to intake of selective caffeinated products were investigated and it can be concluded that the intake of *C. arabica* and *C. robusta* blend based coffee and coke imparted many physiological and histopathological variations in the body weight, diameter and thickness of blood vessels, glomeruli, bile duct, liver lobule and necrotic area. Comparatively more side effects were observed in experimental group treated with selected composition having coffee than coke and the intake of this blend induces synergistic but damaging effects on liver and kidney of mice. The way, mammalian model of current study faced side effects; similarly, the regualr consumers of these local caffinated products may also suffer from hepatic and renal physiologocal disturbances which are usually overlooked yet due to lack of general

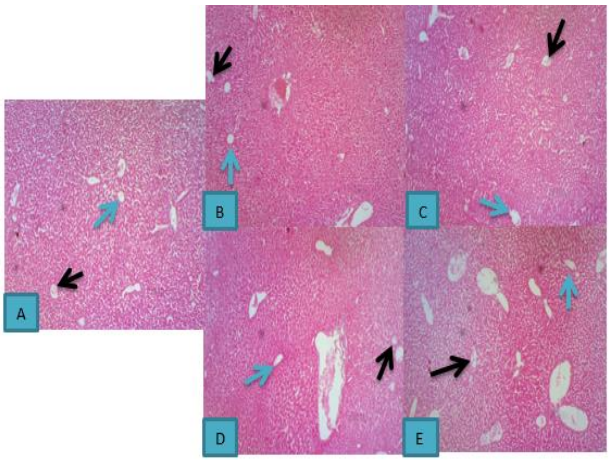


**Fig. 3.** Diameter of blood vessels of liver, A = Control group, B = group A at the end of 1st week, C = group B at the end of 1st week, D = group A at the end of 4th week, E = group B at the end of 4th week presenting the diameter of vein (µm) with black arrows while blue arrows are indicating diameter of artery (µm). All microphotographs are taken at 100X magnification, H & E staining was used.

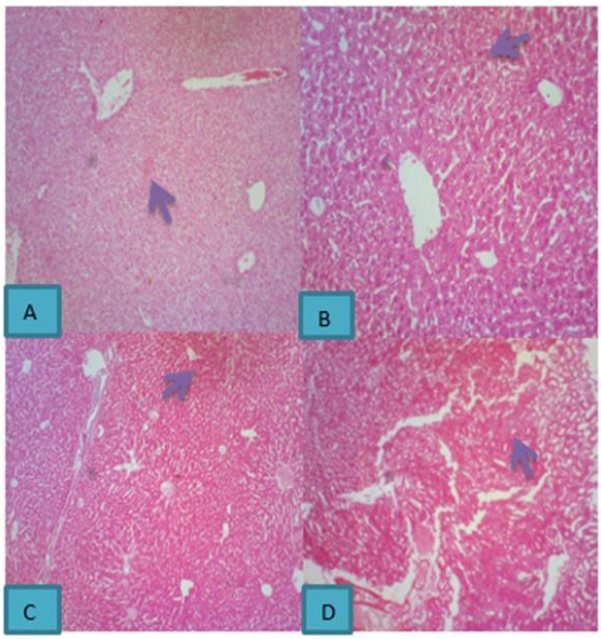


**Fig. 4.** Diameter of necrotic area of liver, A = group A at the end of 1st week, B = group B at the end of 1st week, C = group A at the end of 4th week, D = group B at the end of 4th week presenting the diameter of necrotic area (µm) with purple arrows. All microphotographs are taken at 100X magnification, H & E staining was used.

public awareness and such health related awareness should be provided to consumers through different research and elctronic media.

## 5. ACKNOWLEDGEMENTS

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## 6. ETHICAL STATEMENT

The experimental protocols and procedures used in this study were approved by the Ethical Committee of the Directorate of Academics, Minhaj University Lahore, Pakistan with reference number: MUL/DA/11356.

## 7. CONFLICT OF INTEREST

All authors have no conflict of interest.

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