# A STUDY OF MODULATING EFFECTS OF CAFFEINE AGAINST HEPATIC DAMAGE IN ACUTE PARACETAMOL INTOXICATED MALE ALBINO RATS

BY

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## ABSTRACT

*Coffee is the most frequently consumed beverage worldwide that affects several body organs and systems. This work was designed to evaluate the possible modulating effects of caffeine on hepatotoxicity induced by acute paracetamol (PCM) intoxication in rats. Ninty adult male albino rats were classified into 4 main groups: Group 1 (negative control) contains 10 rats, Group 2 (positive control) contains 40 rats which is divided equally into 4 subgroups: [2a] received distilled water, [2b] receiving 30 mg/kg caffeine, (2c) receiving 100 mg/kg caffeine, [2d] receiving PCM 2 g/kg, Group 3 contains 20 rats which is divided equally into 2 subgroups: [3a] Rats were treated on the 14th day only with caffeine 30 mg/kg plus 2 g/kg PCM, [3b]: Rats were treated on the 14th day only with caffeine 100 mg/kg plus 2 g/kg. PCM, Group 4 contains 20 rats which is divided equally into 2 subgroups: [4a]: Rats were treated daily with 30 mg/kg caffeine then on the 14th day rats were given also 2 g/kg PCM and [4b]: Rats were treated daily with 100 mg/kg caffeine then on the 14th day rats were given also 2 g/kg PCM. Blood samples were obtained for estimation of liver enzymes then rats were sacrificed and samples of liver tissue were used for measurements of oxidative stress markers malondialdehyde and advanced oxidation protein products and antioxidants superoxide dismutase and catalase also, the remaining tissue was subjected to histopathological and immunohistochemical localization of Bcl2. Paracetamol induced acute hepatotoxicity which were evident biochemically and confirmed by histopathology (massive necrosis, hemorrhage and inflammation), and immunohistochemistry (marked expression of Bcl2 indicating severe necrosis). The use of single dose 30 mg/kg of caffeine did not improve the liver, but repeated caffeine 30 mg/kg, partially opposed the toxic effect of PCM through some improvement in all previous parameters. However, increasing the dose of caffeine to 100 mg/kg either in a single dose or in repeated doses enhanced the ability of caffeine to provide nearly complete improvement of hepatotoxicity. Caffeine can modulate hepatic damage induced by acute PCM intoxication.*

***Keywords:*** *Paracetamol, caffeine, acute hepatotoxicity, MDA, AOPP, antioxidants and Bcl2 protein.*

## *INTRODUCTlON*

Paracetamol (PCM) is used worldwide for its analgesic and antipyretic action. Although PCM is generally safe at usual therapeutic doses, it may cause hepatic toxicity in case of chronic or acute overdose and even in therapeutic doses in susceptible individuals (Graham et al., 2013).

The first reports of PCM poisoning in humans describing hepatic necrosis provoked a series of animal studies which demonstrated that acute centrilobular hepatic necrosis with collapse of the reticulum frame work could be produced in some species (Ekam and Ebong, 2007).

Apoptosis plays a significant role in the course of hepatocytes death in acute and chronic hepatitis. Antiapoptotic protein Bcl2 prevents apoptosis induced by various treatments (Mochizuki et al., 1999).

The hepatic cell injuries caused the leaking of cellular enzymes into the blood stream and thus can be measured in the serum (Yahya et al., 2013). Lactate dehydrogenase localized in the cytoplasm is extruded into the serum and increases in damaged or necrotic liver cells, thus considered as a sensitive indicator of acute liver damage in addition to ALT along with the AST and ALP that are routinely assessed to monitor liver function (Kim et al., 2001; Avila et al., 2011).

Acute liver injury can be induced experimentally by many chemicals such as carbon tetrachloride (CCL4), thiocetamide (TAA), and ethionine. In addition, many experimental models of toxic liver injury were induced by PCM. Thus, it may be useful to evaluate the effects produced by many compounds such as coffee and/or caffeine (Manibusan et al., 2007).

Coffee is the most frequently consumed beverage worldwide, mainly due to the psychoactive properties of caffeine and its moderate intake has been considered to be beneficial for human health due to its effects on several body organs and systems (Butt and Sultan, 2011).

Caffeine consumption, particularly from regular coffee, above a threshold of approximately two coffee-cups equivalents per day, was associated with less severe hepatic fibrosis (Modi et al., 2010).

Caffeine (1, 3, 7-trimethylxanthine), a white crystalline xanthine alkaloid, is found mainly in coffee. Additionally, several analgesic drugs contain caffeine associated with PCM or with anti-inflammatory drugs, which in many occasions are sold without prescription (Goldstein et al., 2006).

Despite various epidemiological and experimental studies that have reported beneficial effects of coffee and/or caffeine

in the prevention of liver disease and the benefit of rapid analgesic effect due to combination of caffeine with PCM, some studies have suggested potential health risks with coffee consumption and its protective role on chemically induced liver injury are still inconclusive and controversial (Ita et al., 2009).

As coffee is the most popular beverage in the world and combination preparations or products of PCM with caffeine are very common, many researches swing between health benefits and risks against PCM- induced hepatic toxicity, so the aim of this work was to study the possible modulating effects of caffeine on liver damage induced by acute paracetamol (PCM) toxicity and detect the role of Bcl2 expression as an immune marker in adult male albino rats.

## *MATERIAL and METHODS*

**Material:**

**1. Chemicals**

**A- Paracetamol (PCM):** It was obtained from Sigma Chemical Company (St. Louis, USA) in the form of white powder. It was dissolved in distilled water and given orally to rats in a single toxic dose (2 g/kg BW) (Ekor et al., 2006 ; Prabu et al., 2011).

**B- Caffeine:** It was obtained from Sigma Chemical Company (St. Louis, USA) in the form of white powder. It was dissolved in distilled water. Freshly prepared caffeine solutions were administrated orally at a volume of 2 ml/day at doses 30 and 100 mg/kg body weight (Demirtas et al., 2012).

**2. Animals**

This study was carried out on 90 adult male albino rats, their weights ranged from 180-220 g. The animals were obtained from the experimental animal house of faculty of veterinary medicine, Zagazig University. All animals were left for one week of passive preliminaries in order to adapt themselves to their new environment. They were maintained on a standard diet and allowed water ad-libitum. The animals were divided equally into four groups (two control and two treated):

**Group 1** [Negative control group], ten rats were given no medications to measure the basic parameters.

**Group 2** [Positive control group], forty rats were divided equally into 4 subgroups:

- Group 2a: Each rat was given 2 ml of distilled water by gavage for 14 days.
- Group 2b: Each rat was given caffeine 30mg/kg B.W. by gavage for 14 days.
- Group 2c: Each rat was given caffeine 100 mg/kg B.W. by gavage for 14 days.

- Group 2d (PCM-induced toxicity model): Each rat was given single oral dose of PCM 2 g /kg B.W. on the 14$^{th}$ day only.

**Group 3** [Single caffeine dose plus PCM], twenty rats were divided equally into 2 subgroups:

- Group 3a: Rats were given on the 14$^{th}$ day only a single dose of combined low caffeine dose 30 mg/kg plus toxic PCM dose 2 g/kg B.W.

- Group 3b: Rats were given on the 14$^{th}$ day only a single dose of combined high caffeine dose 100 mg/kg plus toxic PCM dose 2 g/kg B.W.

Group 4 [Repeated caffeine doses plus PCM], twenty rats were divided equally into 2 subgroups:

- Group 4a: Rats were given repeated daily dose of 30 mg/kg caffeine for 14 days before combined treatment of PCM 2 g/kg plus caffeine 30mg/kg which was given on the 14$^{th}$ day.

- Group 4b: Rats were given repeated daily dose of 100 mg/kg caffeine for 14 days before combined treatment with PCM 2 g/kg and caffeine 100 mg/kg that was given on the 14$^{th}$ day.

Food was withdrawn before PCM intoxication for 12 hours to trigger acute liver damage. Animals were sacrificed 24 hours after PCM administration then all rats were subjected to the tested parameters.

**Methods**

**1. Biochemical parameters:**

Venous blood samples were taken from animals by means of capillary glass tubes from the retro- orbital plexus, under light ether anesthesia (Semler, 1992) and 3 ml of blood was collected in non- heparinized capillary tube and used for assessment of liver function, including alanine amino-transferase (ALT) and aspartate amino-transferase (AST) according to Reitman and Frankel (1957), alkaline phosphatase (ALP) according to Kind and King (1954), and lactate dehydrogenase (LDH) according to Kim et al. (2001).

**2. Tissue parameters:**

Rats were anaesthetized with ether and sacrificed. Liver tissues were immediately dissected and grossly inspected to access any gross abnormalities then washed out with cold normal saline.

***[A] Determination of oxidative stress markers in liver tissues:***

Tissue samples from liver were immediately preserved in liquid nitrogen at -80 ℃ till analysis to measure malondialdehyde (MDA) according to the guidelines of Ohkawa et al. (1979), advanced oxidation protein products (AOPP) according to Witko-Sarsat et al. (1998) and catalase (CAT) activity according to Aebi et al. (1974).

The remaining clear supernatants were

used to measure superoxide dismutase (SOD) according to Yi-Sun et al. (1988). All the diagnostic kits assaying hepatic function tests, lipid peroxidation and antioxidants levels were obtained from Sigma Chemical Company (St. Louis, USA).

***[B] Histopathological study:***

The liver tissue was immediately removed and fixed in 10% neutral buffer formalin and embedded in paraffin, sectioned to a thickness of approximately 5-6 μm and stained with Hematoxylin and Eosin (H and E) for histological examination by light microscopy according to Bancroft and Gamble (2008).

***[C] Immunohistrochemical study:***

Immunohistochemical study was conducted using Meyer's hematoxylin counterstain method. Paraffin sections of liver specimens were deparaffinized and rehydrated in decreasing concentrations of ethyl alcohol, and induced overnight with the primary antibody specific for Bcl2 immunostain (Bcl2: code no.: PMD016, monocolonal mouse antibody, DBS, CA, USA). Universal DAB (Diaminobenzidine tetrahydrochloride) kits (Code no.: K0673, DAKO, Carpinteria, CA, USA) were used to demonstrate antibodies expression according to the manufacturer's instructions. Negative control slides were prepared by replacing the primary antiserum with phosphate-buffered saline (PBS) (Ramos-Vera et al., 2014).

**Statistical analysis:**

Data were coded, entered and analyzed using an SPSS (Windows, Version 12.0, SPSS Inc., and USA). Statistical analysis was expressed as mean values and standard deviation (SD). Statistical comparisons among the means of different experimental groups were done using one-way analysis of variance (ANOVA) and least significant difference (LSD). Significance was expressed in terms of P-value and the level of significance was 0.05.

## *RESULTS*

**1- Biochemical results** (liver function tests) (Table 1, 2 and 5):

A non- significant difference ($P>0.05$) in liver enzymes (ALT, AST, ALP and LDH) were found among normal control groups (group 1, 2a, 2b and 2c) (non-PCM-intoxicated control groups), so negative control group (group 1) was used for comparison with other groups.

These enzymes were highly significantly increased ($P<0.001$) in PCM intoxicated group (2d) and in treated group with combined single low caffeine dose 30 mg/kg plus PCM intoxication (group 3a) when compared with group (1). Interestingly, repeated daily dose of 30 mg/kg caffeine plus PCM (group 4a) revealed significant decrease in these enzymes ($P<0.01$) when compared to PCM group alone (group 2d), however showed significant increase

(P<0.01) in the mean values of liver enzymes when compared with group (1).

Single high caffeine dose 100 mg/kg plus PCM (group 3b) showed highly significant decrease (P<0.001) in liver enzymes when compared with single low caffeine dose 30 mg/kg plus PCM intoxication (group 3a). However, repeated high caffeine dose 100 mg/kg plus PCM (group 4b) showed significant decrease (P<0.01) in the mean values of liver enzymes when compared with repeated low caffeine dose 30 mg/kg plus PCM intoxication (group 4a).

There was a non -significant difference (P>0.05) in these enzymes when 100 mg/kg caffeine (either single or repeated) (groups 3b and 4b) was compared with negative control group.

A highly significant decrease in the mean values of liver enzymes (P<0.001) was found between single and repeated low caffeine dose 30 mg/kg B.W (group 3a and 4a). However, no significant difference (P>0.05) was found in all of these values between single and repeated high caffeine dose 100 mg/kg B.W (groups 4a and 4b).

**2- Tissues parameters:**

***[A] Oxidative stress markers in liver tissues*** (Tables 3, 4 and 5):

The liver oxidative stress markers (lipid peroxidation MDA and protein oxidation AOPP) and (antioxidant enzymes SOD and CAT) revealed non- significant difference (P>0.05) among normal control groups (non-PCM-intoxicated groups) (groups 1, 2a, 2b and 2c).

These markers showed highly significant increase in MDA and AOPP with concomitant highly significant decrease (P<0.001) in CAT and SOD enzymes in PCM- intoxicated positive control group (group 2d) when compared to negative control group.

The same previous result in PCM- intoxicated control group (group 2d) was found in treated group with combined single low caffeine dose 30 mg/kg plus PCM (group 3a). However, repeated daily dose of 30 mg/kg caffeine plus PCM (group 4a) revealed significant decrease (P<0.05) in the mean values of MDA and AOPP with concomitant increase in antioxidant enzymes when compared with group (1) and a highly significant decrease in MDA and AOPP (P<0.001) with concomitant highly significant decrease in antioxidant enzymes was detected when compared to PCM group (group 2d).

Single high caffeine dose 100 mg/kg plus PCM (group 3b) showed highly significant decrease (P<0.001) in the mean values of MDA and AOPP at the same time, highly significant increase in antioxi-

dant enzymes when compared with low single caffeine dose 30 mg/kg plus PCM intoxication, also, repeated daily high caffeine dose 100 mg/kg plus PCM (group 4b) showed significant decrease ($P<0.01$) in the mean values of MDA and AOPP with concomitant significant increase in antioxidant enzymes when compared with repeated daily low caffeine dose 30 mg/kg group plus PCM intoxication.

A non -significant difference ($P>0.05$) in the mean values of these markers were detected when 100 mg/kg caffeine (either single or repeated) (groups 3b and 4b) were compared with negative control group.

A highly significant decrease ($P<0.001$) was found in the mean values of MDA and AOPP with concomitant highly significant increase in the mean values of SOD and CAT between single and repeated low caffeine dose 30 mg/kg (group 3a and 3b). However, no significant difference ($P>0.05$) was found in all of these values between single and repeated high caffeine dose 100 mg/kg (groups 4a and 4b).

***[B] Histopathological changes in the liver:*** (Figure 1)

All normal control groups (Non-PCM intoxicated liver in groups 1, 2a, 2b and 2c) showed normal lobular architecture (A). Sections of PCM intoxicated liver (group 2d), exhibited massive necrosis, hemorrhage and inflammatory infiltrates involving mainly centrilobular zone together with dilated central veins causing pressure atrophy on the adjacent hepatocyte leading to necrosis around central vein (centrizonal) in addition to focal piecemeal necrosis in the periportal area. Sinusoids are dilated and filled with RBCs (B and C).

Liver specimens of (group 3a) treated with low single caffeine dose 30 mg/kg plus PCM showed the same picture described previously with PCM alone (D). However, these changes were reduced to nearly normal picture with increasing the single dose of caffeine to 100 mg/kg plus PCM (group 3b) (only slight congestion with mild inflammation, no hemorrhage or necrosis) (F).

Liver specimens of (group 4a) treated with repeated daily low caffeine dose 30 mg/kg plus PCM revealed mild cell injury appeared as cloudy swelling, slight congestion in central vein with localized inflammatory infiltrates resulted in random areas of necrosis (F), that appeared nearly normal with increasing the dose of repeated daily caffeine to 100 mg/kg (group 4b) (Figure G).

***[C] Immumohistochemical changes:*** (Figure 2)

All normal control groups (non-PCM-intoxicated liver in groups 1, 2a, 2b and

2c) showed negative Bcl2 staining (A), while sections of PCM intoxicated liver (group 2d), exhibited positive cells with marked expression of bcl2 (brown discoloration of Bcl2 immunotain) (B and C). Liver specimens of (group 3a) treated with low single caffeine dose (30 mg/kg plus PCM), showed the same picture of marked Bcl2 expression as PCM intoxication alone (group 2d) (D). However, these Bcl2 expressions were reduced to nearly normal picture with increasing the single caffeine dose to100mg/kg plus PCM (group 3b) (E).

Liver specimens of (group 4a) treated with repeated daily caffeine 30 mg/kg plus PCM, revealed few scattered expression of Bcl2 (F) that appeared nearly normal through negative Bcl2 staining with increasing the repeated daily caffeine dose to100 mg/kg plus PCM (group 4b) (G).

**Table (1) :** Comparison between control and treated groups with single caffeine dose plus PCM as regard liver function tests (n: 70).

| Groups n=10 / Parameters | Control groups | | | | | Treated group with single caffeine dose plus PCM | | |
|---|---|---|---|---|---|---|---|---|
| | Negative Control group 1 Mean ± SD | Positive control group 2a (Distilled water) Mean ± SD | Positive control group 2b (30 mg/kg caffeine) Mean ± SD | Positive control group 2c (100 mg/kg caffeine) Mean ± SD | Positive control group 2d (2 g/kg PCM) Mean ± SD | Treated group 3a (PCM with 30 mg/Kg caffeine) Mean ± SD | Treated group 3b (PCM with 100 mg/Kg caffeine) Mean ± SD | P |
| ALT (IU/L) | 66.05±7.91 | 66.11±7.88 | 66.06±7.89 | 66.13±7.94 | 170.94±8.96 a | 170.88±8.99 a | 65.92±7.82 b | <0.001* |
| AST (IU/L) | 91.88±2.97 | 91.93±2.89 | 91.92±2.91 | 91.89±2.92 | 155.03±8.11 a | 154.98±8.13 a | 92.01±3.04 b | <0.001* |
| ALP (IU/L) | 71.63±6.22 | 71.96±6.88 | 71.99±6.89 | 71.98±6.86 | 127.82±9.33 a | 127.85±9.29 a | 72.03±6.01 b | <0.001* |
| LDH (IU/L) | 110.26±7.11 | 110.11±7.17 | 110.12±7.12 | 110.15±7.14 | 182.03±9.96 a | 181.98±10.06 a | 109.98±7.57 b | <0.001* |

SD : standard deviation, n: number, PCM: paracetamol, *Significant (P< 0.05); a: significant compared with negative, positive control groups (2a, 2b and 2c), b: significant compared to PCM and PCM with 30 mg caffeine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.

**Table (2) :** Comparison between control and treated groups with repeated caffeine doses plus PCM as regard liver function tests (n: 70).

| Groups n=10 / Parameters | Control groups | | | | | Treated groups with repeated caffeine dose plus PCM | | |
|---|---|---|---|---|---|---|---|---|
| | Negative Control group 1 Mean ± SD | Positive control group 2a (Distilled water) Mean ± SD | Positive control group 2b (30 mg/kg caffeine) Mean ± SD | Positive control group 2c (100 mg/kg caffeine) Mean ± SD | Positive control group 2d (2 g/kg PCM) Mean ± SD | Treated group 4a (PCM and 30 mg/Kg caffeine) Mean ± SD | Treated group4b (PCM and 100 mg/Kg caffeine) Mean ± SD | P |
| ALT (IU/L) | 66.05±7.91 | 66.11±7.88 | 66.06±7.89 | 66.13±7.94 | 170.94±8.96 a | 120.18±7.99 b | 66.01±7.89 c | <0.001* |
| AST (IU/L) | 91.88±2.97 | 91.93±2.89 | 91.92±2.91 | 91.89±2.92 | 155.09±8.11 a | 114.54±5.14 b | 92.14±2.86 c | <0.001* |
| ALP (IU/L) | 71.63±6.22 | 71.96±6.88 | 71.99±6.89 | 71.98±6.86 | 127.82±9.33 a | 91.85±7.32 b | 72.03±6.34 c | <0.001* |
| LDH (IU/L) | 110.26±7.11 | 110.11±7.17 | 110.12±7.12 | 110.15±7.14 | 182.03±9.96 a | 122.28±8.53 b | 110.04±7.15 c | <0.001* |

SD: standard deviation, n: number, PCM: paracetamol, *Significant (P< 0.05); a: significant compared with negative, positive control groups (2a, 2b and 2c) and treated groups (4a and 4b), b: significant compared to negative, positive control groups (2a, 2b and 2c) and treated group (4b), c: significant compared to PCM and PCM +30 mg caffeine. ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.

**Table (3) :** Comparison between control and treated groups with single caffeine doses plus PCM as regard liver oxidative stress markers (n: 70).

| Groups N=10 / Parameters | Control groups | | | | | Treated groups with single caffeine dose plus PCM | | |
|---|---|---|---|---|---|---|---|---|
| | Negative Control group1 Mean ± SD | Positive control group 2a (Distilled water) Mean ± SD | Positive control group 2b (30 mg/kg caffeine) Mean ± SD | Positive control group 2c (100mg/kg caffeine) Mean±SD | Positive control group 2d (2 g/kg PCM) Mean ± SD | Treated group 3a(PCM with 30 mg/Kg caffeine) Mean ± SD | Treated group 3b(PCM with 100 mg/Kg caffeine) Mean ± SD | P |
| MDA (nmol/g) | 105.68±7.46 | 105.56±7.27 | 105.61±7.54 | 105.5±7.24 | 123.17±9.42 a | 122.29±9.11 a | 106.02±7.53 b | <0.001* |
| AOPP (µmol/mg) | 60.96±3.12 | 60.94±3.13 | 60.93±3.11 | 60.95±3.09 | 85.65±4.04 a | 85.22±4.01 a | 61.15±3.03 b | <0.001* |
| SOD (U/g) | 8.85±2.06 | 8.84±1.98 | 8.89±1.95 | 8.86±2.07 | 5.82±1.87 a | 5.53±1.76 a | 8.93±2.11 b | <0.001* |
| CAT(U/g) | 280.01±29 | 280.05±29 | 280.11±28 | 280.09±29 | 217.43±32 a | 217.52±39 a | 279.96±29 b | <0.001* |

SD: standard deviation, n: number, PCM: paracetamol, *Significant (P< 0.05); a: significant compared with negative, positive control groups (2a, 2b and 2c) and treated groups (3a and 3b), b: significant compared to negative, positive control groups (2a,2b and 2c) and treated groups (3b), MDA: malondialdehyde, AOPP: advanced oxidation protein products, SOD: superoxide dismutase, CAT: catalase.

**Table (4) :** Comparison between control groups and treated groups with repeated caffeine doses plus PCM as regard liver oxidative stress markers (n: 70).

| Groups N=10 / Parameters | Control groups | | | | | Treated groups with repeated caffeine dose plus PCM | | |
|---|---|---|---|---|---|---|---|---|
| | Negative Control group 1 Mean ± SD | Positive control group 2a (Distilled water) Mean ± SD | Positive control group 2b (30 mg/kg caffeine) Mean ± SD | Positive control group 2c (100 mg/kg caffeine) Mean ± SD | Positive control group 2d (2 g/kg PCM) Mean ± SD | Treated group 4a(PCM with 30 mg/Kg caffeine) Mean ± SD | Treated group 4b(PCM with 100 mg/Kg caffeine Mean ± SD | P |
| **MDA (nmol/g)** | 105.68±7.46 | 105.56±7.27 | 105.61±7.54 | 105.5±7.24 | 123.17±9.42 a | 113.37±8.61 b | 104.92±7.51 c | <0.001* |
| **AOPP (µmol/mg)** | 60.96±3.12 | 60.94±3.13 | 60.93±3.11 | 60.95±3.09 | 85.65±4.04 a | 74.73±3.71 b | 61.05±3.23 c | <0.001* |
| **SOD (U/g)** | 8.85±1.66 | 8.84±1.98 | 8.89±1.95 | 8.86±1.67 | 5.82±1.87 a | 7.03±1.5 b | 8.94±2.03 c | <0.001* |
| **CAT(U/g)** | 280.01±29 | 280.05±29 | 280.11±28 | 280.09±29 | 217.43±32 a | 258.29±25 b | 280.22±33 C | <0.001* |

SD: standard deviation, n: number, PCM: paracetamol, *Significant (P< 0.05), a: significant compared with negative, positive control groups (2a,2b and 2c) and treated groups (3a and 3b), b: significant compared to negative, positive control groups (2a,2b and 2c) and treated groups (3b)., c: significant compared to PCM and PCM +30 mg caffeine. MDA: malondialdehyde, AOPP: advanced oxidation protein products, SOD: superoxide dismutase, CAT: catalase.

**Table (5) :** Comparison between single and repeated caffeine doses either in low dose 30 mg/kg or high 100 mg/kg as regard liver function tests and oxidative stress markers.

| Groups (N=10) / Parameters | Treated groups with 30 mg/kg caffeine plus PCM | | | Treated groups with 100 mg/kg caffeine plus PCM | | |
|---|---|---|---|---|---|---|
| | Single Mean±SD | Repeated Mean±SD | P | Single Mean±SD | Repeated Mean±SD | P |
| **ALT (IU/L)** | 170.88±8.99 | 120.18±7.99 | <0.001 | 65.92±7.82 | 66.01±7.89 | >0.05 |
| **AST (IU/L)** | 154.98±8.13 | 114.54±5.14 | <0.001 | 92.01±3.04 | 92.14±2.08 | >0.05 |
| **ALP (IU/L)** | 127.85±9.29 | 91.85±7.32 | <0.001 | 72.03±6.01 | 72.03±6.34 | >0.05 |
| **LDH (IU/L)** | 181.98±10.06 | 122.28±8.53 | <0.001 | 109.98±7.57 | 110.04±7.15 | >0.05 |
| **MDA (nmol/g)** | 122.29±9.11 | 113.37±8.61 | <0.05 | 106.02±7.53 | 104.92±7.51 | >0.05 |
| **AOPP (µmol/mg)** | 85.22±4.01 | 74.73±3.71 | <0.001 | 61.15±3.03 | 61.05±3.23 | >0.05 |
| **SOD (U/g)** | 5.53±1.76 | 7.03±1.5 | <0.05 | 8.93±2.11 | 8.94±2.03 | >0.05 |
| **CAT(U/g)** | 217.52±39 | 258.29±25 | <0.01 | 279.96±29 | 280.22±33 | >0.05 |

SD: standard deviation, n: number, P>0.05: non-significant difference, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, MDA: malondialdehyde, AOPP: advanced oxidation protein products, SOD: superoxide dismutase, CAT: catalase.

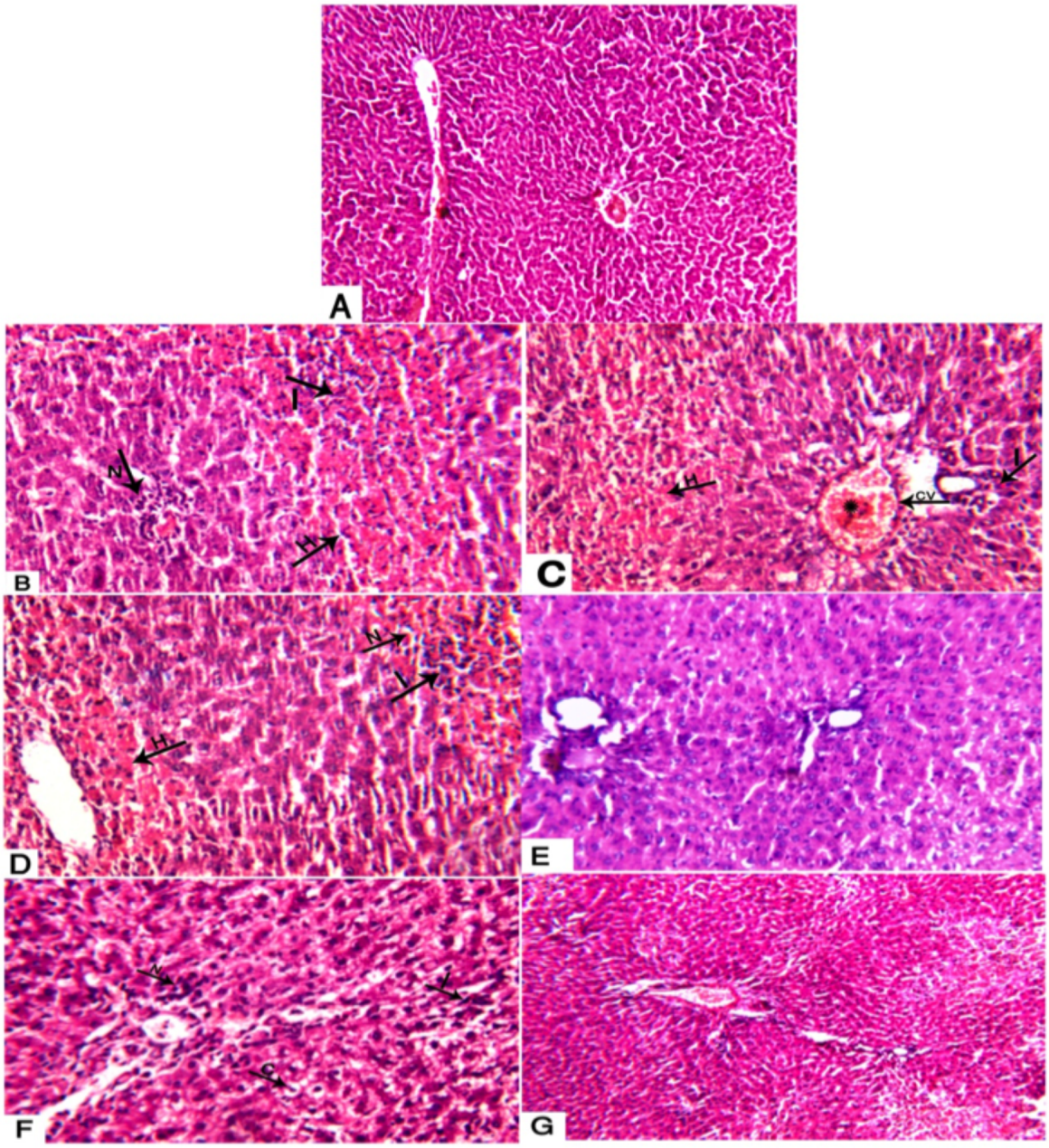


**Figure (1) :** Hematotoxlin and Eosin stained sections of the liver in control group (A), PCM intoxicated group 2 g/kg B.W. showing massive hepatic necrosis, hemorrhage and inflammation, in addition to piecemeal necrosis in the periportal areas (B and C), combined PCM with single low caffeine dose 30 mg/k showing the same picture as Figs. B and C (D), combined PCM with single high caffeine dose 100 mg/kg showing nearly normal hepatic cells (E), combined PCM with repeated daily low caffeine dose 30 mg/kg showing some improvement manifested by few random necrosis, Hge and inflammation (F), and combined PCM with repeated daily high caffeine dose 100 mg/kg showing nearly normal hepatic cells (G).

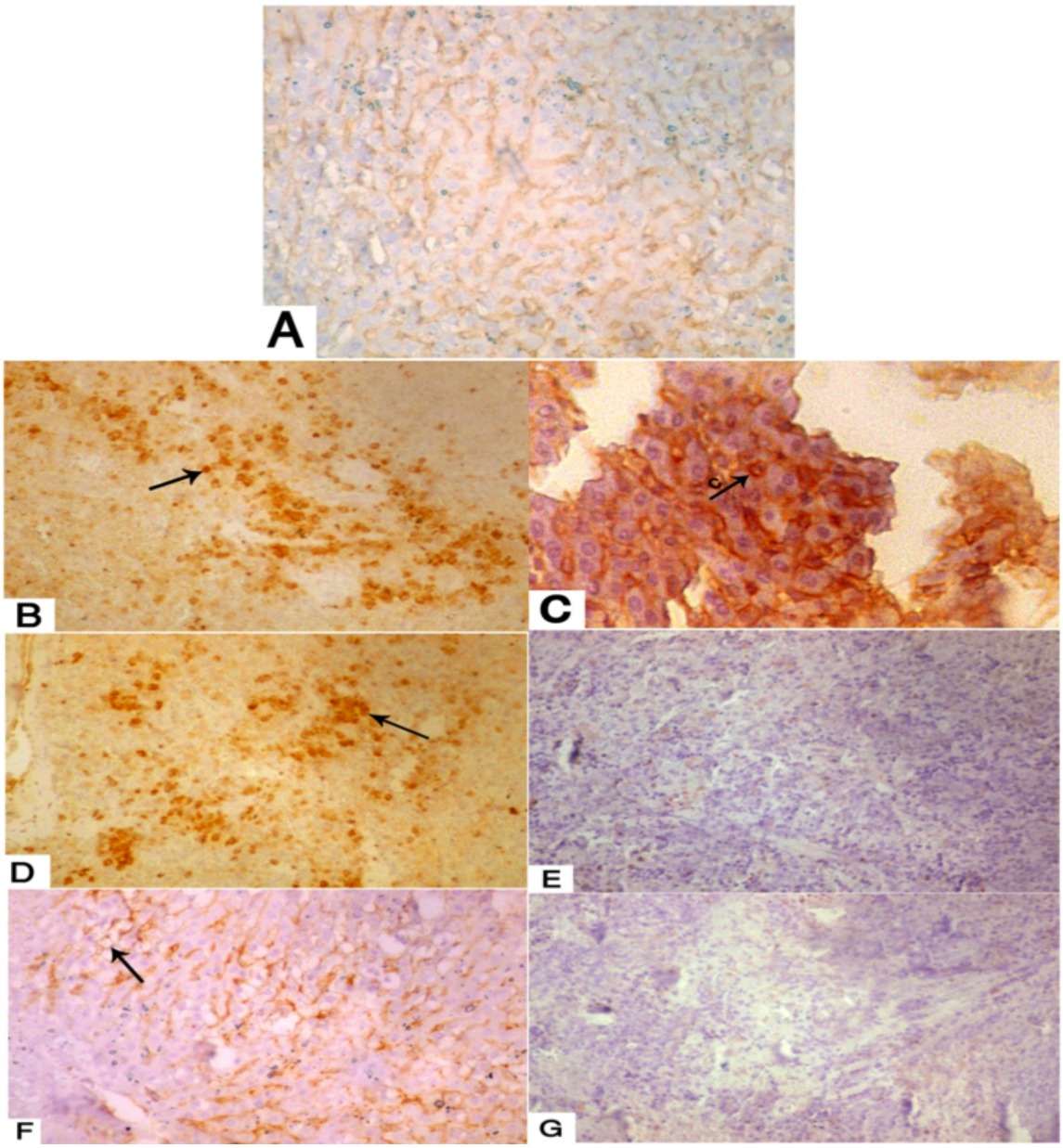


**Figure (2) :** Immunolocalization of Bcl2 (arrow) in liver specimens reveals no cytoplasmic reaction in hepatocytes of the control group (A), Positive Bcl2 reaction with marked expression in PCM intoxicated group 2 g/kg B.W. indicating severe necrosis and apoptosis (B and C), combined PCM with single low caffeine dose 30 mg/kg showing the same picture as Figs. B and C (D), combined PCM with single high caffeine dose caffeine 100 mg/kg showing negative bcl2 reaction due to nearly normal hepatic cells (E), combined PCM with repeated daily low caffeine dose 30 mg/kg showing moderate expression of Bcl2 reaction with few scattered positive stained hepatocytes (F) and combined PCM with repeated daily high caffeine dose 100 mg/kg showing negative Bcl2 reaction due to nearly normal liver cells (G).

## *DISCUSSION*

The liver is the main organ for xenobiotic detoxification therefore; it is very susceptible to being damaged by hepatotoxic agents. Drug induced liver disorders occurred frequently could be life threating and mimic all forms of liver diseases (Watkins and Seef, 2006). Paracetamol, an over-the counter medication, is a commonly used antipyretic and analgesic which can lead to liver damage if taken in overdose and in severe cases may progress to acute liver failure and need for liver transplantation (Yahya et al., 2013).

The PCM-induced toxicity model is used to study the hepatoprotective effect of many herbal substances (Zakaria et al., 2011). In the present study, PCM- induced hepatotoxicity is used to evaluate the modulating effect of caffeine (either single or repeated) in two different doses (low and high non-toxic dose) on this hepatic damage. As expected, PCM showed marked elevation of serum levels of liver enzymes, lipid peroxidation and protein oxidation, in addition to marked decrease in liver antioxidant enzymes when all were compared with negative control group. These biochemical studies were confirmed by both histopathological and immunohistochemical studies.

The results of acute PCM intoxication in the current study are similar to many previous studies used the same dose (2 g/kg PCM) to induce this intoxication in rats. Ekor et al. (2006) and Prabu et al. (2011) induced hepatotoxicity using 2 g/kg PCM and recorded highly significant increase in liver enzymes with increased lipid peroxidation and associated reactive oxygen species leads to collapse in membrane integrity and other pathological changes in liver.

Excess PCM is oxidatively metabolized by hepatic cytochrome P450 (CYP 450) system to a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Yen et al., 2007). Also, the toxic dose of PCM caused depletion of GSH resulting in accumulation of NAPQI, which then covalently binds to the cysteinyl sulfhydryl groups of cellular proteins forming NAPQI-protein adducts (Subramanian et al., 2013). This can lead to generation of reactive oxygen species (ROS) including the hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2$), and hydroxyl (OH) radical that affect the cellular membrane to induce lipid peroxidation and cause hepatic necrosis (Chen et al., 2009).

In the present study, PCM intoxicated rats with single low caffeine dose (30 mg/kg) showed the same results of hepatotoxicity as with PCM intoxicated rats alone.

Early research reports stated that simultaneous administration of caffeine potentiates PCM-induced hepatotoxicity more than in rats taking PCM only (Sato et al.,

1985). This was confirmed by Sato and Izumi (1989) who stated that caffeine can interact with an enzyme that cause increasing the formation of toxic metabolite of PCM causing more depletion in hepatic glutathione.

Persistence of liver damage by low caffeine dose in this study seems to be controversial with many epidemiological studies which strongly suggest the beneficial effects of caffeine consumption on chronic liver diseases including cirrhosis but, the mechanism for these beneficial effects of caffeine in chronic liver inflammation may be different from tissue damaging effects of coffee/caffeine during acute inflammation (Ruhl and Everhart, 2005).

A new marker of protein oxidation, AOPP, has recently attracted the attention of various investigators. The oxidative damage done to proteins is marked by an increase in the levels of protein carbonyls and a decrease in the levels of protein thiols (Çakatay et al., 2003). This protein marker is more accurate than markers of lipid peroxidation due to their relatively early stability and longer life span (Perrone et al., 2007).

Pasaoglu et al. (2011) investigated for the first time the effect of caffeine on AOPP levels in rat's liver, which showed a decrease in AOPP levels even with 30 mg/kg caffeine, and this may reflect the cell's ability to protect against the oxidative injury imposed by caffeine, which is dose dependent, while in this study this dose doesn't exhibit any improvement on liver damaged by PCM intoxication. The increased formation of lipid peroxidation MDA and protein oxidation AOPP with associated reactive oxygen species leads to collapse in membrane integrity that was responsible for the pathological changes in liver (Prabu et al., 2011).

Ohta et al. (2007) demonstrated the enhancement and exacerbation of acute inflammatory liver injury by two small doses of caffeine (10 and 20mg/kg) and, this effect was induced using two independent hepatitis models other than PCM. Liver damage was due to the inhibition of the tissue-protecting adenosine-A2AR pathway so, caffeine enhanced pro-inflammatory cytokine induction and subsequently tissue destruction as a mechanism of the exaggeration of tissue damage and this can be done by the administration of low caffeine dose during ongoing acute inflammation.

In people who do not consume coffee, caffeine half-life is twice as high, which explains a higher incidence of intoxication. As caffeine increases PCM bioavailability, it may increase their plasma concentrations and consequently the likelihood of adverse effects, thus, it explained the persistence of liver damage with low dose

caffeine in this study (Sawynok, 2011). This was expressed by marked increase in lipid peroxidation and protein oxidation with marked decrease in the antioxidants and confirmed by histopathological and immunohistochemical pictures.

In this study, treatment with 100 mg/kg caffeine (either single or repeated) plus PCM intoxication was significantly reduced the elevated levels of those enzymes, oxidative stress markers in liver tissue towards the normal values. Also, this dose elevates the decreased levels of antioxidant enzymes to normal values and improves the histopathological and immunohistochemical pictures.

In parallel with this study, Ohta et al. (2007) found that high dose of caffeine (100 mg/kg) provided strong protection against acute liver damage because high dose of caffeine suppressed tissue damage which was explained by the inhibition of pro-inflammatory cytokine responses (TNF, IL4, and IL12, p53 levels) and the induction of an anti-inflammatory cytokine IL10. So, single caffeine dose (either low or high) could have opposite effect on liver inflammation (proinflammatory versus anti-inflammatory). But repeated caffeine consumption for 14 days before triggering acute liver inflammation by PCM reduce liver damage induced by PCM intoxication in a dose dependent manner.

Demirtas et al. (2012) reported that oral caffeine administration at low dose (30 mg/kg) for a short time interval (14 days) showed a decrease in liver MDA level and increase in SOD, catalase and GPx enzyme activities.

The present study was in accordance with Mukhopadhyay et al. (2003) who stated that caffeine administration to rats in low dose (20 mg/kg for 30 days) caused augmentation of hepatic catalase and SOD activities and a reduction in MDA levels. Additionally, Noschang et al. (2009) concluded that chronic administration of repeated daily dose of caffeine led to increased activity of antioxidant enzymes. However, these effects were not observed in the stressed animals.

These results could be explained by Johansson et al. (1997) and Fredholm et al. (1999) who indicated the development of tolerance by the effect of caffeine on the immune system just as tolerance to caffeine develops in the CNS. Thus, after long-term caffeine treatment, up-regulation of adenosine receptors including A2AR may have increased sensitivity to the anti-inflammatory action of adenosine and consequently reduced the liver damage. Also, it is interesting that caffeine is detected in habitual caffeine-consuming humans at concentration capable of antagonizing A2AR in moderate to heavy caffeine consumers (Cook et al., 1996).

Our data showed that high dose of caffeine (either single or repeated) does not seem to have any pro-apoptotic properties on hepatocytes in vivo but, on contrary caffeine produce anti-apoptotic properties, and this is in consistence with Corsetti et al. (2008) who reported that even a single very high dose of caffeine does not seem to have any pro-apoptotic properties on cardiomyocytes in vivo.

Caffeine in high dose decreased liver enzymes and these biochemical restorations could be due to its ability to inhibit the cytochrome P450, also, coffee ingestion modulates phase II of the hepatic biotransformation system improving the hepatic detoxification enzyme system against toxic xenobiotic like PCM (Abreu and Moraes-Santos, 2011).

Yukawa et al. (2004) suggested that consuming 150 ml (8 g) of coffee 3 times a day for 7 days resulted in reduction in the level of MDA in the serum indicating the benefit of high caffeine dose. The previous results together with the results of Pasaoglu et al. (2011) who reported that reduced level of MDA in liver is especially sensitive to dosage so, provide high liver protection with the use of high caffeine dose 100 mg/kg, in addition to our results that revealed high degree of liver protection by using the same high caffeine dose, suggesting that the effect of caffeine may be dose dependent .

The histopathological results revealed nearly complete protection of liver after treatment with 100 mg/kg caffeine plus PCM intoxication that can be explained by Ekam and Ebong (2007) who reported that the molecular mechanism by which caffeine reduce liver damage may be due to their ability to maintain liver cell integrity and triggering immune system. even in the presence of hepatotoxic agent such as PCM.

In contrast to the present study, Ita et al. (2009) reported that high dose of caffeine increase risk of PCM hepatotoxicity marked by high levels of liver enzymes, so used herbal extract to protect rats from PCM 600 mg/kg and caffeine 100 mg/kg. Also, Johnkennedy and Adamma (2011) revealed that 600 mg/kg PCM plus 100mg caffeine for 14 days elevated serum enzyme activities such as AST, ALT, and ALT higher than the PCM group only.

There are very little data regarding the apoptosis after acute caffeine consumption in vivo. However, in vitro studies, caffeine has been reported to affect cell cycle function, including programmed cell death or apoptosis and perturbing key cell cycle regulatory proteins. Therefore, the family of Bcl-2 related proteins plays a key role in the regulation of apoptosis (Bode and Dong, 2007).

Normal liver sections showed no evi-

dence of Bcl2 protein expression by hepatocytes and this was consistent with several reports who demonstrated that Bcl2 is not expressed in normal hepatocytes (Mochizuki et al., 1999) while in PCM intoxicated rats marked Bcl2 expression in liver indicate massive hepatic necrosis.

Caffeine can reduce hepatic necrosis, and promote cell survival, so, this study provides a new insight into the mechanism by which caffeine dose can modulate the hepatoprotection. Thus, decrease in expression of Bcl-2 may determine survival following the apoptotic stimuli of PCM and augments the anti-apoptotic effect of Bcl-2 gene. Thus Bcl2 is important and critical in cell fate decisions, and in detection of abnormal cells in cancer and apoptosis.

Conclusion: Repeated low caffeine doses or high caffeine dose (either single or repeated) appears to help the liver to become more resistant to damage caused by toxins as PCM. The antioxidant activity exhibited by caffeine and its hepatoprotective action against acute PCM toxicity make it a potential agent against liver diseases and other pathologies associated with oxidative stress.. In combined drugs, paracetamol and caffeine, it is recommended to increase the dose of caffeine while decrease the dose of PCM. Therefore, a dose response study may be necessary to identify the optimal dose range at which caffeine is beneficial. Also, chronic caffeine consumption even at low doses can protect against PCM hepatotoxicity. Other studies must be performed to evaluate the effect of caffeine on other body organs and systems.

## *REFERENCES*

**Abreu, R.V. and Moraes-Santos, T. (2011):** "The protective effect of coffee against paracetamol-induced hepatic injury in rats". Journal of Food Biochemistry, 35: 1653-1659.

**Aebi, H. (1974):** Catalase. In: Methods of Enzymatic Analysis. Bergmeyer, H.U. (Ed), Academic Press, New York and London, P.P. 673-677.

**Avila, D.S.; Palma, A.S.; Colle, D.; et al. (2011):** "Hepatoprotective activity of a vanylic telluride against acute exposure to acetaminophen". European Journal of Pharmacology, (1-3): 92-101.

**Bancroft, G.D. and Gamble, M. (2008):** Theory and practice of histological techniques. 6th ed., Churchill Livingstone. New York, London, Philadelphia. P.P. 125-138.

**Bode, A.M. and Dong, Z. (2007):** "The enigmatic effects of caffeine in cell cycle and cancer". Cancer Lett., 247:26-39.

**Butt, M.S. and Sultan, M.T. (2011):** "Coffee and its consumption: benefits and risks". Crit. Rev. Food Sci. Nutr., 51: 363-373.

**Çakatay, U.; Telci, A.; Kayah, R.; et al. (2003):** "Relation with aging with oxidative protein damage parameters in the rat skeletal muscle". Clinical Biochemistry, 36:51-55.

**Chen, Y.H.; lin,F.Y.; Liu, P.L.; et al. (2009):** "Antioxidative and hepatoprotective effects of magnolol on acetaminophen-induced liver damage in rats". Archives of Pharmacol. Research, (2): 221-228.

**Cook, D. G.;Peacoch, J.L.; Feyerabend, I.M. et al. (1996):** "Relation of caffeine intake and blood caffeine concentrations during pregnancy to fetal growth: Prospective population based study". B. M. J., 313: 1358-1362.

**Corsetti, G.; Pasini, E.; Assanelli, D. and Bianchi, R. (2008):** "Effects of acute caffeine administration on NOS and Bax/ Bcl-2 expression in the myocardium of rat". Pharmacol. Res., 57:19-25.

**Demirtas, C.; Ofluoglu, E.; Hussein, A. and Pasaoglu. H. (2012):** "Effects of caffeine on oxidant-antioxidant mechanisms in the rats liver". Gazi. Med. J., 23:13-18.

**Ekam, V. S. and Ebong, P.E. (2007):** "Serum proteins and enzyme levels in rats following administration of antioxidant vitamins during caffeinated and non-caffeinated paracetamol induced hepatotoxicity". Niger. J. Physiol. Sci., 22 (1-2): 65-68.

**Ekor , M.; Adepoju, G.K.A. and Epoyun, A.A. (2006):** "Protective effect of the methanolic leaf extract of Persea Americana (Avocado) against paracetamol-induced acute hepatotoxicity in rats". Intr. J. Pharmacol., 2(4): 416-420.

**Fredholm, B.B.; Battig, K.; Holmen, J.; et al. (1999):** "Actions of caffeine in the brain with special reference to factors that contribute to it its widespread use". Pharmacolgy Reviews, 51:84-125.

**Goldstein, J.; Silberstein, S. D.; Saper, J. R.; et al. (2006):** "Acetaminophen, aspirin and caffeine in combination versus ibuprofen for acute migraine: results from a multicenter, double-blind, randomized, parallel-group, single-dose, placebo- controlled study". Headache, 46:444-453.

**Graham, G. G.; Davies, M. J.; Day, R. O.; et al. (2013):** "The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharma-cological findings". Inflammopharmacology, 21(3): 201-232.

**Ita, S.O.; Akpanyung, E. O.; Umoh, B. I.; et al. (2009):** "Acetaminophen induced hepatic toxicity: protective role of Ageratum conyzoides". Pak.J. Nutr., 8(7): 928-932.

**Johansson, B.; Georgiev, V.; Lindstrom, K. and Fredholm, B.B. (1997): "**A1 and A2A adenosine receptors and A1 mRNA in mouse brain: effect of long-term caffeine treatment". Brain Res., 762: 153-164.

**Johnkennedy, N. and Adamma, E. (2011):** "The protective role of Gongronema latifolium in acetaminophen induced hepatic toxicity in Wister rats". Asian Pacific Journal of Tropical Biomedicine, 1 (Suppl. 2): 151-154.

**Kim, K. A.; Lee. W.H.; Kim, J. K.; et al. (2001):** "Mechanism of refractory ceramic fibre-and rock wool-induced cytotoxicity in alveolar macrophages". Arch. Occup. Health, 74: 9-15.

**Kind, P.R. and King, E.J. (1954): "**Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antpyrin". J. Clin. Pathol., 7 (4): 322-326.

**Manibusan, M. K.; Odin, M. and Eastmind, D. A. (2007):** "Postulated carbon tetra-chloride mode of action: a review". J. Environ. Sci. Health C. Environ. Carcino. Ecotoxicol. Rev., 25: 185-209.

**Mochizuki, K.; Hayashi, N.; Hiramatsu N.; et al (1999):** "Fas antigen expression in liver tissue of patients with chronic hepatitis". B. J. Hepatol., 24:1-7.

**Modi, A.A.; Feld, J.J.; Kleiner, D.E. et al. (2010):** "Increased caffeine consumption is associated with reduced hepatic fibrosis". Hepatology, 51 (1): 201-209.

**Mukhopadhyay, S. Mondal, A. and Poddar, M.K. (2003):** "Chronic administration of caffeine; effect on the activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice". Indian Journal of Experimental Biology, 41:283-289.

**Noschang, C.G.; Krolow, R.; Pettenuzzo, L. F.; et al. (2009):** "Interactions between chronic stress and chronic consumption of caffeine on the enzymatic antioxidant system". Neurochem. Res., 34:1568-1574.

**Ohkawa, H.; Ohishi, N. and Yagi, K. (1979):** "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". Analytical Biochemistry, 95:351-358.

**Ohta, A.; Lukashev, D.; Edwin, K.; et al. (2007):** "1,3,7-Trimethylxanthine (Caffeine) may exacerbate acute inflammatory liver injury by weakening the physiological immunosuppressive mechanism". J. Immunol., 179: 7431-7438.

**Pasaoglu, H.; Ofluoglu, E.; Demirtas. C.; et al. (2011):** "The effect of caffeine on oxidative stress in liver and heart tissues of rats". Turk. J. Med. Sci., 41:665-671.

**Perrone, S.; Mussap, M.; Longini, M.; et al. (2007):** "Oxidative kidney damage in preterm newborns during perinatal period". Clinical Biochemistry, 40: 656-660.

**Prabu, K.; Kanchana, N. and Sadiq, A. M. (2011):** "Hepatoprotective effect of eclipta alba on paracetamol induced liver toxicity in rats". J.Microbiol. Biotech. Res., 1(3): 75-79.

**Ramos-Vera, J. A. and Miller, M. A. (2014):** "When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry-the red, brown, and blue technique". Veterinary Pathology, 51 (1): 42-87.

**Reitman, S. and Frankel, A.S. (1957):** "Cloromertic method for the determination of serum glutamic oxaloacetic pyruvic transaminase". Am. J. Clin. Pathol., 28:53-56.

**Ruhl, C.E. and Everhart, J.E. (2005):** "Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States". Gastroenterology, 128:24-32.

**Sato, C. and Izumi, N. (1989):** "Mechanism of increased hepatotoxicity of acetaminophen by the simultaneous administration of caffeine in the rat". J. Pharmacol. Exp. Ther., 248: 1243-1247.

**Sato, C. N.; Izumi, T.; Nouchi, Y.; et al. (1985):** "Increased hepatotoxicity of acetaminophen by concomitant administration of caffeine in the rat". Toxicology, 34: 95-101.

**Sawynok, J. (2011):** "Methylxanthines and pain". Handb. Exp. Pharmacol., 200:311-329.

**Semler, D.E. (1992):** The rat toxicology. In: Animal Models in Toxicology. Gad, S. C. and Chengelis, C.P. (Eds.). Chapter 2, Marcl Dekker, Inc., New York, Basel and Hong Kong, P.P. 21-75.

**Subramanian, M.; Balakrishnan, S.; Chinnaiyan, S.K.; et al. (2013):** "Hepatoprotective effect of leaves of Morinda Tinctoria Roxb. Against paracetamol induced liver damage in rats". Drug Intervention Today, 3: 223-228.

**Watkins, P.B. and Seef, L.B. (2006):** "Drug induced liver injury: Summary of a single topic research committee". Hepatology, 43: 618-631.

**Witko-Sarsat, V.; Friedlander, M.; Khoa, T.N.; et al. (1998):** "Advanced oxidation protein products as novel media-

tors of inflammation and monocyte activation in chronic renal failure". The Journal of Immunology, 161:2524-2532.

**Yahya, F.; Mamat, S.S.; Kamarolzaman, M. F. F.; et al. (2013):** "Hepatoprotective activity of methanolic extract of Bauhinia purpurea leaves against paracetamol-induced hepatic damage in rats". Evidance-Based and Complimentry Alternative Medicine, Article ID 636580, 10 pages.

**Yen, F. L.; Wu, T.H.; Lin, L.T. and Lin, C.C. (2007):** "Hepatoprotective and antioxidant effects of Cuscuta chinensis against acetaminophen-induced hepatotoxicity in rats". Journal of Ethopharmacology, (1): 123-128.

**Yi-Sun, S.; Oberly, L.W. and Li, Y. (1988):** "A simple method for clinical assay of superoxide dismutase". Clin.Chem., 34:497-500.

**Yukawa, G.S.; Mune, M.; Otani, H.; et al. (2004):** "Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans". Biochemistry, 69: 70-74.

**Zakaria, Z.A.; Rofiee, M.S.; Somchit, M. N.; et al. (2011):** "Hepatoprotective activity of dried-and fermented-processed virgin coconut oil". Evidence-Based Complementray and alternative Medicine. Article ID 142739, 8 pages.

# دراسة التأثيرات المعدلة للكافيين ضد الأضرار الكبدية الناتجة عن التسمم الحاد بالباراسيتامول في ذكور الجرذان البيضاء

المشتركون فى البحث

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تعد القهوة المشروب الأكثر استهلاكاً في العالم حيث أنها تؤثر على جميع أعضاء وأجهزة الجسم. دراسة التأثيرات المعدلة للكافيين على الأضرار الكبدية الناتجة من التسمم الحاد بالباراسيتامول في ذكور الجرذان البيضاء البالغة وأيضا دراسة مدى دلالة مضاد الموت المبرمج للخلايا البي سي ل ٢ على هذه التأثيرات . تم استخدام ٨٠ جرذ قسمت إلى ٤ مجموعات رئيسية متساوية: المجموعة الأولى: (المجموعة الضابطة السالبة)، المجموعة الثانية: (المجموعة الضابطة الموجبة) والتي قسمت إلى ٤ مجموعات فرعية متساوية: (٢ أ) أعطيت الماء المقطر، (٢ ب) أعطيت ٣٠ مللجم/كجم كافيين لمدة ١٤يوم (٢ج) أعطيت ١٠٠مللجم/كجم كافيين لمدة ١٤يوم (٢ د) أعطيت الجرعة المسببة للتسمم الحاد بالباراسيتامول ٢جم/كجم وذلك في اليوم الرابع عشر فقط، المجموعة الثالثة (مجموعة مركب الباراسيتامول والكافيين ولم تعطى الكافيين مسبقاً أي جرعة واحدة) والتي قسمت إلى مجموعتين فرعيتين متساويتين (٣ أ) أعطيت ٣٠ مللجم/ كجم من الكافيين مع الباراسيتامول في اليوم الرابع عشر فقط والأخرى (٣ ب) أعطيت ١٠٠ مللجم/ كجم من الكافيين مع الباراسيتامول في اليوم الرابع عشر فقط، المجموعة الرابعة (مجموعة مركب الباراسيتامول والكافيين وقد أعطيت الكافيين بتكرار يومياً لمدة ١٤ يوم) والتي قسمت إلى مجموعتين فرعيتين متساويتين (٤ أ) أعطيت ٣٠ مللجم/ كجم من الكافيين المعتاد مع الباراسيتامول في اليوم الرابع عشر والأخرى (٤ب) أعطيت ١٠٠مللجم/ كجم من الكافيين المعتاد مع الباراسيتامول في اليوم الرابع عشر. تم ذبح الجرذان والحصول على عينات من الدم لقياس وظائف الكبد كما تم أخذ عينات من أنسجة الكبد لتحليل دلالات أكسدة الدهون والبروتينات بها وكذلك فحص الأنسجة بواسطة المجهر الضوئي باستخدام صبغة الهيماتوكسلين والأيوسين وأيضاً الفحص الهستوكيميائي المناعي من خلال استخدام بروتين البي سي ل ٢ . أدى التسمم الحاد بالباراسيتامول إلى إرتفاع ذو دلالة إحصائية في إنزيمات الكبد بالدم وودلالات أكسدة الدهون والبروتينات في أنسجة الكبد أما الفحص المجهري فقد أظهر وجود إلتهاب كبدي حاد في صورة نخروموت الكثير من خلايا الكبد مع وجود نزيف شديد بها وبالفحص المناعي يتضح أعلى ظهور إيجابي لبروتين البي سي إل ٢ والتي لم تتأثر عند إعطاء جرعة واحدة صغيرة من الكافيين مع الباراسيتامول في حين لوحظ تحسن واضح في كل الفحوص السابقة عند استخدام نفس الجرعة من الكافيين يومياً لمدة ١٤يومَ. وعند استخدام جرعة كبيرة من الكافيين سواء جرعة واحدة أو متكررة فقد لوحظ تحسن ملحوظ في خلايا الكبد يقترب من الطبيعي مع حدوث تفاعل سلبي للبي سي ل ٢. أظهرت النتائج أن الكافيين له آثار إيجابية على الكبد وإنزيماته عند التعرض للتسمم الحاد بالباراسيتامول بالإضافة إلى تحسن دلالات أكسدة الدهون والبروتينات وكذلك الصورة الباثولوجية والهستوكيميائية المناعية وذلك عند تكرار أخذه ولو بجرعات صغيرة. أما عند استخدامه بجرعات كبيرة نجد سرعة عودة خلايا الكبد إلى طبيعتها سواء كان هناك تكرار أخذ الكافيين مسبقاً أم لا.

لذا في ضوء نتائج الدراسة الحالية، نوصي باعتياد تناول الكافين بجرعات صغيرة مع عمل متابعة مستمرة لوظائف الكبد كما ننصح بزيادة نسبة الكافيين وتقليل نسبة الباراسيتامول، كما نوصي بإجراء أبحاث أخرى لمعرفة تأثيرالكافيين على أجهزة الجسم المختلفة.