

THE EFFECT OF SOME PLANT OILS ON LIVER EFFICIENCY AFTER EXPOSURE TO CEMENT IN RATS

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ABSTRACT

This study aimed at investigating possible protective effects of some plant oils on cement induced liver toxicity in rats. Methods: Forty Sprague Dawley rats divided into five groups (8 rats/group; four males and four females). Animals fed for 4 weeks the following diets: (I) basal/standard diet, negative control; (II) basal diet contains cement (1.5g/Kg body weight), positive control; (III, IV, and V) basal diet contains 10% (coconut oil or flaxseed oil or olive oil) plus the same dose of cement, as treatment groups.

Results: Cement exposure resulted in a significant elevation in liver enzymes (Alanine transaminase (ALT), Aspartate transaminase (AST), lactate dehydrogenase (LDH), and along with elevating the level of hepatic tissue malondialdehyde (MDA). Plant oils reinstated most of the altered measured parameters. Conclusion: Supplementation of diet with coconut oil, flaxseed oil, and olive oil was effective in modulating some aspects of cement induced liver toxicity.

Keywords: cement; liver efficiency; olive oil; flaxseed oil; coconut oils; rats.

INTRODUCTION

Cement is widely used construction materials. Anyone who uses, responsible for managing or supervising its use cement (or mixtures containing it) should be aware that it may be a hazard to health. Safe working practices must be used to minimize risk (Mehraj, *et al.*, 2013). The wide use

of cement enhances the probability of exposure via several routes such as inhalation, oral ingestion and the dermal route (Farheen *et al.*, 2017). The gastrointestinal tract could be a significant route for the absorption of cement. Entrances of cement to the body with contaminated food or water or via eating with contaminated hands suggest a potentially high incidence of oral exposure to cement (Hughes and Ferrett, 2015 and Richard *et al.*, 2016). Many studies have revealed that the increased exposure to cement could cause an inflammatory reaction, oxidation damage and serious damage to the liver, kidney, and lung (Emmanuel *et al.*, 2015 and Richard *et al.*, 2016). Based on the key role of oxidative stress in cement induced toxicity, natural antioxidant has attracted the attention of many researchers in this field as adjuncts or alternative synthetic drugs. Previous studies have shown antioxidant and anti-inflammatory effects of coconut oil, flax seed oil, and olive oil (Cline, 2015).

Olive oil is rich in polyphenol compounds that possess anti-inflammatory and antioxidant properties and exerts preventive effects of immune-inflammatory (Aparicio Soto *et al.*, 2017).

Flax seed oil contains a good amount of Linolenic acid (ALA), omega-3 fatty acid, protein (arginine, aspartic acid, and glutamic acid), (Ganorkar and Jain, 2013). Flax seeds have three different types of Phenolic compounds– Phenolic acids, flavonoid, and lignin's (Kajla *et al.*, 2015). These Phenolic compounds are well-known for anti-oxidative stress. Wherefore flax seed oil possesses anti-hyperlipidemia and anti-inflammatory activities and may

reduce the risk factors of cardiovascular disease (Elimam and Ramadan, 2018).

Coconut oil has antioxidant properties due to its high polyphenols which prevented lipid peroxidation and increased the antioxidant enzymes in the osteoporosis rat model (Abujazia *et al.*, 2012). According to this knowledge, possible beneficial effects of these plant oils on cement induced toxicity in rats have not yet been tested.

Therefore, this study aimed at investigating the potentially hazardous effect of oral exposure to cement on the liver in rats, and also, to study the possible ameliorative effect of some plant oils against induced toxicity.

MATERIALS AND METHODS

Materials:

Chemicals: Cement purchased from the local markets. The addition of cement to diet by dose (1.5 g/ kg) was according to the LD₅₀ test. Pure olive oil, flaxseed oil and coconut oil purchased from the official markets of the Ministry of Agriculture (Dokki, Cairo, Egypt). Kits for measurements of AST, ALT and LDH obtained from analytic on (Germany). All other chemicals were of analytical grades.

Experimental animals: Forty albino rat's male and female "Sprague Dawley strain" weighing 175±25g obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Helwan, Cairo, Egypt.

Diets: Standard diet prepared according to Reeves *et al.* (1993).

Methods:

Biological Experiments: All rats were allowed to be adapted to the new environment (*i.e.*, temperature, humidity, light, and dark as well as diet and water) for one week before the onset of the experiment and kept individually in wire cages under standard and hygienic conditions. Food and water provided ad-libitum and checked daily.

Acute Toxicity Test: The acute toxicity of cement dust was measured using the classical LD₅₀ method as described by Gabriel *et al.* (2008).

Experimental design: The rats were divided into five groups (8 rats/groups, four male plus four females) fed on 10% from diet plant oils as follows:

- Negative/Normal control group (G1): fed on basal diet.
- Positive/Sufferer control group (G2): fed on basal diet containing cement (1.5 g/ kg diet).
- Coconut oil group (G3): fed on basal diet contain cement (1.5 g/ kg diet) and 10% coconut oil instead of corn oil.
- Flaxseed oil group (G4): fed on basal diet contain cement (1.5 g/ kg diet) and 10% flaxseed oil instead of corn oil.
- Olive oil group (G5): fed basal diet contains cement (1.5 g/ kg diet) and 10% olive oil instead of corn oil.

Biological evaluation: Feed intake was calculated as g/day /rat by weighing the diets three times per week. All rats were individually weighed. The difference between the initials and final weights were calculated to determine body weight change (g).

Bodyweight gain (BWG) = Final weight (g) – Initial weight (g)

Feed efficiency ratio (FER): was calculated according to the following equation as mentioned by Eggum *et al.* (1973)

FER = Body weight gain (g) / Feed intake (g)

At the end of the experimental period (4 weeks), animals were overnight fasted and allowed free access to water only then rats were sacrificed under anesthesia. The blood samples were collected directly from the portal vein into non-heparinized centrifuge tubes. Serum aliquots were separated by centrifugation at 4000 rpm for 15 minutes and it frozen at -20°C for subsequent biochemical analysis. Immediately after sacrificing rats, livers excised from rats, washed in cold saline, blotted in filter paper and stored at – 20°C for the determination of MDA.

Biochemical analysis: The level of AST, ALT and LDH activities were estimated by spectrophotometric methods according to Bergmeyer *et al.*, (1986); Schumann *et al.*, (2002) and Gay *et al.*, (1968) respectively. Lipid peroxidation was estimated in liver homogenates by measuring malondialdehyde production formed in the thiobarbituric acid reaction according to the method of Uchiyama and Mihara (1978).

Statistical analysis: Statistical analysis was carried out using the program of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 11; Untitled–SPSS Data Editor). The results were expressed as mean ± standard deviation (mean ± SD). Data were analyzed using one-way classification, analysis of variance (ANOVA). The differences between

means were tested for significance using the least significant difference (LSD) test at $p < 0.05$. Independent T-test was also used to determine the statistical difference between two means and percentage change (Snedecor and Cochran, 1979)

RESULTS AND DISCUSSION

Table (1): Effect of oils on feed intake (FI), body weight gains (BWG) and feed efficiency ratio (FER).

| Groups | Negative control (G1) | Positive control (G2) | Coconut oil (G3) | Flaxseed oil (G4) | Olive oil (G5) | P value |
|------------------------------|------------------------|------------------------|-------------------------|------------------------|-------------------------|---------|
| FI (g/day/rat) | 14.1±0.5 ^a | 10.4±0.6 ^c | 12.5±0.6 ^b | 12.4±0.8 ^b | 11.6±0.7 ^b | <0.05 |
| % of change | | | | | | |
| Compared to negative control | -- | -26.2% | - 11.4% | -12.1% | -17.7% | |
| Compared to positive control | 35.6% | -- | 20.2% | 19.2 | 11.5 | |
| BWG (g/4weeks) | 84.0±6.0 ^a | 36.9±2.2 ^c | 52.8±2.9 ^b | 51.2±7.1 ^b | 48.0±4.7 ^b | <0.05 |
| % of change | | | | | | |
| Compared to negative control | -- | -56.1% | -37.1% | -39.1% | -42.9% | |
| Compared to positive control | 127.6% | -- | 43.1% | 38.8% | 30.1% | |
| FER | 0.20±0.04 ^a | 0.11±0.01 ^c | 0.14±0.01 ^{bc} | 0.17±0.0 ^{ab} | 0.18±0.01 ^{ab} | <0.05 |
| % of change | | | | | | |
| Compared to negative control | -- | -45% | -30% | -15% | -10% | |
| Compared to positive control | 82% | -- | 27% | 55% | 64% | |

These values are expressed as mean ±SD and percent change (n=8 rats /group).

The different small letters in row means that there is a significant difference between groups at ($P < 0.05$), the same letters mean that there are no significant differences between groups.

Data presented in table (1) showed that feed intake, body weight gain, and feed efficiency ratio were significantly ($P < 0.05$) decreased in rats exposed to cement as compared to the negative control. This decrease was 26.2%, 56%, and 45%, respectively. Feed intake and BWG diminution may be due to the presence of some elements in the cement such as (silica, clay, organic matters or hydrocarbons) which affect the taste and may lead to loss of appetite and followed by weight loss. This may be due to regulatory mechanisms that control the feeding and feeding behavior of animals. Some of these factors include gastrointestinal factors, feedback from fat, and glucostatic mechanisms which are all considered internal factors (Dauda *et al.*, 2016). Also, toxic contents of cement (i.e. heavy metals) can cause severe damage through inhibition of enzymes, and interference with normal metabolic pathways in vital organs leading to high free radical generation (Koh *et al.*, 2011). Also, table (1) indicated an augmentation in feed intake by 20.1%, 19.2%, and 11.5%; increase in body weight gain by 43%, 38.7%, and 30% that lead to boost in feed efficiency ratio by 27.3%, 54.5%, and 63.6% in the groups fed on coconut oil, flaxseed oil and olive oil, respectively, as compared with positive control. There were non-significant between three oil groups. The best improvement for FI and BWG was in the coconut oil group, followed by the flaxseed group, but FER was in the group received olive oil. These results were as well may be coconut oil contents approximately 86.75% saturated fatty acids (Handayani *et al.*, 2009 and Elsayed *et al.*, 2015)

as long-chain saturated fatty acid, which caused improve loss of weight by fed cement, this due to increase BWG and still lower than normal control.

Coconut oil is high lauric acid content (48.5%) as medium-chain fatty acids (MCFAs) helps to boost metabolism (Thijssen, and Mensink. 2005; Handayani *et al.*, 2009). MCFAs are burned up immediately after consumption and therefore the body uses them immediately to make energy (Enig, 1996); so maybe intension FI. Flaxseed oil has health benefits besides the nutrition due to its high content of polyunsaturated fatty acids as (α -linolenic acid (ALA), (Goyal *et al.* 2014); so maybe ameliorate palatability. Olive oil contains polyphenols have been a beneficial impact on health (Gorzynik-Debicka *et al.*, 2018)

Table (2): Effect of coconut oil, flaxseed oil and olive oil on serum ALT, AST and LDH in cement exposed rats' group

| Groups | Negative control (G1) | Positive control (G2) | Coconut oil (G3) | Flaxseed oil (G4) | Olive oil (G5) | P-value |
|------------------------------|--------------------------|--------------------------|----------------------------|---------------------------|--------------------------|---------|
| ALT (U/L) | 40.3±8.3 ^a | 76.1±10.2 ^b | 51.7±13.9 ^a | 45.0±8.5 ^a | 52.8±6.9 ^a | <0.05 |
| % of change | | | | | | |
| Compared to negative control | -- | 88.8% | 28.3% | 11.7% | 31% | |
| Compared to positive control | -47% | -- | -32.1% | -40.9% | -30.1% | |
| AST (U/L) | 121.7±7.8 ^a | 209.9±29.5 ^c | 177.7±18.4 ^{bc} | 145.5±12 ^{.3ab} | 146.0±14 ^{.1ab} | <0.05 |
| % of change | | | | | | |
| Compared to negative control | | 72.5% | 46% | 19.6% | 20% | |
| Compared to positive control | -42% | -- | -15.3% | -30.7% | -30.4% | |
| LDH (U/L) | 1162.8±16.3 ^a | 1804.0±49.6 ^c | 1296.5±23 ^{5.5ab} | 1403.7±1 ^{48.8b} | 1483.8±5 ^{3.1b} | <0.05 |
| % of change | | | | | | |
| Compared to negative control | -- | 55.1% | 11.5% | 20.7% | 27.6% | |
| Compared to positive control | -35.5% | -- | -28.1% | -22.2% | -17.8% | |

These values are expressed as mean ±SD (n=8 rats /group)

The different small letters mean that there are significant differences between groups (p <0.05), the same letters means that there is no significant difference between groups

The findings recorded in the table (2) demonstrated that cement exposure resulted in significant (P<0.05) elevation in serum activities of ALT, AST,

and LDH by 88.8%, 72.4%, and 55.2% respectively, as compared to the negative control. These results were compatible with those reported by Vitaglione *et al.*, (2005); Tirajumalau *et al.*, (2016), and Ojevwe and Osadolor, (2017) who found elevation in the levels of hepatic enzymes (ALT, AST) with consuming cement. These results may be appeared in the liver probably because liver mainly responsible for detoxification of foreign compounds in the body. The serum level of these enzymes is very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the bloodstream. This can be quantified to assess the type and extent of liver injury (Mayer and Kulkarni, 2001; Thapa and Walia, 2007). Also, LDH is especially concentrated in the heart, liver red blood cells, kidney, muscles, brain and lungs, thus the damage of any these organs could elevate LDH in serum (AL-Hayali 2009).

Regard to table (2) showed that all the tested oils had a hepatoprotective effect as evidenced by a significant reduction in serum activities of ALT, AST, and LDH. As coconut oil significantly ($p<0.05$) reduced their activities by 32%, 15.3% and 28.1%, while flaxseed oil significantly ($p<0.05$) reduced their activities by 40.8%, 30.6% and 22.2%, and olive oil significantly ($p<0.05$) reduced their activities by 31%, 30.4%, and 17.8%, respectively Coconut oil, flaxseed oil, and olive oil have level improvement of hepatic enzymes ALT, AST and LDH this due to all the tested oils there rich in antioxidants (polyphenols and tocopherols).

Table (3): Effect of coconut oil, flaxseed oil and olive oil on hepatic tissue MDA in cement exposed rats

| Groups | Negative control (G1) | Positive control (G2) | Coconut oil (G3) | Flaxseed oil (G4) | Olive oil (G5) | P-value | |
|------------------------------|------------------------|------------------------|--|----------------------------|----------------------------|---------|--|
| MDA (U / mg liver tissue) | 0.56±0.25 ^d | 2.16±0.97 ^a | 0.83±0.37 ^c _d | 0.97±0.4 3 ^c | 1.29±0.5 8 ^b | <0.05 | |
| % of change | | | | | | | |
| Compared to negative control | -- | 285.7% | 48.2% | 73.2% | 130.4% | | |
| Compared to positive control | -74.1% | -- | -61.6% | -55.1% | - 40.3% | | |

These values are expressed as mean ±SD (n=8 rats /group)

The different small letters mean that there are significant differences between groups (p < 0.05), the same letters mean that there is no significant difference between groups

Table (3), evidenced that oral cement exposure promotes lipid peroxidation by increasing the level of hepatic tissue MDA by 285.7 % as compared to the negative control. This elevation in hepatic tissue MDA could be because cement induced formation of free radicals, and also through exhaustion of antioxidant leading to oxidative stress (Prabu *et al.*, 2012). The three plant oils were all found to offer significant protection against the hepatotoxic effects of cement. Besides contributing to the maintenance of the membrane integrity of hepatocytes, as inferred from lower serum activities of the measured liver enzymes, these oils were also able to markedly reduce the production of MDA. The level of hepatic tissue MDA was significantly (p<0.05) reduced by 61.5%, 55.1% and 40.3% in all groups fed the three oil,

respectively. These results strongly support that these oils have powerful antioxidant and hepatoprotective properties against cement induced free radicals damage in the liver.

The free radicals scavenging effects of these oils could be attributed to their higher vitamin E, polyphenols and flavones contents (Cline, 2015; Hussein *et al.*, 2016; Kamisah *et al.*, 2016; Tirajrumalau *et al.*, 2016 and Ojevwe and Osadolor, 2017). The presence of these antioxidant compounds in the tested oils reduces the hepatic lipid peroxidation. Vitamin E helps to protect cells from oxidative stress. It is one of the most important lipophilic antioxidants found in nature and has an important role in preventing lipid peroxidation of cellular membranes and lipoproteins (Piroddi *et al.*, 2017).

Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials. The antioxidant activity of polyphenol is mainly due to their redox properties, which allow their act as reducing agents, hydrogen donor, and singlet oxygen quenchers. And also flaxseed oil contains α -Linolenic acid (ALA) which as anti-inflammatory agent and omega -3 fatty acids. These results are in accordance of Amamou *et al.*, (2015); Senphan and Benjakul, (2016) and Aparicio-Soto *et al.*, (2017).

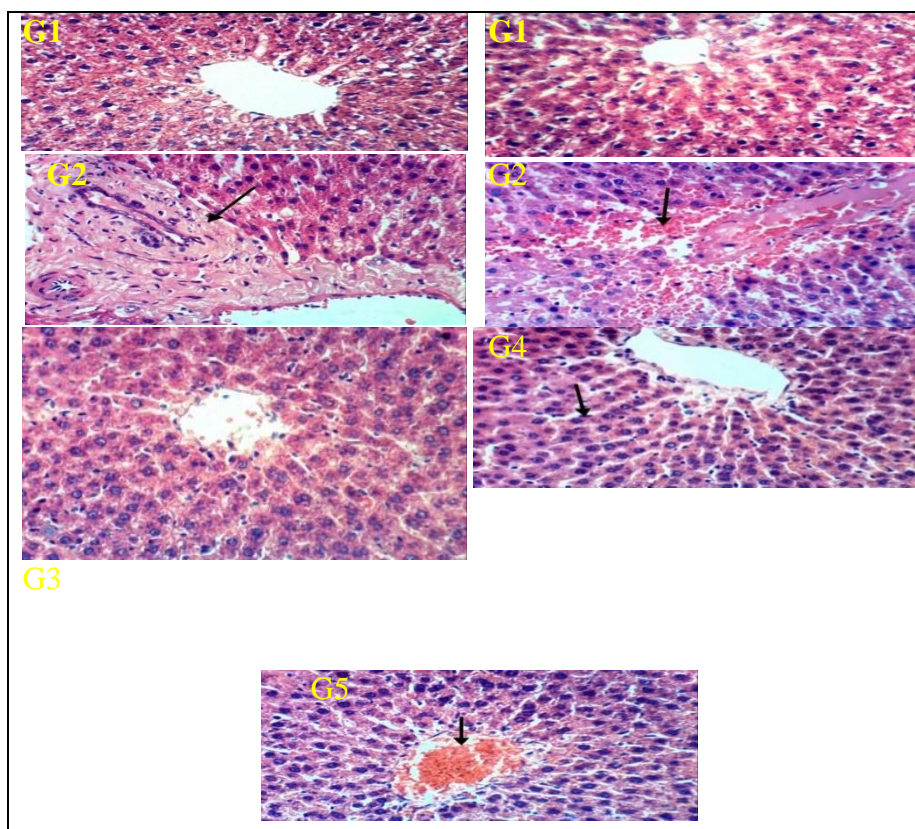


Fig. (1): Histological examination

Figure (1) shows Liver of rat from group (1) showing the normal histological structure of hepatic lobule (H & E X 400). Liver of rat from group (1) shows the normal histological structure of hepatic lobule (H & E X 400). Liver of rat from group (2) shows fibrosis in the portal triad (H & E X 400). Liver of rat from group (2) shows focal hepatic hemorrhage (H & E X 400). Liver of rat from group (3) shows apparent normal hepatic lobule (H &

E X 400). Liver of rat from group (4) shows Kupffer cells activation (H & E X 400). Liver of rat from group (5) shows congestion of central vein (H & E X 400).

Microscopically In contrary, liver of rats from group 2 showed fibrosis in the portal triad and focal hepatic hemorrhage the results are in agreement with Garcia et al. (2017) who observed that the histological finding showed the damage histologically in liver.

Some examined sections from group 3 showed apparent normal hepatic lobule the results also are consistent with Zakaria et al. (2011) who observed preserved hepatic lobular when treated with coconut oil. Kupffer cells activation was the only histopathological finding observed in liver of rats from group 4. The results also are in consistent to Alkhatib et al. (2017) who found that the presence of excess Kupffer cells with a diet rich in flaxseed oil. However, liver of rats from group 5 showed congestion of central vein the results also are in consistent to, Elsayed et al., (2015) who found showing congestion of central vein.

CONCLUSION

In conclusion, the results of the present investigation revealed that cement is capable of producing obvious changes in liver status. Clinical chemistry data showed elevations of ALT, AST, LDH, and MDA. The results obtained in this study illustrate the beneficial effects of the studied oils on improving the antioxidant status and reducing the oxidation damage in the liver.

Accordingly, it is recommended to supplement food with these oils. In this study, was found that flaxseed oil is better than coconut oil and olive oil as the flaxseed oil is rich in omega 3 fatty acids, α -Linolenic acid (ALA) which is an anti-inflammatory agent and Phenolic compounds are well-known for anti-oxidative stress.

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تأثير بعض الزيوت النباتية على كفاءة الكبد بعد التعرض للإسمنت في الجرذان

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المستخلص

يهدف هذا البحث إلي دراسة التأثيرات الناتجة عن التدخل الغذائي ببعض الزيوت النباتية علي حاله الكبد في الجرذان التي تعرضت لتناول وجبة تحتوي علي جرعه من الاسمنت قدرها 1.5 جم/كجم من الوجبه. فقسمت الجرذان الي خمس مجموعات: الاولى تغذت علي الوجبة القياسية/الاساسية كمجموعة ضابطة سالبة. ومجموعة اضيف الاسمنت للوجبة كمجموعة ضابطة موجبة. وثلاث مجموعات تم علاجها باستبدال زيت الذرة بزيت جوز الهند أو زيت الزيتون أو زيت الكتان. أظهرت النتائج زيادة واضحة في إنزيمات الكبد ALT, AST وكذلك LDH في مصل الدم. كما ظهرت الزيادة في أكسدة الدهون (MDA) بانسجة الكبد بالمجموعة الضابطة الموجبة وتحسنت تلك القياسات بالمجموعات التي تغذت علي الزيوت قيد الدراسة. الخلاصة: أوضحت هذه الدراسة أن هذه التغيرات في وظائف الكبد يمكن أن تكون ذات صلة بتطور تلف أنسجة الكبد نتيجة للتعرض لجرعة من الأسمنت، ويمكن التحسن عن طريق إضافة بعض الزيوت النباتية. الكلمات المفتاحية: الإسمنت، كفاءة الكبد، زيت (الزيتون - الكتان - جوز الهند)، الجرذان.