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Effect of Jojoba Oil and Extra Virgin Olive Oil on Genetic Expressions and DNA Damage in Induced CCl_4 Toxicity in Rats

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ABSTRACT

The present study was carried out to investigate the effects of jojoba oil and extra virgin olive oil in normal and in carbon tetrachloride (CCl_4) induced genetic toxicity in male albino rats.

A total of sixty male albino rats (120-140 g) were used in this study. Rats were divided into 6 groups of ten animals each. Group one was kept as a negative control and fed on basal diet only, while the second group kept as positive control and was fed on basal diet and injected with CCl_4 to induce genetic and hepatic toxicity. The third group was fed on basal diet mixed with jojoba oil. The fourth group was fed on basal diet mixed with extra virgin olive oil. The fifth group was fed on basal diet mixed with jojoba oil and injected with CCl_4. The sixth group was fed on basal diet mixed with extra virgin olive oil and injected with CCl_4.

At the end of experimental period, blood samples were collected from each rat for biochemical analysis and rats were then sacrificed to elucidate the effect on genetic and hepatic toxicity.

Subcutaneous injection of CCl_4 caused a significant increase in serum levels of AST, ALT, ALP, triglyceride, total cholesterol, LDL-c and lipid peroxidation (MDA) but it caused a significant decrease of HDL-c and reduced glutathione (GSH).The smear of hepatic DNA on agarose gel had been observed in CCl_4 treated groups indicating random DNA fragmentation and a hallmark of necrosis. The genetic expression of hepatic CYP3A2 and GADD153 genes showed marked up regulation in CCl_4 group. Jojoba oil significantly restored the serum levels of biochemical parameters directed toward normal as compared with the positive control group except for ALT and genetic expression. The extra virgin olive oil showed better amelioration for biochemical and genetic parameters when compared with the positive control group.

INTRODUCTION

CCl_4 is biotransformed in the liver forming reactive metabolic trichloromethyl radicals (¯CCl3) which reacts with excess O2 producing reactive free radicals. These free radicals begin peroxidation of membrane polyunsaturated fatty acids and covalently bind microsomal lipids and proteins producing lipid peroxides leading to cellular disorders and pathological damage **Hiraganahalli et al. (2012); (Nazima and Manoharan, 2014)**

Natural plant oils and food-derived antioxidant vitamins and phenolic phytochemicals have received considerable attention because they are known to function as chemo preventive agents against oxidative damage **(Pérez-Bonilla et al., 2006)**

Jojoba oil is rich in protein, phenolic compounds, phytic acid, and considerable amounts of simmondsin **(Abdel-Wahhab et al., 2016)**

The cis-ll-eicosenoic (jojobenoic) acid is the major acid (71%) in jojoba seed extract also has antioxidant activity and has the ability to bind metal ions **(Bouali et al., 2008). (Zheng and Wang, 2001; Francis et al., 2002)** the higher total phenolic content of jojoba oil suggested another mechanism for its antioxidant activity. **(Aly et al., 2008)** jojoba was rich in saponin which was well known to stimulate the cell-mediated immune system, as well as to enhance antibody production.**(Wang et al., 2006)** jojoba oil content of active polyphenol components such as flavonoids and phenolic acids possess antioxidant activities.

Olive oil, is rich in mono- unsaturated fatty acid content, including oleic acid as well as it contains high amounts of several micronutrient compounds, including polyphenolic compounds such as hydroxytyrosol, tyrosol and oleuropein **(Berrougui et al., 2015).**

Four major classes of polyphenols can be found in extra virgin olive oil including flavonoids, lignans, simple phenols and secoiridoids. The last two groups can be found exclusively in olive oils **(Corona et al., 2009)**.

MATERIALS AND METHODS

Plants oils:

Crude Jojoba oil (wax):

It was purchased from local market from the Egyptian company for plant oils (Egypt)

Extra virgin olive oil:

It was purchased from local market from the colavita co, Italy

Chemicals:

Carbon tetrachloride (CCl_4):

From Sigma Chemical Company It was used as 50% in 1 propylene glycol

Animals:

Sixty white male Albino rats of an average body weight 120- 140 g were used for the experiment. Animals were acclimatized to laboratory condition before being used. Rats were fed on standard diet supplying the essential vitamins, trace elements and water, was supplied given add libitum.

Methods:

Experimental design: groups of rats were as follows:

G1: Negative control group that fed the basic diet for the whole experimental period 30 days.

G2: Positive control group : fed the basic diet and injected with CCl_4 mixed with propylene glycol (1:1) and injected to the rats by subcutaneous rout at medial aspect of the thigh twice / week for two successive weeks by dose (0.1 ml / 100g.bwt) **(Borah et al., 2004).**

G3: Jojoba group: that fed the basic diet mixed with 2.5 % of jojoba oil **(Hanan et al., 1998)** for the whole experimental period 30 days.

G4: Extra Virgin Olive Oil group: that fed basic diet mixed with 5 % of extra virgin olive oil **(Bies, 2006)** for the whole experimental period 30 days.

G5: Jojoba oil group with CCl_4 injection: that fed basic diet mixed with 2.5 % of jojoba oil for 30 days with injection of CCl_4 mixed with propylene glycol (1:1) and injected to the rats by subcutaneous rout at medial aspect of the thigh twice / week for two successive weeks by dose of 0.1 ml / 100g.bwt.

G6: EVOO group with CCl_4 injection: that fed basic diet mixed with 5 % of extra virgin olive oil for 30 days with injection of CCl_4 mixed with propylene glycol (1:1) and injected to the rats by subcutaneous rout at medial aspect of the thigh twice / week for two successive weeks by dose of 0.1 ml / 100g.bwt.

The animals had free access to water and feed.

Blood samples

Blood samples were collected from fasting rats from retro orbital venous plexus by introduction of capillary tube in median canthus of eye on clean plain tubes without EDTA for separation of serum and another tube with EDTA for GSH analysis **(Beutler et al., 1963)**.

Serum:

Clear serum samples used for analysis of hepatic enzymes AST and ALT **(Lorentz, 1998)**, and ALP **(Tietz et al., 1983)**), lipid profile (triglycerides (TG) **(Fossati and Prencipe, 1982)**, total cholesterol (TC) **(Richmond, 1973; Allain et al., 1974)**, high density lipoprotein cholesterol (HDL-c) **(Burstein et al., 1970; Lopes-Virella et al.,**

1977), low density lipoprotein cholesterol (LDL-c) **(Friedewald et al., 1972)**, total plasma protein (TP) **(Gornall et al., 1949)**, albumin plasma protein **(Doumas et al., 1971)**, globulin plasma protein **(Doumas et al., 1972)**, alpha fetoprotein (AFP) **(Wisdom, 1976)**, and malondialdehyde (MDA) **(Ohkawa et al., 1979)**.

Tissue specimens:

Liver: Small piece of hepatic tissue from each animal was cut and kept immediately in liquid nitrogen (-196 C) for mRNA expression **(Riesgo et al., 2012).**

Primers and probe nucleotide sequence specific for used for real time PCR

Gene	Name	Sequence (5'-3')
GAPDH **Chen et al., 1994)**	Fr.	CGGAGTCAACGGATTTGGTCGTAT
	Rev.	AGCCTTCTCCATGGTTGGTGAAGAC
CYP3A2 **(Wauthier et al., 2006**	Fr.	AGTAGTGACGATTCCAACATAT
	Rev.	TCAGAGGTATCTGTGTTT
GADD153 **Chen et al., 1994)**	Fr.	TCTGCCTTTCGCCTTTGAG
	Rev.	GCTTTGGGAGGTGCTTGTG

And another small piece of hepatic tissue from each animal was cut and kept in the freezer **(Seutin et al., 1991)** for DNA fragmentation test on gel.

Statistical analysis:(Miller and Miller, 2005)

Comparison were carried by means using analysis of variance "F test" (ANOVA) using least significant difference "LSD". The level of statistical significance was taken as $P<0.05$.

RESULTS AND DISCUSSION

The present study was carried out to elucidate the effect of jojoba oil and extra virgin olive oil in normal and on carbon tetrachloride (CCl4) induced DNA, damage genetic expression and some serum parameters using one concentration of each oil alone for 30 days in rats.

Effect of jojoba oil and extra virgin olive oil on biochemical analysis of male albino rats with and without CCl_4 injection

CCl_4 injection caused a significant increase in hepatic enzymes (AST, ALT and ALP) while the use of jojoba oil in rats injected with CCl_4 showed a significant decrease in both AST and ALP hepatic enzymes in compare with the positive control group. They expressed a significant increase when compared with the negative control group. These results were in agreement with **(Alkreathy et al., 2014; Abdel-Wahhab et al., 2016; Debib et al., 2016; Hanan et al., 2016)** treated rats**.** While in concern of ALT hepatic enzyme the jojoba oil groups showed a significant increase in relation to the negative control group and caused a decrease in ALT enzyme when compared with the positive control without significant difference **table (2).**

Our finding indicated that jojoba oil not completely reversed CCl_4 hepatotoxicity also the jojoba oil caused elevation of hepatic enzymes **(Stalder et al., 1985; Verschuren, 1988)** and these results may be due to its content of traces of simmondsin and erurcic acid.

The extra virgin olive oil group injected with CCl_4 revealed a significant decrease of hepatic AST, ALT and ALP enzymes in correlation to the positive control group and tended them toward the negative control values. The extra virgin olive oil group didn't show any significant changes in hepatic AST, ALT and ALP enzymes in comparison to the negative control group in agreement with **(Naglaa and Mona, 2015; Debib et al., 2016)** as shown in **table (1).**

The ameliorative effect of extra virgin olive oil may be attributed to the presence of omega-3 fatty acids of virgin olive oil which caused liver regeneration effect **(Owen et al., 2000)**.

Both the jojoba oil and extra virgin olive oil groups injected with the CCl_4 revealed a significant increase in HDL-c in compare with the control +ve group but they showed a significant decrease in cholesterol, LDL-c and TG in comparison to the positive control group (**table 2)**.

Our results for jojoba oil agreed with**(Abdel-Wahhab et al., 2016).** These results may be due to jojoba oil is rich in phytic acid and omega-3 fatty acid. Phytic acid is well known to have anti-radical effects **(Khattab et al., 2010) (Bouali et al., 2008)** also jojoba oil has a high concentration of jojobenoic acid which is mono unsaturated acid. While results of extra virgin olive oil agreed **(Naglaa and Mona, 2015**) and may be due to abundant concentration of the monounsaturated fatty acids, oleic acid in olive oil which has effects on lipid profiles and peroxidation and polyphenolic constituents **(Servili et al., 2013; Debib et al., 2016)**

The CCl_4 expressed a significant increase in cholesterol, LDL-c and triglycerides in comparison with the negative control group. And also had a significant decrease in HDL-c as compared with negative control group. The previous findings were matched with **(Alkreathy et al., 2014)** as reported in **table (2).**

Both jojoba oil and extra virgin olive oil groups injected with CCl_4 showed a significant increase of GSH and a significant decrease of MDA in comparison with the positive control group **table (3).** The results of jojoba oil matched with **(Hanan et al., 2015; Abdel-Wahhab et al., 2016; Hanan et al., 2016)** and may be due to the content of jojoba oil of flavonoids and lignans which have antioxidant effect **(Abdel-Mageed et al., 2014)** also the jojoba oil contain glucoside which has antioxidants activity. The results of extra virgin olive oil matched with **(Naglaa and Mona, 2015; Saber et al., 2015)** and may be due to extra-virgin oil active component is oleuropein **(Owen et al., 2000)**. Oleuropein is able to chelate metal ions which catalyse free radical generation reactions **(Andrikopoulos et al., 2002)**. Oleuropein and its metabolite, hydroxytyrosol, both possess the structural requirement (a catechol group) needed for optimum antioxidant and/or scavenging activity **(Al-Azzawie and Alhamdani, 2006)**.

The CCl_4 also showed a significant decrease in GSH in comparison with negative control group. And this agreed with **(Alkreathy et al., 2014)** while The CCl_4 also showed a significant increase in MDA in comparison with positive control group. And this study was the same as **(Jiang et al., 2015; Debib et al., 2016) table (3).**

Both the jojoba oil and extra virgin olive oil groups injected with CCl_4 showed a significant decrease AFP (tumor marker) in compare with the positive control group **table (6)** results of jojoba oil may be due to the content of jojoba oil of flavonoids and lignans which have antioxidant effect **(Abdel-Mageed et al., 2014)** while results of extra virgin olive oil is due to extra-virgin oil active component is oleuropein **(Owen et al., 2000)**.

The CCl_4 also showed a significant increase in AFP, tumor marker, in comparison with the positive control. And this result matched with **(Akamatsu et al., 2001)**

Effect of jojoba oil and extra virgin olive oil on DNA fragmentation and genetic expressions of livers of male albino rats with and without CCl_4 injection

Targets for free radicals are lipids, DNA and proteins **(Sies, 2007).**

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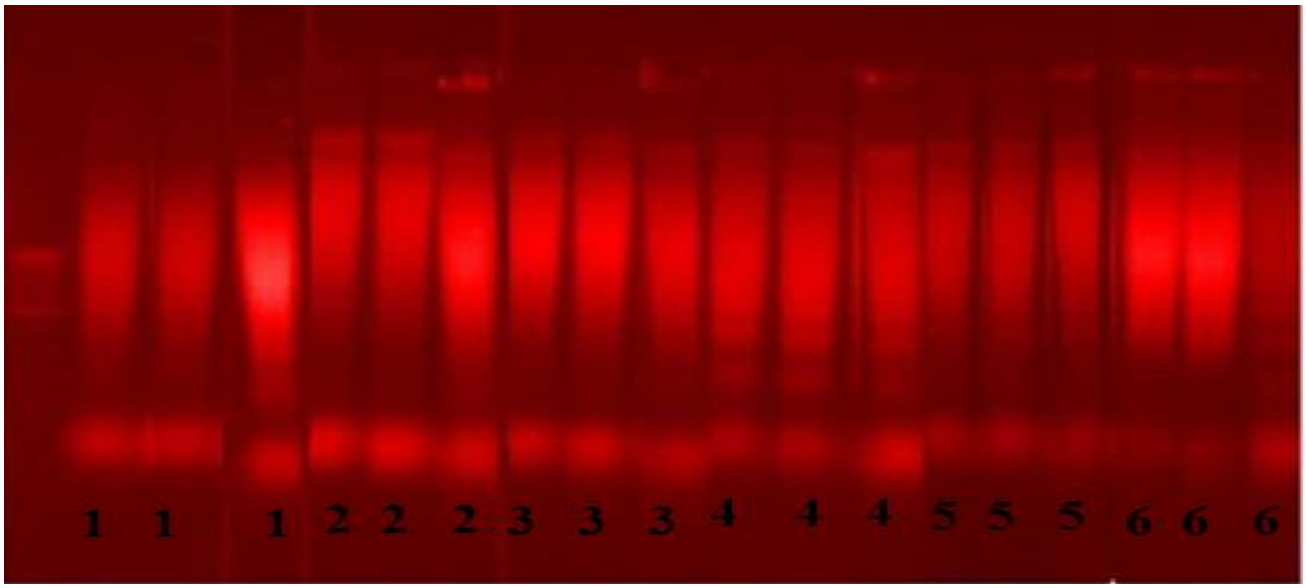
Figure 1: **Agarose gel electrophoresis of undigested DNA stained by ethidium bromide. The DNA was extracted from liver samples of male albino rats :(1) control, (2) CCI4 treated, (3) jojoba oil group, (4) extra virgin olive oil, (5) jojoba oil with CCl_4 injection and (6)extra virgin olive oil with CCl_4 injection. (M) 1 kilo base pair marker, 50 bP ladder size.**



Both jojoba oil and extra virgin olive oil showed a protection against DNA damage of CCl_4 with superiority to extra virgin olive oil. These results may be due to the content of jojoba oil of flavonoids and lignans which have antioxidant effect **(Abdel-Mageed et al., 2014)** glucoside **(Van Boven et al., 2000)**. **(Bouali et al., 2008)** reported that jojoba is rich in phytic acid .Phytic acid is well known to have anti-radical effects by chelating iron. **(Khattab et al., 2010)** Phytic acid was also shown to have anti-cancer property **(Norazalina et al., 2010)**.

The efficacy of olive oil to decrease the DNA damage may be due to its phenolic content that acts as a metal ion chelator and enhances the endogenous antioxidant defense system either by induction of phase II enzymes or by stimulating mitochondrial biogenesis. These effects may also be due to the DNA repair system and protection of DNA repair genes **(Owen et al., 2000; Fabiani et al., 2008).** In agreement to our results of extra virgin olive oil **(Salas-Salvadó et al., 2008; Saber et al., 2015)**

The CCl_4 showed fragments of DNA of rats' livers on agarose gel when compared to the negative control group. The same results were obtained by **(Alkreathy et al., 2014).**

Genetic expressions

Gene expression analysis is sensitive enough to detect early organ damage, even though there were no remarkable changes in the serum chemistry parameters or histopathological findings **(Inadera et al., 2010).** Previous studies showed that in CCl_4 toxicity Genes involved in DNA damage and repairs and Genes involved in different metabolic pathways, including some , were altered in the treated rats **(Carver and Clancy, 2000; Fountoulakis et al., 2002)**

(Jiang et al., 2004) reviewed that acute administration of CCl_4 to rats caused significant changes in gene expression profiles **(Waring et al., 2001; Fountoulakis et al., 2002)** these included DNA damage and stress-related genes such as GADD153 indicating that the regulatory mechanisms that increased by CCl_4 treatment remained in an active state even after CCl_4 cessation. These genes might serve as markers to monitor the recovery of liver injury.

In this study we investigated the effect of CCl_4 on the gene expression in albino rat livers because the gene and protein expression changes after exposure to a toxic compound might help elucidate its mechanism of action and may provide newly applied toxicity markers genes **(Fountoulakis et al., 2002)**.

Effect of jojoba oil and extra virgin olive oil on CYP3A2 gene and GADD153 gene expressions of livers of male albino rats with and without CCl_4 injection

The extra virgin olive oil didn't cause expression changes of CYP3A2 and GADD153 genes in compare with the negative control group but the jojoba oil caused a marked expression up regulation of CYP3A2 gene and no expression changes of the GADD153 gene as compared with the negative control **table (5).**

The extra virgin olive oil group injected with CCl_4 showed a marked down regulation of both CYP3A2 and GADD153 genes in compare with the positive control. While the jojoba oil group injected with CCl_4 showed up regulation of CYP3A2 gene in compare to the positive control group but it showed down regulation of GADD153 expression in compare to the positive control group but still up regulated when compared to the negative control group **table (5).**

The CCl_4 groups showed a marked up regulation of both CYP3A2 and GADD153 genes in compare with the negative control group **table (5).**

(Malekinejad et al., 2010) stated that the hepatic CYP3A2 gene expression increased in case of body response to exposure to some toxic chemicals.

Hepatic CYP3A2 gene of male rats was up regulated in response to paraquat (1, 10-dimethyl-4, 40-bipyridilium dichloride) toxicity. This data suggest the increased expression of CYP3A2 may induce detoxification processes **(Malekinejad et al., 2010)**. So we expect that the over expression of the CYP3A2 gene in CCl_4 may be due to the induction of detoxification activity in which it participates.

(Carver and Clancy, 2000; Fountoulakis et al., 2002) stated that the cytochromes expression was altered due to CCl_4. **(Nomura et al., 1998; Matsunaga et al., 2001)** stated that Cytochrome (CYP3a9, CYP3a18, CYP2J3 Cytochrome 2C11 are reduced in CCl_4 toxicity but CYP2E1 is increased. In contrast **(Waring et al., 2001; Jiang et al., 2004)** have shown that acute treatment with CCl_4 resulted in down-regulation of CYP2E1.

Our result of increasing the CYP3A2 expression may be due to its role in detoxification of CCl4 or it may help in recovery mechanisms of injured tissues.

GADD153 gene expression is increased in case of body response to a stress state and also increased in case of DNA damage and DNA toxicity **(Fountoulakis et al., 2002; Inadera et al., 2010).** In this study the mRNA expression of GADD153 gene in CCl_4 group was up regulated in comparison to the positive control group. And these result was matched with **(Fountoulakis et al., 2002; Inadera et al., 2010).**

These obtained results of up-regulated GADD153 expression were matched with the biochemical analysis, antioxidant system evaluation, DNA fragmentation test which showed the stress and damaged DNA and hepatotoxicity findings in CCl4 groups.

The current results are matched with the hepatic enzymes analysis which showed increased serum level of AST, ALT and ALP and the There weren't any previous publications discussing the effect of neither the jojoba oil nor the extra virgin oil on CYP3A2 and GADD153 genes expressions, so according to our results we expect that the extra virgin olive oil expressed a better effect on its expression against CCl_4 toxicity rather than the jojoba oil.

The jojoba oil group and the jojoba oil group with CCl_4 injection showed altered gene expression of both hepatic CYP3A2 and GADD153 genes while the GSH, MDA and AFP, lipid profile and kidneys enzymes showed a normal profiles may be due to the Gene expression analysis is sensitive enough to detect early organ damage, even though there were no remarkable changes in the serum chemistry **(Inadera et al., 2010)**. So the gene expression may be applied as early toxicity markers.

Table 1: Mean activities of liver enzymes in the serum of rats fed diets containing Jojoba oil and extra virgin olive oil with or without injection of CCl_4 (n=10)

groups / parameters	ALT (U/L)	AST (U/L)	ALP (U/L)
C –ve	44.33 ± 2.96^{b}	181.67 ± 2.41^{c}	91.67± 4.41^{c}
C +ve	66.00 ± 4.36^{a}	313.0 ± 2.00^{a}	161.7± 5.20^{a}
JO	56.33 ± 2.96^{a}	205.00 ± 7.64^{bc}	126.00± 1.00^{b}
EVOO	41.67 ± 4.91^{b}	175.00 ± 2.77^{c}	93.67 ± 4.10^{c}
CCl4 + JO	58.00 ± 3.61^{a}	229.33 ± 3.84^{b}	130.67 ± 2.90^{b}
CCl4 + EVOO	46.67 ± 2.73^{b}	203.3 ± 2.60^{bc}	104.67 ± 6.20^{c}

Values are means ± SE

Means carrying different superscripts considered significant ($P < 0.05$).

Table 2: showing lipid profile in the serum of rats fed diets containing jojoba oil and extra virgin olive oil with or without injection of CCl_4 (n=10)

Groups / parameters	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
C –ve	51.00 ± 1.53^{c}	25.00 ± 0.58^{b}	26.00 ± 1.73^{c}	83.33 ± 2.5^{b}
CCl_4	71.00± 0.57^{a}	14.33 ± 0.87^{c}	57.67 ± 1.33^{a}	101.67 ± 1.28^{a}
JO	60.33 ± 1.7^{b}	32.33 ± 1.2^{a}	28.00 ± 1.58^{c}	83.33 ± 2.01^{b}
EVOO	58.67 ± 1.76^{b}	34.33 ± 1.29^{a}	24.34 ± 1.2^{c}	79.00 ± 2.4^{b}
CCl_4 + JO	60.33 ± 1.8^{b}	20.33 ± 1.87^{b}	40.00 ± 1.26^{b}	84.67 ± 2.10^{b}
CCl_4 + EVOO	61.04 ± 1.20^{b}	21.37 ± 0.87^{b}	39.67 ± 0.67^{b}	82.67 ± 2.2^{b}

Values are means ± SE

Means carrying different superscripts considered significant ($P < 0.05$).

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Table 3: showing the mean erythrocytic GSH concentration and serum MDA of rats fed diets containing jojoba oil and extra virgin olive oil with or without injection of CCl_4 (n=10)

Groups parameters	GSH (mg/dl)	MDA (mg/dl)
C –ve	22.63 ± 0.24^{a}	3.43 ± 0.1^{c}
CCl_4	11.13 ± 1.18^{c}	6.40 ± 0.22^{a}
JO	22.47 ± 0.68^{a}	3.65 ± 0.22^{c}
EVOO	23.10 ± 0.58^{a}	3.70 ± 0.05^{c}
CCl_4 + JO	19.13 ± 0.61^{b}	5.10 ± 0.1^{b}
CCl_4 + EVOO	18.13 ± 0.55^{b}	5.00 ± 0.1^{b}

Values are means ± SE
Means carrying different superscripts considered significant ($P < 0.05$).

Table 4: showing the mean alpha feto protein activity in serum of rats fed diets containing jojoba oil and extra virgin olive oil with or without injection of CCl_4 (n=10)

Groups prameters	**AFP** (mg/dl)
C –ve	**3.03 ± 0.52^{c}**
CCl_4	**6.45 ± 0.953^{a}**
JO	**3.60 ± 0.61^{c}**
EVOO	**3.13 ± 0.64^{c}**
CCl_4 + JO	**4.7 ± 1.05^{b}**
CCl_4 + EVOO	**4.6 ± 0.87^{b}**

Values are means ± SE
Means carrying different superscripts considered significant ($P < 0.05$).

Table 5: showing the fold change in CYP3A2 gene and GADD 153 gene expression in relation to GAPDH gene in hepatic tissues of rats fed diets containing jojoba oil and extra virgin olive oil with or without injection of CCl_4 (n=10)

Groups / parameters	CYP3A2 fold change/ GAPDH	GADD 153 fold change / GAPDH
C –ve	1	1
CCl_4	198.32	23.47
JO	104.45	9
EVOO	1.05	0.19
CCl_4 + JO	380.48	17.88
CCl_4 + EVOO	89.06	6.67

Values are means ± SE

Means carrying different superscripts considered significant ($P < 0.05$).

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