Potential effects of white and dark chocolate in hyper cholesterolemic rats

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Abstract:

Hypercholesterolemia is a condition where the body has an un usually high level of fats in the blood. It is an important risk factor for atherosclerotic cardiovascular disease. Approximately 18% of strokes and 56% of heart attacks are caused by high blood cholesterol. So, the aim of this study was the effects of dark chocolate and white chocolate on some biological, biochemical, and histological factors of hyper cholesterolemic rats. This research aimed to study the effects of white chocolate and dark chocolate on hyper cholesterolemic rats. Thirty-six adult male albino rats were distributed into two main groups. The first main group fed on a basal diet as a negative control group. The second main group was induced in by feeding a high-cholesterol diet [4% cholesterol (w/w) and 1% cholic acid (w/w)] for 8-weeks divided into five subgroups, the first subgroup fed on a basal diet as a positive control group. The other four subgroups were hyper cholesterolemic rats given dark chocolate at 5%, 10%, white chocolate at 5% and 10%, respectively, for 28 days. Results showed a significant decrease in most of lipid profile indices, liver function, antioxidants, and blood lipid indices. On the other hand, results showed a significant increase in some lipid profile indices, antioxidant and the hematological parameters (WBC, RBC, HGB, PLT) were counted. Biochemical results supported by the histopathological examination for heart and liver.

Key words: hypercholesterolemia – cholesterol – dark chocolate – white chocolate.

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Introduction:

Hypercholesterolemia is a condition that refers to a metabolic disorder that may result in elevated concentration of plasma low-density lipoprotein (LDL) cholesterol **(Adekiya *et al.*; 2018; Mu *et al.*; 2017).** Lipoprotein disorders, linked to atherogenesis and atherosclerotic cardiovascular disease, can be reduced by lowering cholesterol and statin drugs. High triglyceride levels increase acute pancreatitis risk. However, the effectiveness of lipid-reducing drugs remains a concern, necessitating timely evaluation and treatment **(Radaelli *et al.*; 2018; Zawacki *et al.*; 2018; Wiegman, 2018).**

Cocoa and dark chocolate contain flavonoids, known as procyanidins, which inhibit cholesterol absorption and LDL cholesterol receptor expression, resulting in a reduction in LDL and TC levels **(Matsui *et al.*; 2005)**. **Kollar *et al.*; (2002)** found that flavonoids in cocoa significantly reduce total cholesterol in hyper cholesterolemic rats' serum **(Baba *et al.*; 2007)**. The observed reduction in cholesterol levels may be due to flavonoids found in cocoa and dark chocolate, which are believed to inhibit cholesterol absorption and LDL cholesterol receptor expression **(Matsui *et al.*; 2005).**

White chocolate has significantly lower polyphenol content and antioxidant activity compared to milk and dark chocolate **(Meng *et al.*; 2009)**. **Chen *et al.*; (1989)** showed that the fatty acid recovered from thoracic lymph and the absorption of cholesterol were lower in rats given cocoa butter when compared with rats receiving corn oil. Thus, the bioavailability of cocoa butter is low **(Weisburger, 2001)**. In studies conducted on rats, cocoa butter has been shown to lower cholesterol and triglyceride levels **(Morrissey *et al.*, 1986, Kiitchevsky *et al.*, 1988).** So, this study was carried out to investigate the effect of white chocolate and dark chocolate on hyper cholesterolemic rats.

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MATERIALS AND METHODS:

Thirty-six adult normal male albino rats strain weighing 180±10g were obtained from the Medical Insects Research Institute located in Dokki, Cairo, Egypt. Menoufia University's Institutional Animal Care and Use Committee (IACUC) granted ethical approval for this investigation (**Reg. No., MUFHE /S/ NFS / 31/24)**.

Pure white crystalline cholesterol powder and saline solutions were purchased from TECHNOGEN Chem. Co., Egypt.

Dark chocolate was prepared manually. It consists of 118 g cocoa powder, 105 g coconut oil, 85 g honey, 5 g pure vanilla extract, and a pinch of sea salt.

White chocolate was prepared manually. It consists of 80 g cocoa butter, 60 g milk powder, and 30 g stevia.

Hypercholesterolemia was induced in rats by feeding high-cholesterol diet according to **Kames and Sumathi (2012)**.

This experiment involved 36 adult male white albino rats, Sprague Dawley Strain, aged 10 weeks. The rats were fed a basal diet according to **American Institute of Nutrition (AIN) (1993)** for 7 days, and then divided into two main groups. The first main group fed on a basal diet as a negative control group. The second main group was induced in by feeding them a high-cholesterol diet for 8-weeks and divided into five subgroups, divided into five subgroups, the first subgroup was hyper cholesterolemic rats fed on basal diet as a positive control group. The other four subgroups were hyper cholesterolemic rats given dark chocolate at 5%, 10%, white chocolate at 5%, and 10%, respectively, for 28 days. The experiment involved estimating body weight and food intake of rats, observing their behavior, and slaughtering them after 28 days. At the end of experiment, Blood samples were collected according to the method described by **Schermer (1967).** At the same time, the

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organs: heart, liver, kidney, lung, spleen, and pancreas were removed, washed in saline solution, dried by filter paper, weighed, and stored frozen in formalin solution 10% for histopathological testing both the heart and liver were stored for histopathological testing. The experiment aimed to understand rats' behavior and health.

Biological evaluation of the different diets was carried out by determination of body weight gain (BWG) % and food efficiency ratio (FER) according to **Chapman *et al*.; (1959).**

Serum total cholesterol was determined according to **Thomas (1992)**, serum triglycerides according to **the Young, (1975)** and **Fossati and Prencipe (1982)**, HDL can be determined according to **Lopez (1977),** v-LDL-c and LDL-c were calculated according to **Lee and Nieman (1996),** atherogenic index (A.I) and coronary risk index (CRI) were calculated according to **Mudhaffar (2013).** Cardiac risk ratio (CRR) was calculated according to **Bhardwaj *et al.;* (2013).** Atherogenic coefficient (AC) was calculated according to **Olamoyegun *et al.;* (2016)**. Atherogenic fraction (AF) was calculated according to **Aguilar *et al*.; (2011).** Catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase activity (GPX) were quantified according to **Rorth and Jensen (1967), R. Guillen-Sans and Guzmán-Chozas (1998)** and **Paglia and Valentine (1967),** respectively. Determination of serum ALT, AST, and ALP were carried out according to **Clinica Chimica Acta (1980), Hafkenscheid (1979),** and **Moss (1982),** and the hematological Parameters (WBC, RBC, HGB, PLT) were counted according to **Jacobs *et al*.; (2001).**

Histopathological investigations were carried out according to **Bancroft *et al*.; (1996).** Statistical analysis were done according to **SAS (1988).**

Results and discussion:

The results in table (1) showed that the positive control

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group had the highest BWG, FI, and FER values compared to the negative control group, while the highest BWG value was recorded in group 3 (5% dark chocolate) and the lowest in group 4 (10% dark chocolate). The highest FI was recorded in group 4 (10% dark chocolate), while the lowest was in group 5 (5% white chocolate), with significant differences. Group 3 (5% dark chocolate) had the highest FER, while group 6 (10% white chocolate) had the lowest, with significant differences.

High BWG in group 3 (5% dark chocolate) owing to low concentration of dark chocolate, so rats didn't completely recover. On the other side, a high concentration of dark chocolate in group 4 (10% dark chocolate) made rats completely recover, which led to a decrease in their weight. This finding agreed with **Miller *et al.*; (2006)** who found that the varied doses and forms of cocoa used in the interventions, which resulted in varying amounts of calories, sugar, and fat of the investigated products, could be one reason for the non-significant improvement in anthropometric measurements, as additional calories could have impeded the weight-reduction effects.

The decrease in BWG in group 4 is attributed to dark chocolate, which contains fiber and monounsaturated fats, providing a feeling of fullness for a longer period. This may lead to a reduction in daily calorie intake. According to **Halib *et al.*; (2020),** dark chocolate showed a significant reduction in body weight. On the same line, the smell of chocolate has been reported to suppress appetite, which is inversely related to levels of ghrelin **(Greenberg *et al.*; 2016, Massolt *et al.*; 2010).** Also, the results are in agreement with **Min *et al.*; (2013)** who demonstrated that consumption of a high-fat diet significantly increased body weight while consumption of cocoa powder significant decreased body weight and induced body loss. Consuming cocoa also has an impact on the

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hydrolysis of stored triacylglycerol in adipocytes into free fatty acids and glycerol, a lipolysis marker, which reduces lipid accumulation in adipocytes (**Chatree *et al.*; 2021),** this mechanism leading to reducing weight.

Table (1): Effects of dark and white chocolate on body weight gain, feed intake and feed efficiency ratio of hyper cholesterolemic rat:

	BWG Mean ± SD	**FI Mean ± SD**	**FER Mean ± SD**
(G1) control –ve	$1.3^{b} \pm 0.17$	$18.56^{b} \pm 1.5$	$0.07^{ab} \pm 0.01$
(G2) control +ve	$2.11^{a} \pm 0.16$	$24.37^{a} \pm 1.96$	$0.09^{a} \pm 0.005$
(G3) 5% dark chocolate	$2.05^{a} \pm 0.38$	$23.77^{a} \pm 2.14$	$0.09^{a} \pm 0.02$
(G4)10% dark chocolate	$0.54^{c} \pm 0.18$	$24.34^{a} \pm 2.23$	$0.07^{ab} \pm 0.02$
(G5) 5% white chocolate	$1.13^{b} \pm 0.27$	$22.17^{ab} \pm 1.39$	$0.05^{b} \pm 0.01$
(G6) 10% white chocolate	$1.62^{ab} \pm 0.34$	$23.63^{a} \pm 2.35$	$0.03^{c} \pm 0.007$
LSD	0.47	3.61	0.02

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (2) showed the heart, lung, liver, spleen, pancreas, and kidney weight of the positive control group was higher than that of the negative control group, with no significant differences. In the treated groups, group 6 had the highest organs weight, while group 4 had the lowest, with no significant differences.

A high-fat diet significantly affects the weight of the organs in rats, as shown in Table 2 in Group 2. This leads to various pathological changes in several organs and an increase in organ weights, particularly in the liver, pancreas, spleen, and kidneys, due to fat accumulation and inflammation. This is agreed with **Rusdiana et al.; (2022)**, they found that high-fat

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diets lead to severe steatosis, with up to 95% of hepatocytes showing fat droplet accumulation. This results in increased liver weight and dysfunction. Also, rats on high-fat diets exhibited a 14% increase in pancreas weight and a 9% increase in spleen weight compared to controls **(Rospond *et al.*; 2022).** This suggests an adaptive response to metabolic stress. There was a 5% increase in kidney weight, indicating potential renal stress due to fat accumulation **(Rospond *et al.*; 2022).**

In group 4, a slight decrease in the organs' weight was observed, and this is attributed to the dark chocolate treatment. The antioxidants present in chocolate improve the health of the tissues and cells of the organs, thereby maintaining their weight. This is agreed with **Ratri *et al.*; (2021),** they found in a research of the hepatosomatic index, no significant variations were identified between groups administered with varied doses of chocolate drinks, indicating that cocoa had no negative effect on liver weight in diabetic rats. Cocoa powder supplementation significantly reduced heart enlargement associated with hypercholesterolemia, with decreases in heart weight ranging from 14% to 21% in treated groups. Also, cocoa powder reduced liver enlargement, indicating that it can protect against hypercholesterolemia-induced organ weight alterations **(Nwichi *et al.*; 2012).** While precise data on kidney, spleen, and pancreatic weights were less commonly recorded, cocoa's antioxidant characteristics imply that it may also have protective effects on these organs **(Allotey-Babington *et al.*; 2019, FadlAlla and Faid, 2015).**

white chocolate treatment in groups 5 and 6 also led to improved organ functions and enhanced tissues, resulting in weight improvement. This is attributed to the cocoa butter, which contains antioxidants. This is agreed with **Morrissey *et al.*; (1986),** they found that cocoa butter supplementation has been proven to dramatically lower cholesterol levels, with no

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pathological changes identified in the heart or liver tissues of rats fed cocoa butter diets. Histological studies of the kidney and spleen revealed no harmful effects, implying that cocoa butter does not harm these organs **(Asiedu-Gyekye *et al.*; 2016)**. Although specific statistics on pancreatic weight were not provided, the rats' general health remained stable, indicating no negative effects on pancreatic function **(Morrissey *et al.*; 1986).**

Table (2): Effects of dark chocolate and white chocolate on internal organs weight of hyper cholesterolemic rats:

	ROW Heart Mean ± SD	ROW Lungs Mean ± SD	ROW Liver Mean ± SD	ROW Splee n Mean ± SD	ROW Pancrea s Mean ± SD	ROW Kidne y Mean ± SD
(G 1) control -ve	0.33^{a} ±0.02	0.62^{a} ±0.09	3.27^{a} ±0.29	0.33^{a} ±0.04	0.27^{a} ±0.05	0.61^{a} ±0.12
(G 2) control +ve	0.39^{a} ±0.14	0.57^{a} ±0.17	3.46^{a} ±0.31	0.30^{a} ±0.08	0.51^{a} ±0.28	0.71^{a} ±0.09
(G 3) 5%dark chocolate	0.34^{a} ±0.01	0.61^{a} ±0.02	3.44^{a} ±0.36	0.27^{a} ±0.03	0.5^{a} ± 0.09	0.69^{a} ±0.17
(G 4) 10% darkchocolate	0.33^{a} ±0.03	0.56^{a} ±0.09	3.35^{a} ±0.82	0.26^{a} ±0.04	0.46^{a} ±0.02	0.67^{a} ±0.02
(G 5) 5% whitechocolat e	0.34^{a} ±0.03	0.59^{a} ±0.04	3.37^{a} ±0.16	0.28^{a} ±0.12	0.49^{a} ±0.06	0.69^{a} ±0.03
(G 6) 10% whitechocolat e	0.35^{a} ±0.04	0.61^{a} ±0.06	3.45^{a} ±0.17	0.29^{a} ±0.05	0.51^{a} ±0.09	0.70^{a} ±0.03
LSD	0.11	0.16	0.74	0.12	0.23	0.17

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

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Data given in table (3) showed the positive control group had the highest total cholesterol serum and triglycerides value compared to negative. There is a significant decrease in the treated groups compared to the positive group. The best total cholesterol serum and triglycerides value was recorded in group 4.

Feeding rats on a diet rich in fats and cholesterol leads to liver stress, altering the pathways of cholesterol and triglyceride synthesis, resulting in increased levels in the blood. The decrease in cholesterol and triglyceride levels in group 4 after the treatment period is attributed to dark chocolate treatment, which contains polyphenols and flavonoids that possess cholesterol-lowering properties by inhibiting cholesterol synthesis, an important strategy for reducing all elevated blood lipid level and this is consistent with **Yasuda *et al*.; (2008)** who found that consumption of cocoa powder-derived polyphenol extract produced a hypocholesterolemic effect, and the active ingredients were shown to be oligomeric procyanidins. Additionally, based on the higher amount of fecal sterols voided in vivo and decreased micellar solubility in vitro, the polyphenol extract from cocoa powder may, at least in part, provide the hypocholesterolemic effect by inhibiting the absorption of cholesterol and bile acids. Also, diet containing cocoa powder led to the inhibition of endoplasmic reticulum stress in the liver. Because endoplasmic reticulum stress can lead to hepatic steatosis and altered cholesterol and triglyceride biosynthetic pathways **(Suyama *et al*.; 2014).** Flavonoids in cocoa and dark chocolate significantly lower total cholesterol levels in hyper cholesterolemic rats, attributed to procyanidins in cocoa, which impede cholesterol absorption and LDL cholesterol receptor development **(Koller *et al*.; 2002, Matsui *et al*.; 2005).**

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The decrease in cholesterol and triglycerides in the groups treated with white chocolate (groups 5,6) is attributed to stearic acid in cocoa butter which reduces cholesterol absorption and promotes endogenous cholesterol excretion, leading to lower plasma cholesterol levels and this is consistent with **Schneider *et al*.; (2000)** they reported that dietary stearic acid significantly lowers cholesterol absorption efficiency to 21% compared to 50-55% in other fatty acids, resulting in increased fecal cholesterol excretion. **Stonehouse *et al*.; (2020)** found that the ability of cocoa butter to neutralize cholesterol is widely recognized. Additionally, 33% of cocoa butter is composed of monounsaturated oleic acid, which has been demonstrated to support ideal lipid profile **(Corti *et al*.; 2009).** Also, **Fadlalla and Faid (2015)** found that white chocolate resulted in highly noticeable decreases in T.C and T.G.

Table (3): Effects of dark and white chocolate on total cholesterol and triglycerides of hyper cholesterolemic rats:

	T.C Mg-dl Mean± SD	T.G Mg-dl Mean± SD
(G1) control –ve	96.39^{e} ±1.52	89.41^{e} ± 2.92
(G2) control +ve	200.85^{a} ± 2.45	145.8^{a} ± 2.25
(G3) 5% dark chocolate	147.77^{b} ± 2.37	127.39^{b} ± 3.05
(G4) 10% dark chocolate	113.35^{d} + 1.78	90.91^{e} ± 2.33
(G5) 5% white chocolate	147.38^{b} ± 1.72	122.29^{c} ± 2.99
(G6) 10% white chocolate	129.35^{c} ± 3.11	106.17^{d} ± 1.87
LSD	3.96	4.71

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (4) showed a significant decrease in HDL-c value in positive control group and the treated group. There is a significant increase in the treated groups compared to

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the positive group. The positive control group had the highest LDL-c and v-LDL value compared to negative group, there is a significant decrease in the treated groups compared to the positive group. The best values were observed in group 4.

The observed increase in HDL and decrease in LDL in the groups treated with dark chocolate especially group 4 is due to the flavonoids and procyanidins found in dark chocolate, which improve HDL's ability to remove excess cholesterol and transport LDL from the arteries to the liver for disposal. The findings are consistent with **Matsui *et al*.; (2005)**, who found that cocoa and dark chocolate contain flavonoids, or procyanidins, which can reduce LDL-c and TC levels by preventing cholesterol absorption and developing LDL cholesterol receptors and in the same way, many human studies have shown that feeding polyphenol-rich foods, including cocoa powder, altered and raised HDL-C and decreased LDL-C concentrations. RCT refers to HDL's role as a cholesterol acceptor, transporting excess cholesterol from peripheral tissues to the liver for excretion or formation. Before being excreted, it is converted into bile acids and salts **(Ouimet *et al*.; 2019).** Reverse cholesterol transport (RCT) is a term used to describe the efflux of excess cellular cholesterol from peripheral tissues and its return to the liver for excretion in the bile and ultimately the feces (**Rader *et al*.; 2009)**. The neutral saturated fatty acids found in cocoa fat are mostly palmitate and stearate, and it also has antioxidants. Cocoa fat's antioxidants can prevent free radicals from forming, which can reduce LDL cholesterol (**Nurhafsah *et al*.; 2021).** According to **Mackenbach (2011),** Several research studies the health benefits of dark chocolate on blood lipid levels, such as lowering LDL-C and increasing HDL-C.

Although cocoa butter contains polyphenols and flavonoids fewer than cocoa, it has reduced LDL-c. This is due to stearic

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acid, which the liver can convert into a monounsaturated fat called oleic acid. This acid lowers levels LDL-c and increases levels of HDL-c in groups 4 and 5 that were treated with white chocolate and this agreed with **Kris-Etherton (1999**), who found that Oleic acid in cocoa butter reduces LDL levels by decreasing the risk of coronary heart disease. The average composition of cocoa butter is 33% stearic acid, 25% palmitic acid, and 33% oleic acid, one monounsaturated lipid that reduces LDL cholesterol is oleic acid **(Cleeman *et al.*; 2001)** and while both stearic and palmitic acids are saturated fats, stearic acid reduces LDL cholesterol more than other saturated fatty acids **(Mensink *et al.*; 2003)**. Also **Fadlalla and Faid (2015)** found that white chocolate resulted in highly noticeable decreases in LDL and v-LDL and a noticeable increase in HDL.

Table (4): Effects of dark chocolate and white chocolate on HDL, LDL and v-LDL of hyper cholesterolemic rats

	HDL Mg-dl Mean± SD	LDL Mg-dl Mean± SD	vLDL Mg-dl Mean± SD
(G1) control –ve	$49.18^{e} \pm 1.86$	$29.66^{e} \pm 1.99$	$17.79^{c} \pm 2.36$
(G2) control +ve	$43.99^{b} \pm 1.73$	$127.18^{a} \pm 3$	$28.67^{a} \pm 2.13$
(G3) 5% dark chocolate	$44.18^{b} \pm 1.82$	$59.08^{c} \pm 2.71$	$25.9^{ab} \pm 1.93$
(G4) 10% dark chocolate	$48.01^{ab} \pm 1.68$	$53.43^{d} \pm 1.92$	$18.57^{c} \pm 0.88$
(G5) 5% white chocolate	$47.75^{ab} \pm 2.28$	$72.38^{b} \pm 1.61$	$23.85^{ab} \pm 2.28$
(G6) 10% white chocolate	$44.63^{b} \pm 1.73$	$59.74^{c} \pm 1.8$	$21.46^{bc} \pm 3.35$
LSD	3.31	4.01	4.04

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (5) showed a significant increase in ALT, AST and ALP value in positive group compared to

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negative group. There is a significant decrease in the treated groups compared to the positive group. The best values were found in group 4 (rats fed on 10% dark chocolate).

Due to antioxidants present in cocoa, dark chocolate treatment has protective effects on the liver, especially in reducing serum liver enzyme levels such as ALT, AST, and ALP in group 4, which are believed to be signs of liver damage. It also reduces inflammation and oxidative stress associated with liver damage and improves the condition of damaged hepatic blood vessels. and this is consistent with **Sokpor *et al*.; (2012) and Kilicgun & Altiner (2009),** they found that Polyphenols, which are abundant in cocoa and have strong antioxidant properties, reduce oxidative stress in the liver. Also, consuming cocoa significantly decreased blood ALT and AST levels in animal models, suggesting reduced liver damage. For example, when sick mice consumed cocoa, their ALT and AST levels decreased by 47% and 48%, respectively **(Aidoo *et al*.; 2012)**. Furthermore, cocoa has been demonstrated to reduce ALP levels, which supports its role in improving liver function. These results suggest that cocoa may be a useful dietary supplement for people who are at risk of liver damage from health conditions like infections or alcohol consumption, possibly providing a natural substitute for traditional treatments **(Asiedu-Gyekye *et al*.; 2016, Saleh & Sabahelkhier, 2017).** Also, the result have agreement with **Schwenger and Allard (2014),** that found information about the impact of dark chocolate consumption on the decrease in AST levels among patients with non-alcoholic fatty liver disease (NAFLD) by reducing hepatic fat accumulation, inflammation and necrosis (**McKim *et al*.; 2002).**

This study showed that cocoa butter also has protective benefits on liver functions, especially concerning liver enzymes such as ALT, AST, and ALP, by including anti-inflammatory

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properties, reducing oxidative stress, and modulating fat metabolism. According to **(Chang *et al.*; 2022, Saleh & Sabahelkhier, 2017),** cocoa butter significantly reduced ALT and AST levels in rats subjected to chronic ethanol consumption, indicating a protective effect against liver injury.

Table (5): Effects of dark chocolate and white chocolate on ALT, AST and ALP of hyper cholesterolemic rats:

	ALT U-L Mean± SD f	AST U-L Mean± SD f	ALP U-L Mean± SD f
(G1) control –ve	43.57 ± 1.34	93.09 ± 2.5	133.39 ± 2.18
(G2) control +ve	140.15^{a} ± 1.25	204.52^{a} ± 2.14	363.37^{a} ± 2.72
(G3) 5% dark chocolate	122.38^{b} ± 2.19	164.17^{b} ± 2.06	252.14^{c} ± 1.85
(G4) 10% dark chocolate	72.06^{e} ± 4.42	98.56^{e} ± 1.47	214.42^{e} ± 2.05
(G5) 5% white chocolate	86.47^{c} ± 1.52	133.54^{c} ± 2.06	287.6^{b} ± 1.56
(G6) 10% white chocolate	81.08^{d} ± 2.52	104.96^{d} ± 1.93	239.62^{d} ± 1.48
LSD	4.38	3.65	3.7

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (6) showed CAT and GPX values of the positive control group were the lowest compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best CAT and GPX value recorded for group 4 (rats fed on 10% dark chocolate). The study found significant increase in MDA value in positive group compared to negative group. There is a significant decrease in the treated groups compared to the positive group. The best value recorded for group 4.

The notable increase in CAT and GPX and the decrease in

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MDA in Group 4 are attributed to the significant biochemical effects of cocoa powder in dark chocolate, especially in modifying the levels of oxidative stress indicators such as MDA, GPX, and CAT. This is mainly due to the antioxidant properties of polyphenolic compounds in cocoa, which enhance the body's defenses against oxidative damage, delay cell damage, and reduce inflammation levels, thereby maintaining cardiovascular health. this is consistent with **Prenetha et al.; (2022)** they found that dark chocolate consumption has been shown in studies to reduce MDA levels, a marker of oxidative stress, implying a cardiovascular preventive effect. Elevated MDA levels are related with an increased risk of coronary heart disease, whereas GPx is an essential antioxidant enzyme that can reduce oxidative damage **(Ahmad, 2023).** According to **Ramos-Romero *et al.*; (2012)** In arthritic female wister rats, cocoa supplementation has been found to enhance catalase levels. **Jenny *et al.*; (2009)** they found that cocoa administration may boost CAT activity by regulating NF-kB transcription factor and reducing DNA damage, while also decreasing inflammation markers like hs-CRP, IL-6, and TNF-α, potentially reducing MDA levels. This is because there was a connection between MDA and inflammatory markers, as shown in a study by **Parsaeyan *et al.*; (2014)**. **Ramirez Sanchez *et al.*; (2013)** found that the Patients with type 2 diabetes who took cocoa supplements saw lower levels of glutathione and catalase after three months of treatment. Consuming cocoa enhances serum antioxidant capacity, which protects the endothelium from oxidative stress and endogenous ROS (**Kris-Etherton and Keen, 2002).** Cocoa antioxidants can prevent or delay cellular damage by quenching free radicals or chelating transition metal ions, reducing their ability to produce reactive oxygen species. They also demonstrate a variety of physiological features that protect against diseases such as coronary heart disease.

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Cocoa butter plays a significant role in modulating the mechanisms of CAT, MDA and GPX activities, which are important for oxidative stress management. The interaction of cocoa butter with specific phospholipids and its physicochemical properties influences these mechanisms, enhancing the antioxidant capacity of cocoa butter products. According to **Middendorf *et al.*; (2015),** the emulsifying qualities of cocoa butter, especially when paired with substances like PGPR, might stabilize cellular structures and possibly increase CAT activity, which is essential for the detoxification of hydrogen peroxide. Also, **Clercq (2011)** found that the enzymatic activity of GPX, which protects against oxidative damage, can be enhanced by the incorporation of cocoa butter. Its unique triglyceride profile may support the enzyme's function, promoting the reduction of peroxides. Cocoa butter's fatty acid composition can influence lipid peroxidation, leading to MDA production. The presence of antioxidants in cocoa butter may mitigate this process, reducing MDA levels **(Stobbs *et al.*; 2024).** (**Andújar *et al.*; 2012, Arranz *et al.*; 2013). Fadlalla and Faid, (2015)** found that lipid peroxidation was significantly reduced by white chocolate.

Table (6): Effects of dark chocolate and white chocolate on CAT, MDA and GPX of hyper cholesterolemic rats:

	CAT Ng-ml Mean± SD	MDA Nmol-ml Mean± SD	GPX U-ml Mean± SD
(G1) control –ve	$10.54^{a} \pm 1.61$	$0.94^{c} \pm 0.05$	$205.7^{a} \pm 2.08$
(G2) control +ve	$0.86^{d} \pm 0.003$	$9.64^{a} \pm 0.67$	$43.97^{e} \pm 1.03$
(G3) 5% dark chocolate	$2.62^{cd} \pm 0.6$	$2.69^{b} \pm 0.63$	$84.55^{d} \pm 1.79$
(G4) 10% dark chocolate	$5.5^{b} \pm 1.47$	$2.6^{b} \pm 0.73$	$156.59^{b} \pm 1.3$
(G5) 5% white chocolate	$4.59^{bc} \pm 1.11$	$2.83^{b} \pm 0.75$	$109.74^{c} \pm 1.82$

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(G6) 10% white chocolate	$4.25^{bc} \pm 0.99$	$3.95^{b} \pm 0.51$	$112.58^{c} \pm 1.73$
LSD	1.96	1.08	3.19

Each value is represented as values are expressed as mean + SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (7) showed that AI, CRR, CRI, AC and AF values of the positive control group was highest compared to negative group. There is a significant decrease in the treated groups compared to the positive group The best AI, CRR, CRI, AC and AF values was recorded in group 4.

The decreased in AI, CRR, CRI, AC and AF values in group 4 due to treatment with dark chocolate led to an improvement in cardiovascular health and the prevention of arteriosclerosis, due to the flavonoids present in cocoa, such as epicatechin and catechin, which possess anti-inflammatory and antioxidant properties. Flavonoids in general have properties that enhance heart health and cardiac circulation, improve heart muscle function, and prevent blood clots. Consequently, a noticeable improvement occurred in the groups treated with dark chocolate and this is consistent with **Zięba *et al*.; (2019** and **Grassi *et al*.; (2023)** they found that Cocoa flavonoids have considerable antioxidant activity and reduce oxidative stress, which is a major factor to cardiovascular disease. That also have anti-inflammatory characteristics, blocking pro-inflammatory mediators and decreasing lipid peroxidation. Also, cocoa consumption improves endothelial function, encouraging vasodilation and boosting blood flow, which is important for cardiovascular health **(Arranz *et al*.; 2013, Patel & Watson, 2018). Buitrago-Lopea *et al* (2011)** raported that many clinical and observational research suggested that food items containing cocoa may help prevent cardiovascular disease. Consuming cocoa-rich products can positively impact

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cardiovascular parameters like arterial vasodilation, platelet aggregation, myocardial reperfusion, and systemic blood pressure in the short term **(Haber and Gallus 2012)**. Research conducted in vivo revealed that dark chocolate, cocoa powder, and cocoa liquor prevented atherosclerosis and reduced the growth of atherosclerotic plaques **(Vinson *et al.*; 2006).** Clinical studies and experimental studies have mostly examined the impacts of cocoa products and cocoa polyphenols on blood pressure, platelet function, lipid profile, vascular inflammation, endothelium-dependent vasomotor function and arterial flow-mediated dilatation (FMD), oxidative stress and plasma antioxidant capacity, and nitric oxide (NO) metabolism and activity **(Jalil and Ismail, 2008, Cooper *et al.*; 2008).** So, after these effects, studied in clinical and experimental studies, are achieved, it is expected that heart health will improve.

Although cocoa butter has a lower flavonoid content than cocoa powder, it has played a role in improving heart health through biologically active flavonoid compounds. Which contribute to cardiovascular health by improving endothelial function, reducing inflammation, and enhancing blood lipid levels. This is consistent with another study reported that cocoa butter contains flavonoids, which have been proven to increase endothelium vasodilation and reduce oxidative stress **(Garcia *et al.*; 2018, Patel & Watson, 2018).** Also, cocoa butter is high in polyphenolic chemicals, which have strong antioxidant properties and assist to neutralize free radicals and reduce oxidative stress **(Eagappan & Sasikumar, 2014).**

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Table (7): Effects of dark chocolate and white chocolate on AIP, CRR, CRI, AC and AF of hyper cholesterolemic rats

	AIP Mean± SD	CRR Mean± SD	CRI Mean± SD	AC Mean± SD	AF Mean ± SD
(G1) control - ve	0.039^{a} ± 5.77	1.95^{c} ± 0.12	0.59^{c} ± 0.07	0.95^{c} ± 0.12	46.97^{c} ± 6.18
(G2) control +ve	0.05^{a} ± 0.02	4.5^{a} ± 0.43	2.85^{a} ± 0.38	3.5^{a} ± 0.43	156.71^{a} ± 17.3
(G3) 5% dark chocolate	0.043^{a} ± 0.002	3.35^{b} ± 0.36	1.77^{b} ± 0.3	2.35^{b} ± 0.36	103.19^{b} ± 8.78
(G4) 10% dark chocolate	0.04^{a} ± 0.003	2.73^{b} ± 0.07	1.28^{b} ± 0.03	1.7^{b} ± 0.06	78.34^{b} ± 5
(G5) 5% white chocolate	0.041^{a} ± 0.002	2.9^{b} ± 0.34	1.42^{b} ± 0.3	1.9^{b} ± 0.34	96.57^{b} ± 13.35
(G6) 10% white chocolate	0.044^{a} ± 0.004	2.74^{b} ± 0.34	1.3^{b} ± 0.31	1.74^{b} ± 0.34	81.81^{b} ± 2.36
LSD	0.01	0.55	0.48	0.55	19.02

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Data given in table (8) found significant increase in RBC in positive group compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best RBC value was observed in group 4. The study found significant increase in WBC value in positive group compared to negative group. There is a significant decrease in the treated groups compared to the positive group. The best WBC value was found in group 4. The study found significant decrease in HGB value in positive group compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best HGB value was observed in group 4. The PLT value of positive group recorded the lowest value when compared with negative group. There is a significant increase in the treated groups compared to the positive group. The best value of PLT recorded for group 4.

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The increased in RBC and HGB in group 4 due to dark chocolate that contains iron, but in small amounts. However, it still improves the process of hemoglobin synthesis. Additionally, the flavonoids present in dark chocolate protect red blood cells from damage, thereby enhancing hemoglobin synthesis. According to **Yokoi *et al*.; (2008)** and **Yokoi *et al*.; (2011)**, Cocoa powder has a varied effect on hemoglobin levels and iron bioavailability. According to research, cocoa contains iron, however at a moderate bioavailability compared to other sources such as ferrous sulfate. According to studies using the hemoglobin regeneration efficiency method, cocoa powder has a relative biological value for iron of approximately 0.46-0.48, showing that it is a major but less efficient source of iron. Furthermore, cocoa flavonoids including epicatechin and catechin have been demonstrated to increase erythrocyte tolerance to oxidative stress, potentially lowering hemolysis and enhancing overall hemoglobin stability **(Zhu *et al*.; 2005).**

high white blood cell count in the affected group (group 2) can indicate a range of conditions, including infections, inflammation, injury and immune system disorders. Therefore, due to the flavonoids and flavonoids present in dark chocolate, they inhibited white blood cells by locating the site of inflammation or infection in the body and releasing antibodies to eliminate it therefore, white blood cell decreased in group 4 . This is consistent with **Heptinstall *et al*.; (2006)** and **Kenny *et al*.; (2009)** they found that cocoa flavanols can inhibit the activation of monocytes and neutrophils, which are crucial components of the immune response. Also, in vitro studies show that cocoa procyanidins can modulate signaling pathways in polymorphonuclear cells, potentially reducing inflammation **(Kenny *et al*.; 2009).**

Treatment with dark chocolate in group 4 led to increased platelet count due to flavonoids and polyphenols, which modify

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platelet function, thereby enhancing their count. Moreover, it does not stop there; it also maintains their normal level in the blood, which is beneficial in preventing thrombosis. This is consistent with **Peluso *et al.* (2015)** found that cocoa flavones have been observed to suppress platelet aggregation in both healthy individuals and those with cardiovascular problems, indicating a preventive effect against thrombotic events. Also, in patients receiving dual antiplatelet medication, cocoa consumption improved clopidogrel's inhibitory action, resulting in a significant reduction in platelet reactivity **(Seecheran *et al.*; 2022).** Cocoa polyphenols boost endothelial nitric oxide synthase activity, which is important for regulating platelet aggregation and enhancing vascular health **(Kim *et al.*; 2017).** Also, **Heptinstall *et al.*; (2006)** found in vitro and ex vivo studies have shown that cocoa flavanols, notably epicatechin and catechin, significantly reduce platelet aggregation. According to **Addai (2009),** cocoa boosts immunity, which results in extraordinary health. Several diseases are prevented by cocoa powder, especially viral illnesses. **Aborokwah *et al.* (2009)** found a significant increase in white blood cells, red blood cells, and platelets in the experimental group compared to the control group. It is thought that consuming a product high in cocoa, even for just a short period of time, may positively affect several cardiovascular indicators, such as platelet counts **(Haber and Gallus 2012).**

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Table (8): Effects of dark chocolate and white chocolate on red blood cells, white blood cells, hemoglobin and platelets of hyper cholesterolemic rats

	RBC $10^3/mm^3$ Mean ± SD	WBC $10^3/mm^3$ Mean ± SD	HGB G/dl Mean ± SD	PLT $10^3/mm^3$ Mean ± SD
(G1) control -ve	4.57^{b} + 0.59	7.33^{c} ± 1.08	13.69^{a} ± 0.86	851.67^{a} ± 50.08
(G2) control +ve	2.66^{b}±0.59	14.44^{a} ± 1.16	8.36^{b} ± 0.92	318.11^{c} ± 70.08
(G3)5%dark chocolate	3.63^{ab} ± 0.69	11.52^{b} ± 1.08	11.23^{a} ± 0.98	552.89^{b} + 55.12
(G4) 10% darkchocolate	4.39^{a} ± 0.7	8.92^{bc} ± 1.07	13.29^{a} ± 1.04	661.22^{b} ± 81.02
(G5) 5% white chocolate	4.16^{ab}±0.5	10.14^{b} + 1.16	12.59^{a} ± 1.32	596.89^{b} + 111.02
(G6) 10% white chocolate	4.05^{ab}±0.65	9.45^{bc} ± 1.34	12.45^{a} ± 1.04	619.56^{b} + 128.78
LSD	1.11	2.05	1.85	155.65

Each value is represented as values are expressed as mean ± SD; means in the same column with different letter are significantly different (P>0.05).

Histopathological examination of heart:

Examined heart sections of rats from group 1 (negative control group) revealed the normal histological architecture of cardiomyocytes (Figs. 1 & 2). In adverse, heart of rats from group 2 (positive control group) showed severe histopathological changes manifested by severe vacuolization of the sarcoplasm of cardiac myocytes (Figs. 3 & 4),

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intermyocardial edema (Figs. 4, 5 & 6) and mononuclear cell infiltration (Fig. 6). Meanwhile, heart of rats from group 3 (5% dark chocolate) exhibited vacuolization of the sarcoplasm of some cardiac myocytes (Figs. 7 & 8), congestion of cardiac blood vessel (Fig. 8) and slight intermyocardial edema (Fig. 9). On the other hand, heart of rats from group 4 (10% dark chocolate) described no histopathological changes (Figs. 10 & 11) except slight intermyocardial edema in some sections (Fig. 12). Furthermore, some heart of rats from group 5 (5% white chocolate) exhibited congestion of cardiac blood vessels (Fig. 13), whereas other sections showed apparent normal cardiac tissue (Fig. 14). Likewise, heart sections of rats from group 6 (10% white chocolate) revealed histologically normal cardiac tissue (Figs. 15 & 16) except slight congestion of cardiac blood vessel (Fig. 17) was seen in some sections.

In this study, severe vacuolization of the sarcoplasm of cardiac myocytes and intermyocardial edema in hyper cholesterolemic rats owing to high fat diet that causes cardiovascular disorders, Increasing levels of inflammation in heart tissues and deterioration of cardiac contractility. This finding agreed with **Yamamoto *et al*.; (2018)** they found The high-fat diet alters the amounts of SFAs and MUFAs in the membrane phospholipids, which results in varying degrees of detrimental cardiac alterations. Also, according to **Calligaris *et al*.; (2013)** and **Nguyen *et al*.; (2017),** high fat diet causes lipotoxic cardiomyopathy and cardiac hypertrophy.

Dark chocolate treatment that contains bioactive compounds such as flavonoids (catechins, epicatechins, e.g.), methylxanthines (theobromine, caffeine, e.g.), and polyphenols leads to inhibition of inflammation in heart. Additionally, dark chocolate has been shown to have anti-inflammatory and free-radical scavenging properties which leads to the improvement of heart cells and tissues, and this is consistent with another

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study found that cocoa prevent endothelial dysfunction and Cocoa has anti-inflammatory and antioxidant properties due to its high polyphenol content which results in the enhancement of cardiac tissues and cells **(Grassi *et al*.; 2015, Stote *et al*.; 2012, Katz *et al*; 2011).**

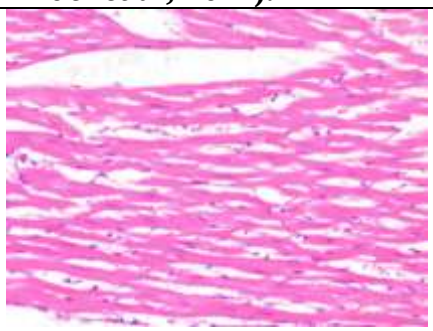

Fig. (1): Photomicrograph of heart of rat from group 1 (negative control group) showing the normal histological architecture of cardiomyocytes (H & E, X 400).

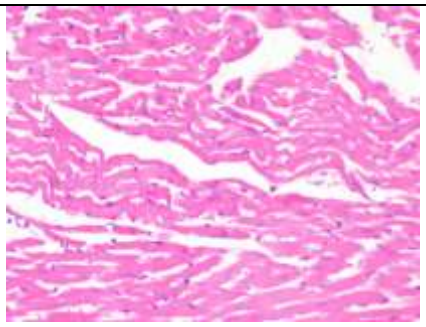

Fig. (2): Photomicrograph of heart of rat from group 1 (negative control group) showing the normal histological architecture of cardiomyocytes (H & E, X 400).

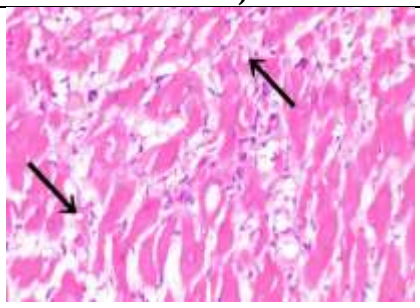

Fig. (3): Photomicrograph of heart of rat from group 2 (positive control group) showing severe vacuolization of the sarcoplasm of cardiac myocytes (black arrow) (H & E, X 400).

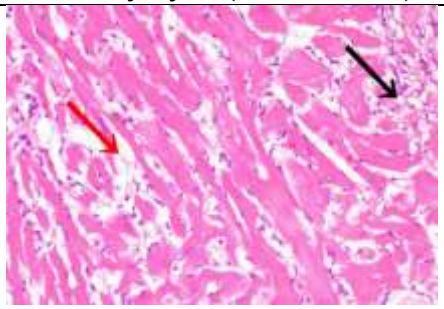

Fig. (4): Photomicrograph of heart of rat from group 2(positive control group) showing severe vacuolization of the sarcoplasm of cardiac myocytes (black arrow) and intermyocardial edema (red arrow) (H & E, X 400).

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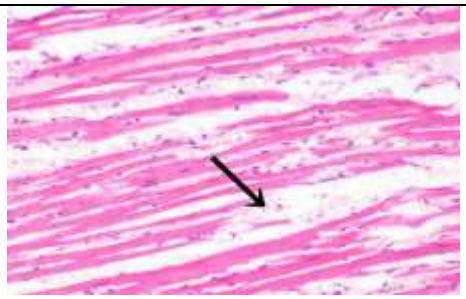

Fig. (5): Photomicrograph of heart of rat from group2 (positive control group) showing intermyocardial edema (black arrow) (H & E, X 400).

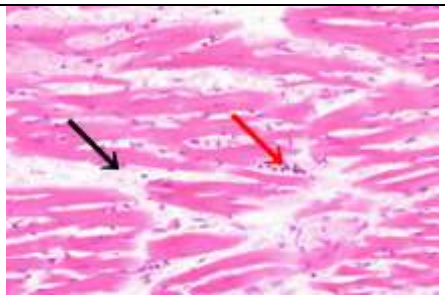

Fig. (6): Photomicrograph of heart of rat from group 2 (positive control group) showing intermyocardial edema (black arrow) with mononuclear cell infiltration (red arrow) (H & E, X 400).

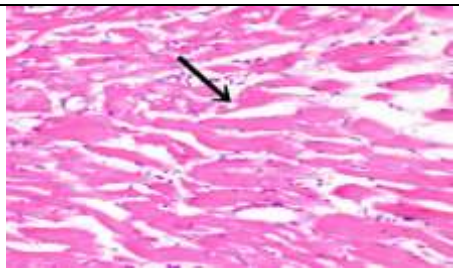

Fig. (7): Photomicrograph of heart of rat from group 3 (5% dark chocolate) showing vacuolization of the sarcoplasm of some cardiac myocytes (black arrow) (H & E, X 400).

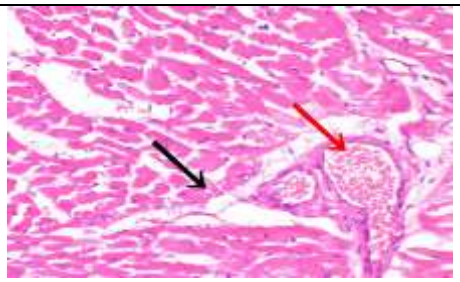

Fig. (8): Photomicrograph of heart of rat from group 3 (5% dark chocolate) showing vacuolization of the sarcoplasm of some cardiac myocytes (black arrow) and congestion of cardiac blood vessel (red arrow) (H & E, X 400).

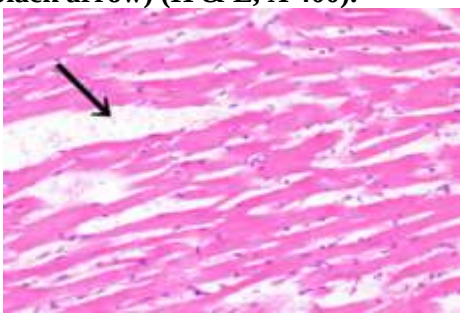

Fig. (9): Photomicrograph of heart of

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rat from group 3 (5% dark chocolate) showing slight intermyocardial edema (black arrow) (H & E, X 400).

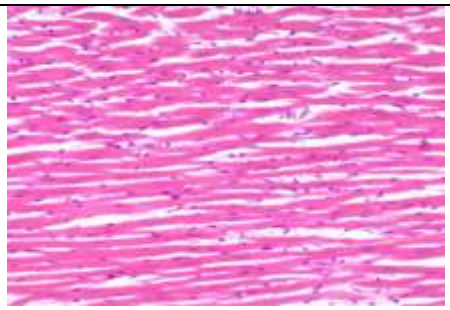

Fig. (10): Photomicrograph of heart of rat from group 4 (10% dark chocolate) showing no histopathological changes (black arrow) (H & E, X 400).

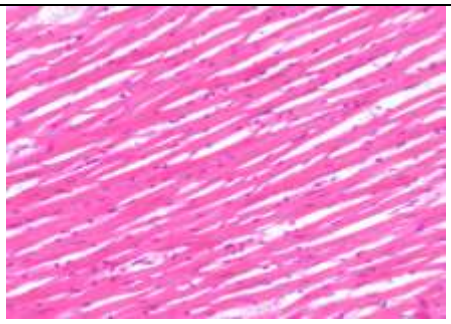

Fig. (11): Photomicrograph of heart of rat from group 4 (10% dark chocolate) showing no histopathological changes (black arrow) (H & E, X 400).

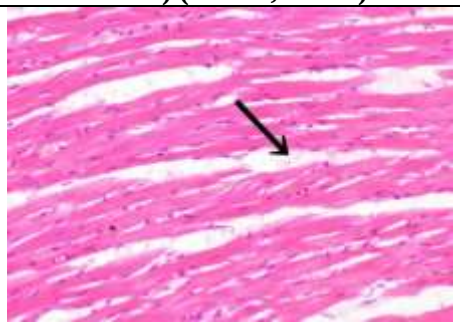

Fig. (12): Photomicrograph of heart of rat from group 4 (10% dark chocolate) showing slight intermyocardial edema (black arrow) (H & E, X 400).

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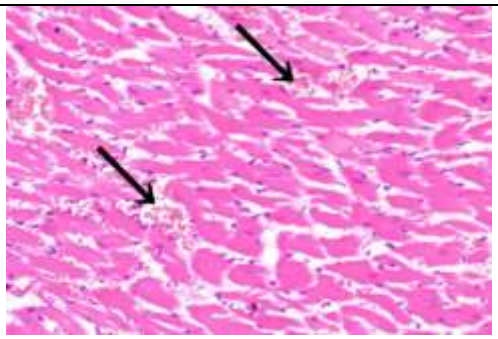

Fig. (13): Photomicrograph of heart of rat from group 5 (5% white chocolate) showing congestion of cardiac blood vessels (black arrow) (H & E, X 400).

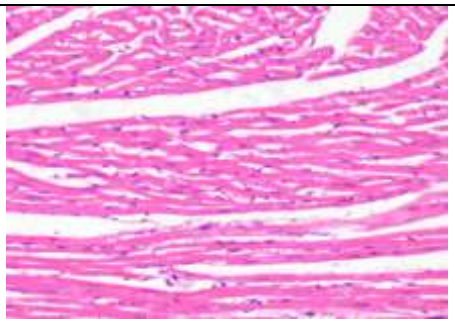

Fig. (14): Photomicrograph of heart of rat from group 5 (5% white chocolate) showing apparent normal cardiac tissue (H & E, X 400).

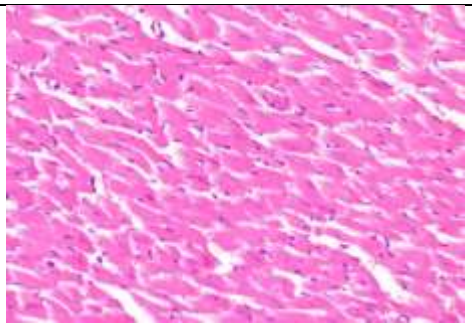

Fig. (15): Photomicrograph of heart of rat from group 6 (10% white chocolate) showing histologically normal cardiac tissue (H & E, X 400).

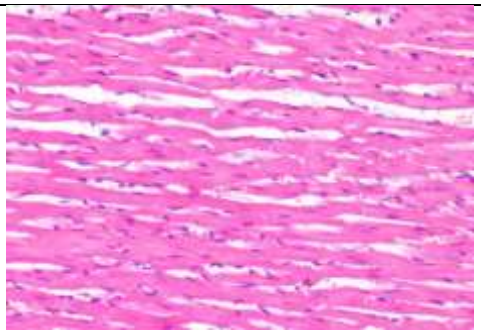

Fig. (16): Photomicrograph of heart of rat from group 6 (10% white chocolate) showing histologically normal cardiac tissue (H & E, X 400).

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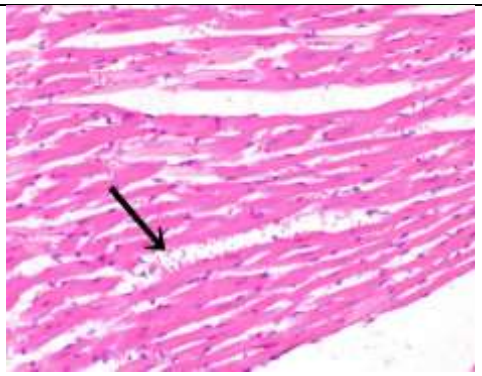

Fig. (17): Photomicrograph of heart of rat from group 6 (10% white chocolate) showing slight congestion of cardiac blood vessel (black arrow) (H & E, X 400).

Histopathological examination of liver:

Histopathological examination of liver sections of rats from group 1 (negative control group) revealed the normal histoarchitecture of hepatic parenchyma (Figs. 1 & 2). In contrast, liver of rats from group 2 (positive control group) showed histopathological damage characterized by remarkable hepatocellular vacuolar degeneration (Figs. 3 & 4) and focal hepatocellular necrosis with inflammatory cells infiltrates (Fig. 5). Otherwise, liver of rats from group 3 (5% dark chocolate) revealed regression of the induced damage; some examined sections from this group exhibited slight hydropic degeneration of some hepatocytes (Fig. 6) and vacuolization of sparse hepatocytes (Fig. 7), whereas other sections revealed vacuolization of centrilobular hepatocytes (Fig. 8). Furthermore, hepatic tissue of rats from group 4 (10% dark chocolate) described only small vacuoles in the cytoplasm of sparse hepatocytes (Figs. 9, 10 & 11). Moreover, some hepatic sections of rats from group 5 (5% white chocolate) demonstrated small vacuoles in the cytoplasm of sparse hepatocytes (Figs. 12 & 13), whereas other sections showed vacuolization of

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centrilobular hepatocytes (Fig. 14). On the other hand, examined sections from group 6 (10% white chocolate) exhibited small vacuoles in the cytoplasm of sparse hepatocytes (Fig. 15) and slight activation of Kupffer cells (Fig. 16). Some examined hepatic sections from group 6 (10% white chocolate) showed hepatocellular vacuolar degeneration around the portal triad (Fig. 17).

Feeding rats on high-fat diet causes liver condition deterioration, fatty liver, fibrosis, and inflammation due to the accumulation of fat in the liver and the liver's failure to process, break down, and eliminate fat properly. This is evident in the histological examination of group 2 in figs. 3, 4, and 5. This is agreed with **Kandhi *et al*.; (2023)** they found that high-fat diet activates hepatic stellate cells, which promote fibrosis via inflammatory pathways and cytokine signaling. High fat diet causes increased lipid buildup in hepatocytes, which results in nonalcoholic fatty liver disease **(Park *et al*.; 2021).**

The regression of the induced damage in figs 6, 7, 8, 9, 10, and 11 in groups 3 and 4 is due to the dark chocolate treatment, which primarily benefits liver cells due to its high polyphenol content, affecting a variety of metabolic and defensive pathways. These processes include regulating lipid metabolism, enhancing the antioxidant response, and activating autophagy, all of which contribute to liver health and protect against disorders such as non-alcoholic fatty liver disease. (NAFLD). According to **Rebollo-Hernanz *et al*.; (2022),** phytochemicals found in cocoa shells activate pathways that limit lipid buildup and fatty acid production, encouraging improved lipid metabolism in hepatocytes. Also, dietary cocoa supplementation in rats boosted antioxidant enzyme activity and decreased lipid peroxides, indicating a protective effect against oxidative damage **(Sun *et al*.; 2021).**

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There was also a slight regression of the induced damage in groups 5 and 6 in figs. 12, 13, 14, 15, 16, and 17 due to cocoa butter, which has protective effects on liver cells. Especially in the context of fat accumulation and inflammation, it can mitigate the harmful effects of liver damage, reducing hepatitis and fat accumulation, thereby enhancing healthy liver function and reducing oxidative stress. According to **Chang et al.; (2022)** in rats fed an alcoholic diet, cocoa butter reduced lipid alterations and inflammatory cell infiltration in liver tissues. It also decreased plasma levels of inflammatory markers such TNF-α and IL-1β, indicating a decrease in liver inflammation.

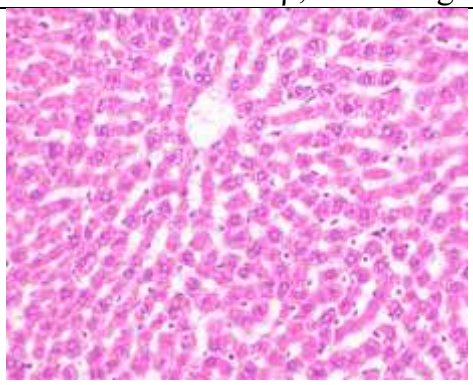

Fig. (1): Photomicrograph of liver of rat from group 1 (negative control group) showing the normal histoarchitecture of hepatic parenchyma (H & E X 400).

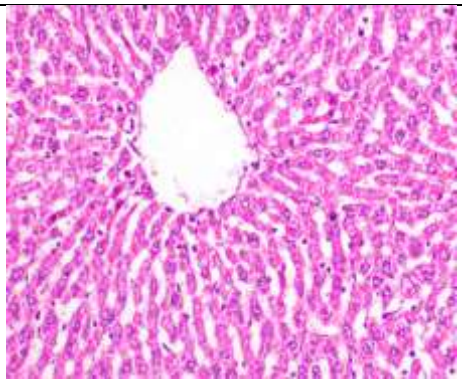

Fig. (2): Photomicrograph of liver of rat from group 1 (negative control group) showing the normal histoarchitecture of hepatic parenchyma (H & E X 400).

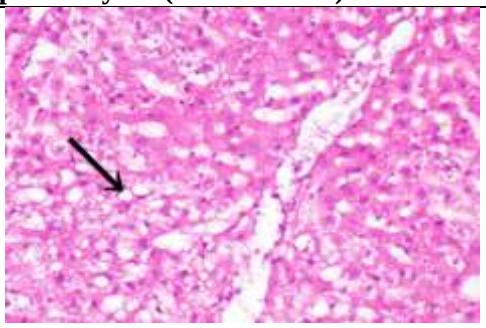

Fig. (3): Photomicrograph of liver of

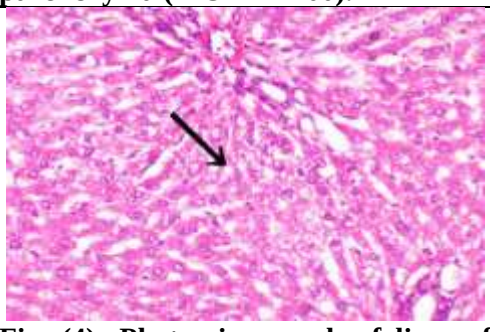

Fig. (4): Photomicrograph of liver of

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rat from group 2(positive control group) showing remarkable hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

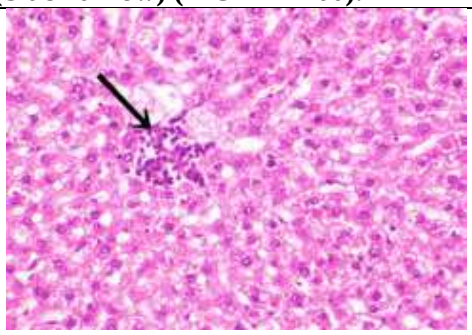

Fig. (5): Photomicrograph of liver of rat from group 2 (positive control group) focal hepatocellular necrosis with inflammatory cells infiltrates (black arrow) (H & E X 400).

rat from group 2 (positive control group) showing remarkable hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

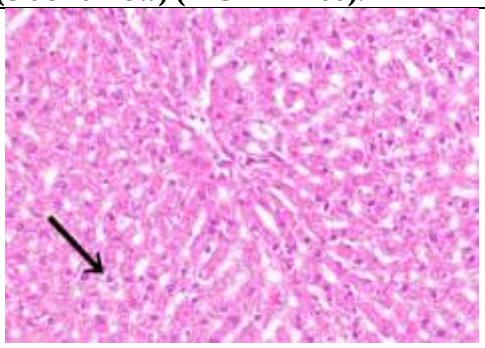

Fig. (6): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing slight hydropic degeneration of some hepatocytes (black arrow) (H & E X 400).

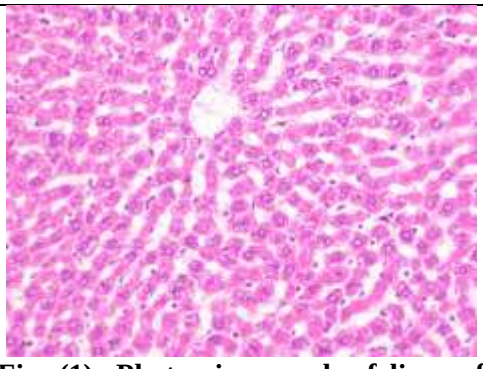

Fig. (1): Photomicrograph of liver of rat from group 1 (negative control group) showing the normal histoarchitecture of hepatic parenchyma (H & E X 400).

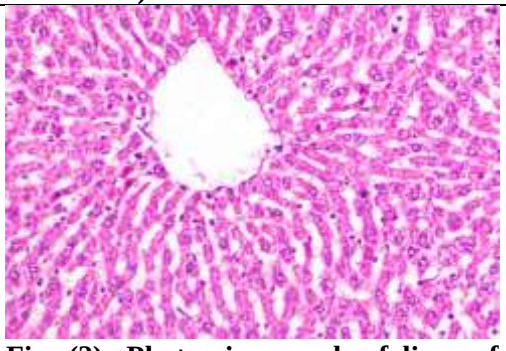

Fig. (2): Photomicrograph of liver of rat from group 1 (negative control group) showing the normal histoarchitecture of hepatic parenchyma (H & E X 400).

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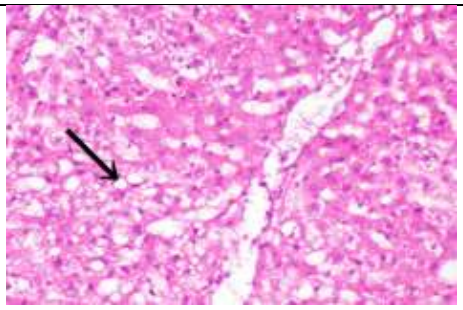

Fig. (3): Photomicrograph of liver of rat from group 2(positive control group) showing remarkable hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

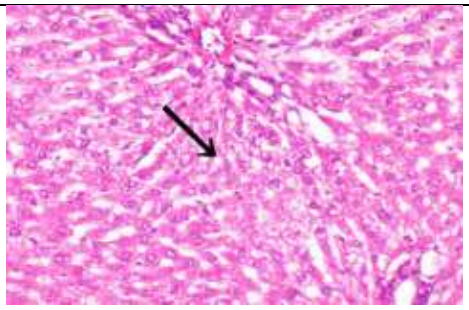

Fig. (4): Photomicrograph of liver of rat from group 2 (positive control group) showing remarkable hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

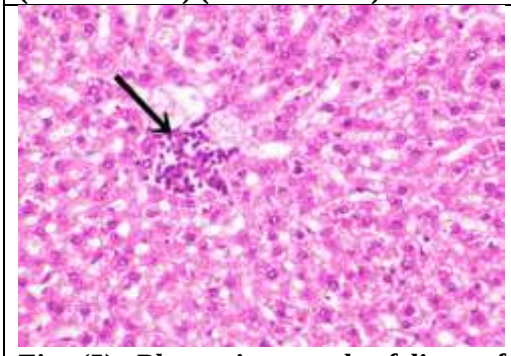

Fig. (5): Photomicrograph of liver of rat from group 2 (positive control group) focal hepatocellular necrosis with inflammatory cells infiltrates (black arrow) (H & E X 400).

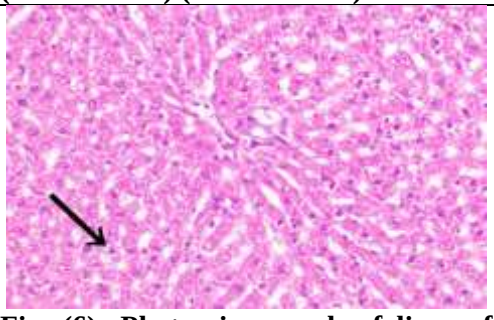

Fig. (6): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing slight hydropic degeneration of some hepatocytes (black arrow) (H & E X 400).

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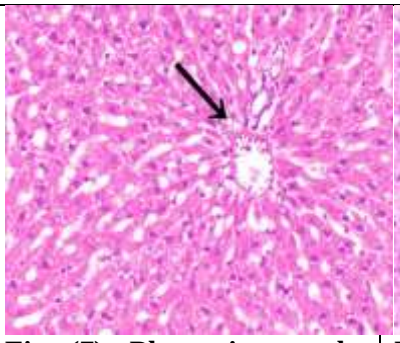

Fig. (7): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing vacuolization of sparse hepatocytes (black arrow) (H & E X 400).

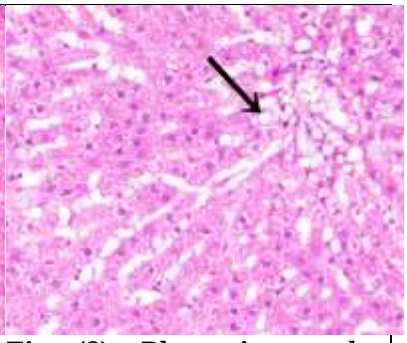

Fig. (8): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing vacuolization of centrilobular hepatocytes (black arrow) (H & E X 400).

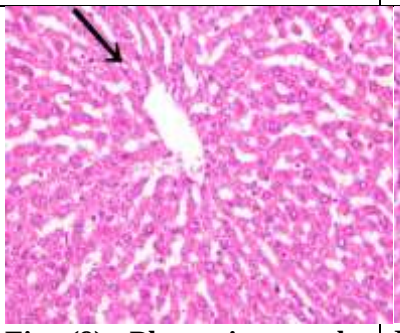

Fig. (9): Photomicrograph of liver of rat from group 4 (10% dark chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

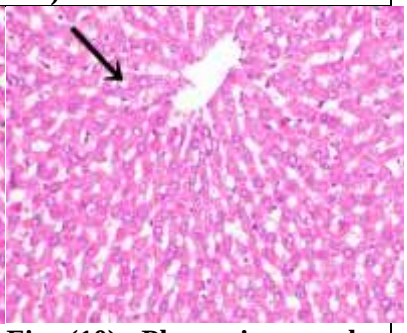

Fig. (10): Photomicrograph of liver of rat from group 4 (10% dark chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

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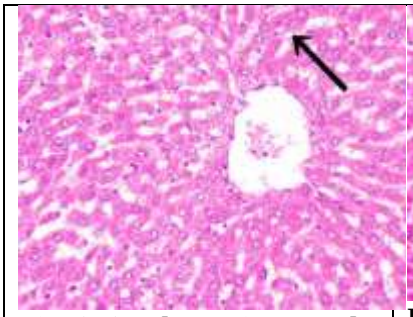

Fig. (11): Photomicrograph of liver of rat from group 4 (10% dark chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

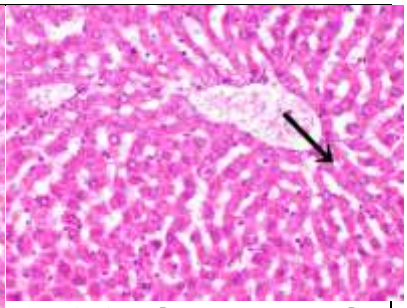

Fig. (12): Photomicrograph of liver of rat from group 5 (5% white chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

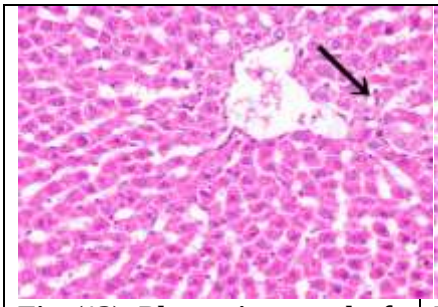

Fig. (13): Photomicrograph of liver of rat from group 5 (5% white chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

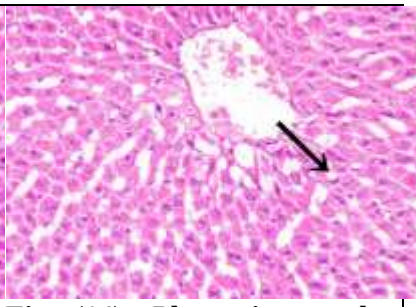

Fig. (14): Photomicrograph of liver of rat from group 5 (5% white chocolate) showing vacuolization of centrilobular hepatocytes (black arrow) (H & E X 400).

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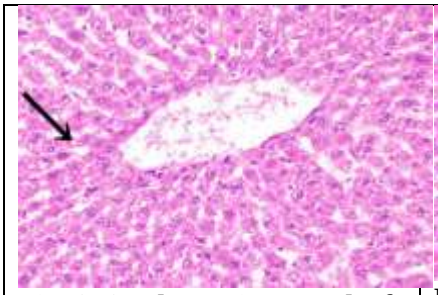

Fig. (15): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

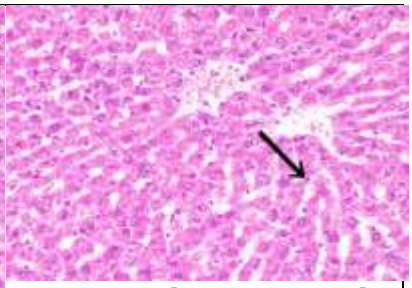

Fig. (16): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing slight activation of Kupffer cells (black arrow) (H & E X 400).

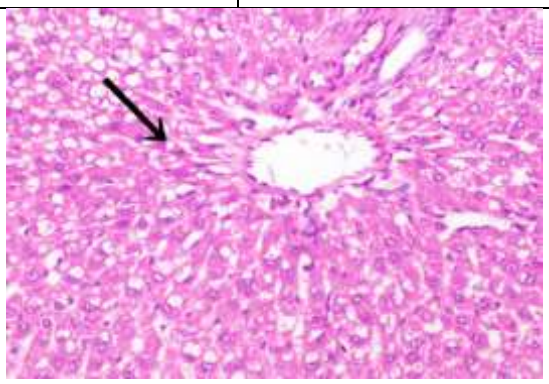

Fig. (17): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing hepatocellular vacuolar degeneration around the portal triad (black arrow) (H & E X 400).

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