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Improvement of liver injury induced by acetaminophen using black cherry (*Prunus serotina* Ehrh) powder and extract in rats

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Abstract

Liver injury is a major health problem .Continuous exposure to drugs, viral and bacterial infections and environmental toxins can trigger liver injury and eventually lead to various liver diseases. The present investigation aims to elucidate the possible hepatoprotective effect of black cherry on liver injury induced by administration of acetaminophen (APAP) (which known as paracetamol) to adult male albino rats. Animals were divided into 5 groups, each of 6 rats. The first group was kept as normal control group received (basal diet only). The other four groups fed on basal diet and daily oral dose of paracetamol (400 mg/kg body weight) by gastric tube for one week to induce hepato injury. The hepato-injury rats were classified into control (+ve) group and three treated rat groups which administered selymarin 100 mg/kg/day, 10% dried black cherry and 500mg/kg/day black cherry extract. The treatment period was designed for 28days. Total antioxidant and total plyphenols and flavonoids for black cherry extract were analyzed. Administration of APAP caused liver injury in rats evidenced by significant increase in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), hepatic malondialdehyde (MDA). In addition, significant decreases in hepatic catalase (CAT) and glutathione (GSH). Total (Phenolic, flavonoids and antioxidant) contents were (4.39 ± 0.33& 6.53 ± 0.46 and 85.94 ± 1.67) in black cherry respectively. Treatment with silymarin , dried black cherry and black cherry extract protect against liver injury as evidenced by significant decreases in liver MDA content, In addition, significant increases in hepatic CAT activity and GSH content. In conclusion**:** black cherry is rich source for polyphenols, flavonoids and total antioxidant sources so it may be have protective role against liver injury in rats.

Key words: - Flavonoids - glutathione-liver enzymes- malondialdehyde

Introduction

The Liver, a paramount organ is Principal for metabolism of nutrients and energy production in the human body. It is also necessary for biotransfirmation and elimination of exogenous drugs and harmful substances via kidney. Hepato-injury

caused by a variety of environmental pollutants, pathogenic micro-organism, viruses, drugs and chemical agents may account for various hepatic diseases such as jaundice, necrosis, hepatitis, fibrosis and cirrhosis etc. **(Shakya, 2020).** Liver diseases are one of the leading causes of illness and death globally. According to WHO estimates about 1.4 million deaths worldwide are due to the liver diseases. Although, modern medicine may treat to hepatic diseases, but they also cause numerous side effects in the human body **(Yang *et al.*, 2010).**

Acetaminophen (N-acetyl-p-aminophenol, APAP) also known as paracetamol is one of the most widely used over the counter antipyretic and analgesic medications. It is safe at therapeutic doses, but its overdose can result in severe hepatotoxicity, a leading cause of drug-induced acute liver failure in the USA. Depletion of glutathione (GSH) is one of the initiating steps in APAP-induced hepatotoxicity **(Khayyat *et al.*, 2016).** More than 50% of all cases of acute liver failure in the United States have been shown to result from exposure to drugs and 40% of these have been attributed to acetaminophen ingestion **(Yoon *et al.*, 2016).**

Recently, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants **(Sridhar and Rajeev, 2007).** Remarkable studies demonstrated that the health promoting effects of bioactive components originated from plants have been frequently attributed to their antioxidant properties and facilitate to increase cellular antioxidant defense system and thereby scavenge free radicals, inhibit lipid peroxidation, augment anti-inflammatory potential, and further protect the liver from damage **(Ganesan *et al.*, 2017).** Proximate analysis showed that black cherry (*Prunus serotina* Ehrh) fruits have high sugar, protein, and potassium contents. The results indicated that black cherry fruits contain phenolic compounds which elicit significant antioxidant and antihypertensive effects. These findings suggest that these fruits might be considered as functional foods useful for the prevention and treatment of cardiovascular diseases**.** Black cherry fruits possess a high content of phenolic compounds and display a significant antioxidant capacity. High-performance liquid chromatography/mass spectrometric analysis indicated that hyperoside, anthocyanins and chlorogenic acid were the main phenolic compounds found in these fruits **(Luna-Vázquez *et al.*, 2013)**.

Recently, there has also been a significant increase in interest in possible pro-health effects associated with the consumption of anthocyanins. It is suggested that fruits rich in anthocyanins or their extracts show a wide range of protective effects.

The consumption of anthocyanins prevents the risk of cardiovascular diseases, oxidative stress and diabetes.The antioxidant activity of cyanidin is 4.4 times higher than that of ascorbic acid. Among edible plants, berries of red, blue or purple color, such as wild cherries, are one of the most important sources of anthocyanins in the diet **(Telichowska *et al.*, 2020)**. Consumption of cherries decreased markers for oxidative stress in 8/10 studies; inflammation in 11/16; exercise-induced muscle soreness and loss of strength in 8/9; blood pressure in 5/7; arthritis in 5/5, and improved sleep in 4/4. Cherries also decreased hemoglobin A1C (HbA1C), Very-low-density lipoprotein (VLDL) and triglycerides/high-density lipoprotein (TG/HDL) in diabetic women, and VLDL and TG/HDL in obese participants. These results suggest that consumption of sweet or tart cherries can promote health by preventing or decreasing oxidative stress and inflammation **(Darshan *et al*, 2018)**. Therefore, this study was performed to examine the role of black cherry (*Prunus serotina Ehrh*) powder and extract in protection against acetaminophen overdose induced liver injury in rats.

Material and methods

Materials:

1- Fruits of black cherry were obtained from local market, Tanta, Egypt.

2- Chemicals: Main chemicals: paracetamol was purchased from agents of Sigma Chemicals Co. All other chemicals, from pharmaceutical industrial grade.

3- Casein (86% protein), cellulose, corn starch, salt mixtures and vitamin mixtures required for preparing diets were obtained from Local market and El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments. Cairo, Egypt

Animals

Thirty normal male albino rats weighing 200±10 g were used in the present study. They were obtained from Ophthalmic Research Institute, Giza, Egypt. Animals were acclimatized to laboratory condition and kept individually in stainless steel metabolic cages at room temperature (25±5 Ċ). Water was given *adlibitum.* Basal diet was prepared according to **Reeves *et al*., (1993).**

Methods:

Preparation of plant materials

Black cherries was washed by tap water and cut into small piece. Black Cherry seeds were removed. Fruits were dried separately in hot air oven electric air drought oven at 40 °C

for 12hours. The obtained dried fruits were ground by Braun grinder and packaged in polyethylene bags, then kept in a deep freezer at -16 °C until used for supplementation.

Preparation of crude ethanol plant extracts

Black berry fruits powder (60 g) were mixed with 80% (*v*/*v*) ethanol solution (300 mL) for 1 hr at 30°C by an overhead stirrer (Wise Stir HS-30D, Daihan Scientific, Wonju, Korea). The extract was filtered with filter paper. The filtrate was concentrated using a vacuum rotary evaporator at low temperature (40°C).

Determination of total phenolic contents in the extract

Total phenolic were determined colorimetric in the extract using Folin-Ciocalteu reagent. Absorbance was measured at 765 nm using visible spectrophotometer. The total phenolic content was expressed as Gallic acid equivalent (GAE) in milligrams per gram extract, according to **Singleton and Rossi, (1965)**.

Estimation of Total Flavonoid Content (TFC)

The amount of TFC in the previous extract was colorimetrically estimated by aluminum chloride assay. A 0.5 ml aliquot of appropriately diluted sample was mixed with 2 ml of distilled water, then with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min., 0.15 ml of a 10% $AlCl_3$ was added and left to stand for 6 min., then 2 ml of 4% NaOH was added to the mix. Immediately, water was added to complete the whole volume to 5 ml, carefully mixed, and left to stand for an additional 15 min. The absorbance of the mix was assessed at 510 nm against prepared water blank. Rutin was utilized as a standard compound for the quantification of total Flavonoid. The TFC was expressed as mg ruting^{-1} dry weight (mg rutin g^{-1} DW) by the calibration curve of Rutin. All samples were measured in triplicates **(Samatha *et al.*, 2012 and Han and May, 2012**).

Antioxidant activity

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation was derived from ABTS [2,20-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 ml) with MnO2 (3 mL, 25 mg/mL) in phosphate buffer solution (10 mM, pH 7,5 mL) After shaking the solution for a few minutes, it was centrifuged and filtered. The Absorbance (A control) of the resulting green-blue solution (ABTS radical solution) was recorded at lmax734 nm. The absorbance (A test) was measured upon the addition of (20 ml of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The decrease in the absorbance is expressed as % inhibition which calculated from this equation:

% Inhibition= Abs (control) – Abs (test)/ Abs (control) ×100

Ascorbic acid (20 ml, 2 mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS. **Induction of liver injury**

Hepato-injury was induced for normal rats by received daily oral dose of paracetamol (400 mg/kg body weight) by gastric tube for one week to induce hepato-injury according to the method described by **Kanchana and Sadiq, (2011).**

Experimental Design

A number of 30 rats were housed in well-aerated cages under hygienic conditions. The first group was kept as normal control group received (basal diet only). The other four groups fed on basal diet and daily oral dose of paracetamol (400 mg/kg body weight) by gastric tube for one week to induce hepato injury. The hepato-injury rats were classified into control (+ve) group and three treated rat groups which administered selymarin 100 mg/kg/day, 10% dried black cherry and 500mg/kg/day black cherry extract as follow:

Group (1): Rats were fed on basal diet and kept as a negative control group without any treatment (- ve control group).

Group (2): Rats were fed on basal diet and kept as a positive control group (+ve control).

Group (3):- Rats were fed on basal diet and received daily oral dose of silymarin (100 mg/kg body weight) by gastric tube according to **Wang *et al*., (2018)**.

Group (4):- Rats were fed on basal supplemented with 10% dry black cherry.

Group (5):- Rats were fed on basal diet and received daily oral dose of black cherry extract (500 mg/kg body weight) by gastric tube.

The study was assigned for 28days. During the experiment, body weight and feed intake were recorded weekly. At the end of experiment, all rats fasted over night before sacrificed. Afterwards rats sacrificed under ether anesthetized and blood samples were collected from hepatic portal vein. Each sample was placed in a dry clean centrifuge tube, and then centrifuged for 10 minutes at 3000 revolutions per minute "r.p.m" to separate the serum. Serum was carefully separated into dry clean eppendorf tubes by using Pasteur pipette and kept frozen at-20C^{o} till analysis. Liver was removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution, dried by filter paper, weighed then kept in 10% formalin for histological examination. At the end of the experimental body weight gain, was calculated according to the following equations **(Chapman *et al*., 1959)**

Body weight gain = Final weight (g) – Initial weight (g)

Biochemical analysis

Hepatic liver functions determination including , alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined according to **Bergmeyer *et al*., (1986),** alkaline phosphates according to King and King, (1954), total protein according to **Sonnenwirth and Jaret , (1980)** and total bilirubin according to **Suzuki and Sakagishi, (1994).**

Lipid peroxidation assay include malondialdhyde (MDA), the extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of **Ohkawa *et al.* (1979).**

The liver content of (GSH) was determined as described by **Bulaj *et al.*, (1998)**. The liver activities of (SOD) and (CAT) enzymes were determined according to **Kakkor *et al, (1984)*** and **Sinha, (1972**) respectively.

Histological Examination

Histological examination of the tissues was conducted after removal of liver tissues from rats. The tissues were gently rinsed with a physiological saline solution (0.9% NaCl) to remove blood and adhering debris. They were then fixed in 5% formalin for 24 h, and the fixative was removed by washing overnight with running tap water. After dehydration through a graded series of alcohols, the tissues were cleared in methyl benzoate and embedded in paraffin. Sections were cut by a microtome at 6 µm thickness and stained with Hematoxylin staining as described by Gabe and counter stained with eosin dissolved in 95% ethanol). After dehydration and clearing, sections were mounted with digital picture exchange and observed under a microscope. The histopathological examination was performed according to **Lobenhofer *et al.*, (2006*).***

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0. Data were given in the form of arithmetical mean ± SE. Differences between groups were evaluated by One-way ANOVA according to **Armitage and Berry, (1987).**

Results and Discussion

Total phenolic, total flavonoid contents and antioxidant activity

Results of total phenolic content, total flavonoids and antioxidant capacity (ABTS) are shown in Table 1. The amount of total phenolics and total flavonoids in black cherry were 4.388 mg GAE/100 g and 6.533 mg QE/ 100 mg dry weight respectively. Another stable free radical cation, ABTS, was used to evaluate the antioxidant activity of black cherry extract. Where, a steady increase was observed in the percentage inhibition of the ABTS radicals by the black cherry extract (table 1).

Total phenolic and total flavonoid represent the total amounts for phenolic and flavonoid compounds in the black cherry. The chemical analyzes of black cherry showed a high content of total phenolic and flavonoids in black cherry. These results are in

agreement with data that point out that the phenolic and flavonoid contents in black cherry fruit were the highest ones compared with those values obtained from plums and grapes **(Luna-Vázquez *et al*., 2013)**. The ABTS systems have been commonly used to measure the total antioxidative status of various biological specimens by measuring radical scavenging through electron donation **(Rufián-Henares and Morales, 2007)**. Plants containing flavonoids have been reported to possess strong antioxidant activities **(Radami *et al*., 2003).** Results obtained indicate that the black cherry fruits are a rich source of natural antioxidants. Also, **Luna-Vázquez *et al*., (2013)** supports our results that black cherry fruit has a good antioxidant capacity, which could be accounted for its polyphenol content. Additionally, this fruit contains compounds such as hyperoside and chlorogenic acid that elicit antioxidant, vasodilator and antihypertensive effects.

Table (1): Total phenolic, total flavonoid contents and total antioxidant activity in black cherry extract

Total phenolic (mg of GAE /100g dry weight)	Total flavonoid (mg of QE/ 100 mg dry weight)	% Inhibition measured by the ABTS
4.39 ± 0.33	6.53 ± 0.46	85.94 ± 1.67

GAE: Gallic acid equivalent; QE: quercetin equivalents.

Biological evaluation:

Table (2) shows the mean value of feed intake, initial weight, final weight, body weight gain, liver weight in different rat groups. Liver injury by paracetamol caused significant reduction in feed intake, final weight and body weight gain, while increased liver weight of rats (+ve) control as compared to (–ve) control group(normal rats). On the other hand, the treatment with silymarin or black cherry (extract or dry) increased final weight, body weight gain and decreased the liver weight in comparison to paracetamol treated rats. Maximum improvement recorded for black cherry extract group.

The observed significant reduction of BWG, FI and FER in rats treated with paracetamol is consistent with previous findings for **Kanchana and Sadiq, (2011)** who concluded that the gain in body weight of paracetamol treated group rats has been reduced compared with normal control group and silymarin treated group. Also, **Dwivedi *et al.*, (2015)** who investigated the efficacy of livartho against paracetamol induced liver injury in adult *sprague dawley* rats. The results showed that a statistically significant decreased in body weight and feed consumption in paracetamol treated group as compared to normal control group.

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The reduction of mean body weight in APAP control group was mainly due to oxidative stress and reactive oxygen species generated in APAP-induced hepatotoxicity and altered metabolism of liver (**Mamat *et al.*, 2013)**. More detailed explanation was offered by **Morrison and Hark, (1999)** who showed that liver disease led to malnutrition and major causes of malnutrition in patients with liver disease are poor dietary intake, maldigestion, malabsorption and abnormalities in the metabolism and storage of macro and micro nutrients. In the present study, black cherry (dried and extract) showed significant the improvement in FI and BWG compared to (+ve). The improvement effects of black cherry may be due to its constituents that improve feed utilization efficiency. For example, in comparison with plums and grapes, black cherry fruit contains high levels of proteins (four times higher) and it has higher levels of carbohydrates than plum. Thus, this fruit is a good source of nutrients **(Luna-Vázquez *et al.*, 2013)**.

From the obtained results, it was increase liver weight had been observed in paracetamol-treated rats compared to rats in control groups. These results are in agreement with the recorded data of **Zakaria *et al.*, (2017)** who found that the rats intoxicated with APAP exhibited significant ($P < 0.05$) increase in liver weight and liver/body weight ratio when compared to rats in the normal control group. The enlargement of livers in paracetamol treated rats signified hepatic lesions and liver injury associated with the toxic effects of paracetamol (**Zakaria *et al.*, 2017**), where the major disorder encountered in APAP-induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition **(Mol and Raja, 2010).**

Table (2): Feed intake, initial weight, final weight, Body weight gain and liver weight in control and different treated groups

Groups	feed intake (g)	Initial weight (g)	final weight (g)	Body weight gain(g)	liver weight (g)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group(1) (-ve) Control	542.00±17.82^{ab}	187.81±5.91^{a}	232.52± 7.50^{a}	46.69±4.61^{a}	9.41 ±0.56^{d}
Group(2) (+ve) Control	413.00± 10.40^{d}	187.02±7.30^{a}	209.00±4.91^{d}	22.13± 6.92^{d}	11.85±0.99^{a}
Group(3) silymarin group	514.00± 9.89^{c}	185.50± 7.52^{a}	220.60 ±7.30^{bc}	35.20± 1.50^{b}	10.98±0.87^{ab}
Group(4) dry black cherry 10%	529.67± 6.92^{b}	188.53 ±7.81^{a}	216.83± 7.93^{cd}	28.51± 1.81^{c}	11.10±0.64^{ab}

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Group(5) black cherry extract (500mg/kg)	548.83± 9.51[a]	187.80±7.86[a]	226.00±5.00[ab]	38.53± 3.43[b]	10.40±0.76[b]

Each value is the mean ±SD. Mean values in the same column having different superscript letters are significantly different at P ≤ 0.05.

Effect of dried and extract of black cherry on liver enzymes, albumin, and total protein in acetaminophen induced hepatotoxicity in rats.

Liver enzymes (AST, ALT and ALP) were raised due to paracetamol administration. Meanwhile treatment with extract and powder of the black cherry or silymarin lowered the levels of liver enzymes. Best treatment for AST was that of the silymarin group followed by black cherry extract, while the best treatment for ALT and ALP were that of black cherry extract followed by silymarin group. Also, there was a significant decrease in total protein and albumin, but increase in Total bilirubin in the serum of (+ve) control group as compared to (-ve) control group (normal rats).

The aforementioned increase of liver parameters in rats treated with paracetamol compared to normal rats indicates that paracetamol induced liver injury, where the deleterious effect of the toxic acetaminophen (APAP) dose on liver function is emphasized by significant elevation in plasma AST, ALT, and ALP activities in APAP rats, as compared to normal group. The activities of (ALT, AST and ALP) used as biochemical markers for evaluation of early hepatic injury **(Abdel Azim *et al.*, 2017).** The liver is involved in the metabolic transformation of drugs, which predisposes it to drug-induced damages. The increase in the activities of AST and ALT in serum of rats treated by paracetamol might be due to increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream **(Rao and Das, 2014).** Additionally, **Alam *et al.*, (2017)** demonstrated that the serum level of hepatic enzymes AST, ALP and ALT were increased, reflecting the hepatocellular damage in the paracetamol (PCM) induced hepatotoxicity in animal model. Data from this study suggested that treated with extract and powder of black cherry improved the level of serum AST, ALT and ALP due to high phenolic content and antioxidant properties. Montmorency tart cherries (*Prunus cerasus* L.) are a rich source of polyphenolic compounds, especially proanthocyanins, anthocyanins, and flavonols, all of which are strong antioxidants (**Ferretti *et al.*, 2010).**

Albumin and globulin are the main components of total protein in the plasma and serum. Albumin is mainly synthesized by the liver. In drug-induced hepatotoxicity, albumin synthesis will be depleted due to cirrhosis and this will lead to a reduction in TP, which may reflect nutritional state, proteins synthesis state and others in liver disease

(Ravichandrab *et al.*, 2013). In the present study, administration of paracetamol caused reduction in TP and albumin compared with control normal group this is in agreement with previous findings by **Alam *et al.*, (2017)** who reported that the serum level of Alb and TP were decreased, reflecting the hepatocellular damage in the PCM induced hepatotoxicity in animal model. Also, **Abdel Azim *et al.*, (2017)** concluded that the total protein and albumin levels decreased in group treated with PCM. These results reflect the incidence of liver dysfunction by paracetamol. Significant decrease in total protein and albumin due to disorder in synthetic function of liver **(Ogutcu *et al.*, 2008),** but the results obtained, referred that treatment of extract and powder of black cherry improve the level of serum total protein and albumin may be due to high content of phenolic and flavonoid compounds in the black cherry. This effect correlated with that of the standard drug silymarin, an antioxidant and a hepatoprotective agent which is well distributed in the body particularly in the red blood cells and in the liver **(Presser, 2000).**

Table (3): Serum liver functions in control and different treated groups

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (mg/dl)	Albumin (mg/dl)	Total bilirubin
	Mean ±SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group(1) (-ve) Control	94.28±9.18^{d}	24.71±5.53^{d}	132.37±8.65^{e}	7.62±0.29^{a}	4.64±0.24^{a}	.26±0.04^{d}
Group(2) (+ve) Control	201.56±10.57^{a}	61.01±3.38^{a}	262.82±9.60^{a}	6.05±0.16^{d}	3.25±0.21^{d}	.69±0.08^{a}
Group(3) silymarin group	131.96±4.66^{c}	41.55±5.15^{c}	203.60±18.58^{c}	7.10±0.22^{b}	4.39±0.19^{ab}	.35±0.04^{c}
Group(4) dry black cherry 10%	156.45±5.86^{b}	49.02±7.06^{b}	234.90±12.0^{b}	6.72±0.28^{c}	3.95±0.15^{c}	.46±0.05^{b}
Group(5) black cherry extract (500mg/kg)	137.16±9.17^{c}	38.88±7.33^{c}	183.58±14.0^{d}	7.08±0.21^{b}	4.22±0.25^{b}	.29±0.03^{cd}

Each value is the mean ±SD. Mean values in the same column having different superscript letters are significantly different at $P \leq 0.05$.

Effect of dried and extract of black cherry on malondialdhyde, GSH level and activities of antioxidant enzyme (SOD and CAT) levels in acetaminophen induced hepatotoxicity rats.

Findings presented in table (4) revealed that paracetamol administration lowered significantly the levels of antioxidant enzymes SOD, CAT and GSH in hepatic tissue while increased hepatic MDA level. Meanwhile treatment with extract and powder of the black cherry or silymarin reversed such changes. The best result recorded by the silymarin group followed by black cherry extract.

Superoxide dismutase (SOD) and catalase (CAT) are the major antioxidant enzymes that stand in the first-line of defense against oxidative damage **(Maturu *et al.*, 2012).** These antioxidants play a key role in scavenging reactive oxygen species(ROS), reduction in hydrogen peroxide and maintaining redox balances in biological system (**Padmavathi *et al.*, 2009).**

Catalase is one of the most important intracellular enzymes in the detoxification of the oxidant hydrogen peroxide. The reduction in the activities of CAT and SOD enzymes in APAP rats was attributed to the increased utilization of these enzymes in scavenging and neutralizing the free radicals and lipid peroxides **(Singh *et al.*, 2011).** where, Following an acute overdose of acetaminophen, there is saturation of the conjugation metabolism pathways in the liver, resulting in more drug being oxidized into the toxic metabolite, N-acetyl-pbenzoquinone imine (NAPQI), by the cytochrome P450 (CYP450) enzyme, CYP2E1 **(Holt and Ju, 2006).** It was found that induction of cytochrome P450 (CYP450) isoforms (CYP2E1, CYP3A4, and CYP1A2), depletion of intracellular glutathione and oxidative stress are the major mechanisms involved in the pathogenesis of APAP induced liver injury **(Bessems and vermeulen, 2001).**

Glutathione (GSH) is the major intracellular redox buffer in the liver and is critical for hepatic detoxification of xenobiotics and other environmental toxins. Hepatic glutathione is also a major systemic store for other organs and thus impacts on pathologies. The most significant site of GSH production is the liver **(Thomas *et al.*, 2015).**

Liver injury induced by paracetamol significantly ($p<0.05$) decreased serum levels of SOD, CAT and GSH in the rats treated with PCM. Similar results were reported by **Moke *et al.*, (2019)** who found that paracetamol caused a significant ($p<0.05$) increase in liver enzymes, significant ($p<0.05$) decrease in antioxidant enzymes (SOD, and CAT) were observed in PCM control group as against those in normal control group. However, there was a significant ($p<0.05$) improvement (increase) in both SOD, CAT and GSH levels in animals treated with extract and powder of the black cherry or silymarin when compared with the PCM control group (Table 4). Increased levels of the antioxidant indices by black cherry extract were very much similar to that of the standard drug silymarin.

Table (4): Liver MDA, SOD, CAT and GSH in control and different treated groups

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Groups	MDA (mmol/dl)	SOD (mmol/dl)	CAT (mmol/dl)	GSH (mmol/dl)
	Mean ±SD	Mean ± SD	Mean ± SD	Mean ± SD
Group(1) (-ve) Control	2.18±0.20^{c}	57.59±**7.77**^{a}	0.78±0.13^{ab}	1.65±0.11^{a}
Group(2) (+ve) Control	6.84±0.98^{a}	32.65±3.07^{d}	0.38±0.07^{d}	0.87±0.15^{d}
Group(3) silymarin group	2.78±0.24^{c}	51.41±4.99^{b}	0.81±0.02^{a}	1.61±0.08^{a}
Group(4) dry black cherry 10%	4.36±0.32^{b}	44.88±3.39^{c}	0.55±0.11^{c}	1.25±0.15^{c}
Group(5) black cherry extract (500mg/kg)	3.76±0.39^{b}	50.18±3.25^{bc}	0.67±0.95^{b}	1.41±0.09^{b}

Each value is the mean ±SD. Mean values in the same column having different superscript letters are significantly different at P ≤ 0.05.

Histopathology examination results

Fig. 1: Microscopic pictures of H&E stained hepatic sections showing normal radially arranged hepatocytes around central vein (CV) with normal sinusoids (s) in control group (a) and group received silymarin only (c). Hepatic sections from group received paracetamol (b) showing hydropic degeneration (black arrow) in hepatocytes, portal congestion (red arrow) and inflammation (yellow arrow) with occluded sinusoids. Marked hepatocytes degeneration (black arrow), portal congestion (red arrow), and occluded sinusoids with milder degrees of portal inflammation (yellow arrow) appear in group received dry black cherry 10 % (d). Mild degrees of hepatocytes degeneration (black arrow) and congestion (red arrow) with partially occluded sinusoids and absent portal inflammation appear in group received black cherry extract (500 mg/kg) (e). These results demonstrated protection of the liver by administration of extract and powder of the black cherry or silymarin from PCM-induced hepatotoxicity in rat's liver. This may be due to antioxidant and hepatoprotective activity of black cherry, which is probably due to presence of phenolic acids and flavanoids, such as chlorogenic acid. According to **Luna-Vázquez *et al.*, (2013).**

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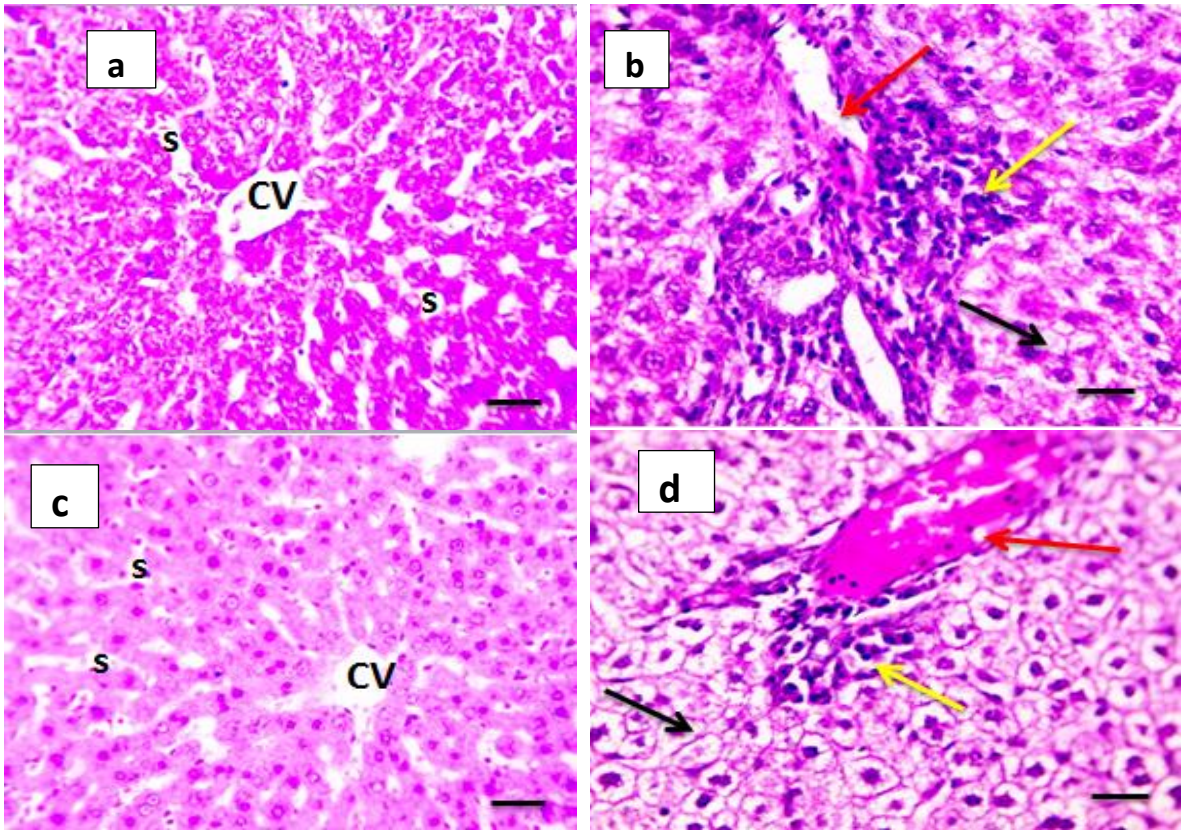

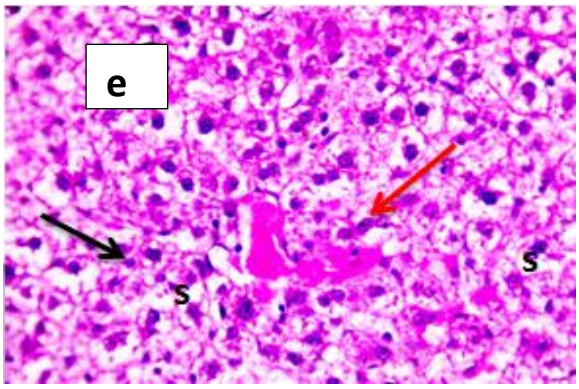

Figure (1) liver histopathology: Normal group (a) paracetamol-treated animals (b), silymarin-treated animals (c), group received dry black cherry 10 % (d) and group received black cherry extract (500 mg/kg) (e).

In conclusion, results obtained indicate that administration of paracetamol at 400 mg/kg to rats induced oxidative stress in the liver. Therefore, the observed hepatoprotective and antioxidant activity of black cherry could be attributed to the presence of polyphenols and flavonoids that can function as an effective free-radical scavenger.

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تحسين الضررالكبدي المحدث بواسطة عقار الاسيتامينوفين باستخدام مسحوق ومستخلص الكرز الأسود في الفئران

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المستخلص العربي

الضرر الكبدي مشكلة صحية كبيرة ، فالتعرض المستمر للأدوية والالتهابات الفيروسية والبكتيرية والسموم البيئية يمكن أن يؤدي إلى إصابة الكبد ويؤدي في النهاية إلى أمراض الكبد المختلفة. يهدف البحث الحالي إلى توضيح التأثير الوقائي للكرز الأسود على الضرر الكبدي الناجم عن إعطاء عقار الاسيتامينوفين(APAP) المعروف باسم (الباراسيتامول) للذكور البالغين من الفئران البيضاء. تم تقسيم الحيوانات إلى خمس مجموعات (كل مجموعة مكونه من ستة فئران). تم الاحتفاظ بالمجموعة الأولى كمجموعة ضابطة سالبة تتغذي على الغذاء الأساسي فقط و تغذت المجموعات الأربعه الأخرى على الغذاء الأساسي والباراسيتامول (400 مجم / كجم من وزن الجسم) عن طريق أنبوب معدي لمدة أسبوع لإحداث الضرر الكبدي. وبعد ذلك صُنفت فئران الإصابة الكبدية إلى المجموعة الضابطة الموجبة وهي المجموعة الثانية وتغذت على الغذاء الأساسي بينما تغذت المجموعة الثالثة والرابعه والخامسه علي التوالي سيليمارين 100 مجم / كجم / يوم و 10٪ كرز أسود مجفف و 500 مجم / كجم / يوم مستخلص الكرز الأسود. استمرت التجربة لمدة 28 يومًا. تم تحليل إجمالي مضادات الأكسدة الكلية والبولي فينول والفلافونويدات في مستخلص الكرز الأسود. تسبب استخدام الباراسيتامول في حدوث زيادة معنوية في مستويات مصل الألانين أمينوترانسفيراز (ALT)والأسبارتات امينوترانسفيراز (AST) مالونالدهيد الكبد .(MDA) ، وانخفاض كبير في الكاتلاز الكبدي (CAT)، الجلوتاثيون (GSH). وقد كان المحتوي الكلي للفينولات والفلافونويدات ومضادات الاكسدة علي التوالي كما يلي (0.33 ± 4.39 , 0.46 ± 6.53 , 1.67 ± 85.94) . واوضحت النتائج ان العلاج بالسيليمارين ، الكرز الأسود المجفف ومستخلص الكرز الأسود يحمي من إصابة الكبد كما يتضح من الانخفاض الكبير في محتوى MDA ، بالإضافة إلى زيادة كبيرة في نشاط CAT الكبد و GSH واستخلصت النتائج ان الكرز الأسود هو مصدر غني للبولي فينول والفلافونويد و مضادات الأكسدة الكلية لذلك قد يكون له دور وقائي ضد إصابة الكبد في الفئران.

الكلمات المفتاحية: الفلافونيدات – جلوتاثيون– إنزيمات الكبد– المالونالدهيد